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**ANALYTIC EXPRESSIONS FOR MAXIMUM LIKELIHOOD
ESTIMATORS IN A NONPARAMETRIC MODEL OF TUMOR
INCIDENCE AND DEATH**

by

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IN A NONPARAMETRIC MODEL OF TUMOR INCIDENCE AND DEATH

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ABSTRACT

PAIGE L. WILLIAMS. Analytic Expressions for Maximum Likelihood Estimators in a Nonparametric Model of Tumor Incidence and Death (under the direction of Dr. Christopher J. Portier)

ABSTRACT: The primary objective of a long-term animal carcinogenicity experiment is the comparison of tumor incidence rates among treatment groups. Complications arise in the statistical analysis of tumor incidence data when the tumor type of interest is not observable. Since reliance on assumptions regarding tumor and treatment lethality is likely to introduce bias, this research focuses attention on the estimation of tumor incidence rates from long-term animal studies which incorporate interim sacrifices. A nonparametric stochastic model is described with transition rates between states corresponding to the tumor incidence rate, overall death rate, and death rate for tumor-free animals. Exact analytic solutions for the maximum likelihood estimators (MLE's) of the discrete hazard rates are presented, and constrained MLE's are derived for a study design with up to three intervals under the imposition of boundary constraints. For a study design with more than three intervals, alternative estimators of the discrete death rates and tumor incidence rate are developed heuristically by pooling together data from adjacent time intervals. The estimators derived in this research are applied to actual data from a long-term animal study, and the results are compared with other published estimates of the tumor incidence rates for the same data. The ability of these estimators to predict the true tumor incidence rate under a variety of study designs is evaluated by Monte Carlo simulation studies.

The availability of closed-form expressions for the MLE's of the tumor incidence rate and death rates represents a dramatic savings in time over the iterative computer algorithms currently used, in addition to avoiding biases from inappropriate assumptions regarding tumor and treatment lethality. The constrained MLE's exhibit only a small degree of bias even within the small samples typically employed in long-term animal studies, and appear to have smaller variances than the unconstrained MLE's. For study designs with

more than three time intervals, estimators derived from intuitively pooled data appear to offer a reliable alternative to the constrained estimators.

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CHAPTER I: STATISTICAL ANALYSIS OF LONG-TERM ANIMAL STUDIES

1.1 Role of Long-Term Animal Studies in Risk Assessment

A rapid increase in the number of chemicals in common use has brought with it an increasing need for qualitative and quantitative methods of evaluating the potential adverse effects from chemical exposure. The assessment of a chemical compound for carcinogenic potential in humans is based upon many sources of information. Epidemiologic observations gathered on human subjects, *in vitro* assays of mutagenicity, study of DNA damage and repair in mammalian cells, examination of chemical structure, pharmacokinetic studies, and short-term and long-term animal carcinogenicity studies all play a part in evaluating the risk of chemical exposure to man.

Recognition of a chemical substance as a human carcinogen has been met with the least controversy when sound epidemiologic evidence of carcinogenic risk factors was available. However, the difficulties inherent in controlling and measuring individual exposures, the long latency period between exposure to the chemical and onset of cancer in humans, and the typically low levels of exposures tend to prevent direct measurement of risk in epidemiologic surveys. Clearly, use of clinical trials with human subjects to perform risk assessment cannot be considered an ethical alternative. The lack of controlled experiments in humans has led to the need to employ animal carcinogenicity studies to predict human risk. In the limited number of chemicals for which both human and animal experimental data are available, there appears to be a good qualitative correlation (Tomatis *et al.*, 1980a).

Although methods for extrapolating animal carcinogenicity studies to human populations have become a center of controversy, long-term animal carcinogenicity studies remain the most commonly used tool for evaluating the carcinogenic potential of compounds in our food, environment, and workplace. Short-term studies can evaluate a large number of chemicals quickly and at a relatively low cost in comparison to long-term animal studies; however, a recent evaluation of the ability of these tests to predict chemical carcinogenicity in rodents demonstrated a lack of both sensitivity and specificity for the four most common short-term tests (Tennant et al., 1987). While the advantages of these short-term methods should not be overlooked, "in the absence of human data, long-term animal tests are still today the only ones capable of providing conclusive evidence of the carcinogenic effect of a chemical" (Tomatis, 1980b).

1.2 Design of Long-Term Animal Studies

The goal of a long-term animal carcinogenicity experiment is to compare animals treated with a particular chemical compound to untreated animals with respect to tumor development. In order to accomplish this goal, the design of a carcinogenicity bioassay must direct attention at the characteristics of the chemical compound, the sex, species, and number of animals to be tested, the dosage levels and route of administration of the test substance, the randomization of animals to dosage levels, the duration of exposure, and the collection of clinical and histopathologic observations. The experimental design must include recruitment of experienced personnel to plan, conduct, and analyze the results of the long-term animal experiment.

The standard animal bioassay guidelines first developed by the National Cancer Institute (NCI, Sontag *et al.*, 1976) and later adopted and refined by the National Toxicology Program (NTP, Haseman, 1983) specify use of both male and female animals of at least two species. Rodents such as mice, rats, and Syrian hamsters are most

commonly employed based on their small size, short lifespan, and availability. In addition to these practical advantages, inbred strains of these rodents have been developed which exhibit fairly uniform responses to chemical insults as a result of their genetic homogeneity.

The levels of doses for a long-term animal study are based on subchronic toxicity studies which determine for each sex and species the maximum tolerated dose (MTD), i.e., the highest dose that can be given that does not cause a significant decrease in survival from effects other than carcinogenicity. Some definitions of the highest dose have also required that it does not appreciably inhibit normal weight gain as compared with control animals (Food Safety Council 1978, IRLG 1979). The number of dose levels employed by the long-term study depends on whether the specific purpose of the long-term carcinogenicity study is the evaluation of the carcinogenic potential of a chemical compound on a qualitative basis (screening assay) or determination of possible dose-response relationship. The original NCI bioassays were designed as carcinogenic screening assays and define only three experimental groups: high dose (MTD), low dose (1/2 or 1/4 MTD), and control (untreated). The MTD is used as the high dose to maximize the probability of observing a carcinogenic effect if one exists, and the lower dose is included to confirm the evidence and protect against early mortality in the high dose group due to toxicity from the MTD. If the purpose of the long-term bioassay is to examine the dose-response relationship, three or more test doses in addition to a control are typically employed; the current NTP guidelines suggest three dose levels in addition to an untreated control (Haseman, 1983). Most bioassay designs recommend a minimum of 50 animals in each sex, species, and dose level cross-classification for testing each potential carcinogen. The route of administration is chosen to most closely mimic the human route of exposure.

The final step before starting a long-term animal study is the randomization of animals to experimental groups. The purpose of randomization is to prevent the bias that

could occur if the predisposition to tumor development is not the same in all treatment groups. Animals are first stratified by factors thought to affect the predisposition to tumor development, such as body weight, litter and age, and then animals are randomly allocated to dose levels within strata. In some study designs, cage locations are also randomized to protect against environmental gradients within the animal facility.

Treatment is initiated a few weeks after the weaning period and continues for an exposure period which constitutes the major portion of the animals' expected lifespan. A commonly used treatment period for mice and rats is 2 years, which constitutes 60-70% of the lifespan of the breeds of mice and rats most commonly used (Portier *et al.*, 1986). The animals are observed daily or weekly for indications of tumor onset (if it is possible to observe this endpoint) or other clinical abnormalities, and a complete necropsy is performed for any animal found dead. If the cause of death can be ascertained, it is recorded along with the time of death. At the end of the treatment period, all surviving animals are subjected to a complete necropsy and histopathologic examination. Specific sites at which tumors are observed are recorded, along with cause of death information, if available.

Occasionally, designs for long-term animal experiments may designate timepoints at which interim sacrifices (planned kills prior to study termination) are to be performed in order to obtain more information on the progression of the tumor over time. Generally, an equal number of animals from each dose group are randomly selected to be sacrificed and necropsied from those animals surviving to the chosen sacrifice time. Since interim sacrifices reduce the number of animals at risk of cancer development at later times, more animals should be included in an animal bioassay design with planned interim sacrifices. Reviews and summaries of the design concepts discussed here are provided by Feron *et al.* (1980), Hamm (1985), Gart *et al.* (1986), and Portier (1988).

1.3 Analysis of Long-Term Animal Experiments

Once a long-term carcinogenicity experiment has been designed and conducted, an analysis of the results must be performed based on the data collected. By convention, a separate analysis of the effect of a chemical compound is performed for each individual tumor site (Peto *et al.*, 1980). Since most carcinogens are believed to have a specific target organ or group of organs, site-specific analyses usually increase the power of detecting a true carcinogenic effect. In fact, a recent comparison of site-specific and overall analyses of NTP tumor incidence data found that less than half of the species- and sex-specific effects regarded as carcinogenic by site-specific analyses remained significant if primary emphasis was given to the overall proportion of animals with primary or malignant tumors (Haseman *et al.*, 1986). Although designs have been proposed in which the cage or litter is the experimental unit, knowledge of these types of clustering effects is still limited and the statistical analysis of most studies continues to treat the individual animal as the experimental unit. This assumption also implies that an animal with a single tumor at a given site is treated the same as an animal with multiple tumors at this site.

1.3.1 Basic Notation and General Statistical Framework

In the construction of a long-term carcinogenicity study, suppose that a total of N animals are randomly assigned to $K+1$ dose groups, and the N_i animals in dose group i receive dose z_i , where $z_0 < z_1 < \dots < z_K$. The control group is indexed by $i=0$ and receives dose $z_0=0$. Let t_{ij} be the time at which the endpoint of interest (e.g., tumor onset or death) is observed for the j -th animal in the i -th dose group, $j=1,2,\dots,N_i$.

For the purposes of summarization and statistical analysis, it is often desirable to divide the total length of the long-term experiment into S time periods with endpoints at t_s , $s=1,2,\dots,S$. For the i -th dose group and the s -th time period, let x_{is} be the number of events (deaths or tumor onsets) and let r_{is} be the number of animals at risk of developing

the response of interest. The data corresponding to the experimental endpoints t_{ij} can be summarized in $S \times (K+1)$ contingency tables of the following form.

Contingency Table for Time Period s

Dose	z_0	z_1		z_K	Total
No. of events	x_{0s}	x_{1s}		x_{Ks}	$x_{.s}$
No. at risk	r_{0s}	r_{1s}		r_{Ks}	$r_{.s}$

In a long-term carcinogenicity experiment, it is assumed that the probability that a single experimental endpoint occurs in a given time period is independent of the probability that the endpoint falls into any other time period for animals in each dose group; the data thus follows the multinomial distribution. Under the null hypothesis of equal tumor incidence rates among dose groups, the probability of observing an event is the same for all dose groups and can be estimated by the ratio of the total number of events to the total number of animals at risk within the specified time period, or $x_{.s} / r_{.s}$. The expected number of events for the i -th dose level and s -th time period computed under the null hypothesis in the multinomial framework is thus $E_{is} = r_{is} * (x_{.s} / r_{.s})$. These expected values can be summed over time periods to obtain the total number of expected events for each dose level, $E_i = \sum E_{is}$.

Several authors have shown that the most commonly used tests for carcinogenicity can be expressed as particular cases of a generic test statistic, Z_G , which is asymptotically distributed as a standard normal variate (Bailer and Portier, 1988), or $X^2 = (Z_G)^2$, which is asymptotically distributed as a chi-square random variable (Gart *et al.*, 1986). The

construction of the test statistic Z_G is accomplished by dividing a "trend" statistic -- the weighted sum of differences between observed and expected number of events of interest over strata defined by dose groups -- by the square root of its corresponding variance under the assumption of independent strata (Mantel and Haenszel, 1959). The weights are taken to be either the actual doses or dose rankings. More specifically, the test statistics can be calculated as shown below:

$$Z_G = \frac{T_G}{V_G}, \quad \text{where} \quad T_G = \sum_{i=0}^K z_i (x_i - E_i),$$

$$V_G^2 = \sum_{s=1}^S \left\{ \left(\frac{x_{.s}}{r_{.s}} \right) \left(\frac{r_{.s} - x_{.s}}{r_{.s}} \right) \left(\frac{r_{.s}}{r_{.s} - 1} \right) \sum_{i=0}^K r_{is} (z_i - \bar{z}) \right\}, \quad \text{and} \quad \bar{z} = (r_{.})^{-1} \sum_{i=0}^K r_{i.} z_i.$$

Although the same generic test statistic can be used for several different types of statistical procedures, both the assumptions and the interpretations of the procedures may differ appreciably. The main differences between many of the statistical procedures to be discussed lie in the response of interest t_{ij} , the construction of the S time-dependent strata, and in the specification of the number of animals at risk, r_{is} . In order to discuss how these criteria are determined, the following indicator variables are defined for each experimental endpoint.

δ_0 = indicator of type of tumor	($\delta_0=0$ if observable, 1 if occult)
δ_1 = indicator of tumor presence	($\delta_1=0$ if not present, 1 if present)
δ_2 = indicator of cause of death	($\delta_2=0$ if sacrifice, 1 if natural death by unknown cause, 2 if natural death by competing causes, and 3 if natural death due to tumor)

In general, δ_{kij} is defined as the value of the indicator δ_k ($k=0, 1$, or 2) for the j -th animal in the i -th dose group. It must be noted that all of the possible values of the indicator variables defined above may not be available to a particular long-term carcinogenicity study. Within the context of a single study, the tumor type will generally remain constant for all experimental endpoints. In many studies, only subsets of the values of the cause of death indicator, δ_2 , will be appropriate.

If $\delta_0=0$ and $\delta_1=1$, then the experimental endpoint t_{ij} is taken to be the time of tumor onset for an observable tumor, i.e., one which can be identified visually or by palpation. In all other cases, t_{ij} is taken to be the time of death. For example, if $\delta_0=1$, $\delta_1=1$, and $\delta_2=1$, then t_{ij} is the time of natural death for which histopathologic examination revealed an occult tumor, i.e., an internal tumor which can be discovered only at necropsy. When a tumor is observed in an animal that died from causes completely unrelated to the tumor (competing causes or sacrifice), the tumor is said to be observed in an "incidental" context. A tumor observed in an animal whose death was directly or indirectly attributable to the tumor of interest is said to be observed in a "fatal" context.

The three indicators δ_0 , δ_1 , and δ_2 together define the amount and types of data available from a long-term animal study, and thus the appropriate type of analysis. Although there are 16 theoretical possibilities for the combinations of the three indicators, there are only nine practical possibilities, as shown in Table 1.3.1.1. For situations in which no tumors of the given type are discovered at the time of sacrifice or death, then the cause of death cannot be due to the tumor, and must therefore be due to competing causes (including sacrifice). The cases in which the triplet $(\delta_0, \delta_1, \delta_2)$ is equal to $(0,0,1)$, $(0,0,3)$, $(1,0,1)$, or $(1,0,3)$ are thus eliminated from consideration. When an observable tumor type is determined to be present, then t_{ij} is the time of tumor onset and no death status indicator is necessary. The four theoretical possibilities $(0,1,0)$, $(0,1,1)$, $(0,1,2)$, and $(0,1,3)$ are

thus combined into the one practical possibility (0,1, .).

Based on the cross-classification of tumor type, tumor status, and death status, statistical analyses of long-term carcinogenicity experiments can be considered to fall into one of four general categories: (1) analysis of observable tumors, (2) analysis of occult tumors when cause of death is known, (3) analysis of occult tumors when cause of death is unknown, but assumptions are made regarding cause of death or interim sacrifices are incorporated, and (4) analysis of occult tumors with unknown cause of death, no assumptions regarding cause of death, and no interim sacrifices. These four categories range from the situation in which the most informative set of data possible is available to the situation when the least information is available regarding tumor incidence.

Table 1.3.1.1

Possible Combinations of Tumor Type, Tumor Status, and Death Status

Group	t_{ij} time of	δ_0 Tumor Type	δ_1 Tumor Status	δ_2 Death Status	Description
(1)	Death	0	0	0	observable tumor not present at sacrifice
(2)	Death	0	0	2	observable tumor not present at death from competing causes
(3)	Tumor Onset	0	1	.	observable tumor present
(4)	Death	1	0	0	occult tumor not present at sacrifice
(5)	Death	1	0	2	occult tumor not present at death from competing causes
(6)	Death	1	1	0	occult tumor present at sacrifice
(7)	Death	1	1	1	occult tumor present at death, cause of death unknown
(8)	Death	1	1	2	occult tumor present at death from competing causes
(9)	Death	1	1	3	occult tumor present at death due to tumor

1.3.2 Case I: Analysis of Observable Tumors

The ideal statistical analysis relies on the primary assumption that the tumor to be studied is detectable either visually or by palpation, so that the time of tumor onset is directly observable. In terms of the three indicator variables, this occurs when $\delta_0=0$. Under the assumptions that the tumor type under study is observable and irreversible, a standard lifetable method of analysis can be used to test for differences in tumor incidence (Tarone, 1975). The observed tumor onset times t_{ij} for group (3) are ordered from lowest to highest to indicate the end of each stratum t_s , and $t_s=t_{ij}$ where $s=\text{rank}(t_{ij})$ among those animals with observable tumors (i.e., $\delta_0=0$ and $\delta_1=1$). For the i -th dose and s -th time period, the number of observed tumors can be calculated by

$$x_{is} = \sum_{j: t_{s-1} < t_{ij} \leq t_s} (1 - \delta_{0ij}) \delta_{1ij} .$$

The number of censored observations, c_{is} , is defined to be those animals dying without the observable tumor, i.e.,

$$c_{is} = \sum_{j: t_{s-1} < t_{ij} \leq t_s} (1 - \delta_{0ij}) (1 - \delta_{1ij}) .$$

The number at risk, r_{is} , is defined to be those animals alive and tumor-free immediately before the stratum delimiter, so that

$$r_{is} = n_i - \sum_{j=1}^{s-1} (x_{ij} + c_{ij}) .$$

McKnight and Crowley(1984) state that the response of interest in the analysis of all long-term carcinogenicity experiments is the tumor incidence rate, or the instantaneous

probability of tumor development in an alive tumor-free animal. Since the analysis of observable tumors is based on the times of tumor onset, the incidence test is directly aimed at testing for differences in tumor incidence rates. In this sense, the incidence test represents the ideal against which all other tests should be compared. However, its practical merit ranks very low among the tests to be discussed, as the times of tumor onset are rarely observable. Nuclear magnetic resonance (NMR) techniques may some day allow accurate identification of the types of internal tumors which are currently regarded as occult. Until these methods have been further developed, the analysis of observable tumors serves primarily as a hypothetical standard for comparison in simulation studies.

1.3.3 Case II: Analysis of Occult Tumors, Known Cause of Death

When the tumor type of interest is occult ($\delta_0=1$), but the cause of death is known ($\delta_2=0, 2$, or 3), then analysis proceeds in a straightforward manner. A review of the statistical methods applicable to the case in which the cause of death is known is provided by Kalbfleisch et al. (1983). Separate analyses are performed for tumors observed in an incidental context ($\delta_2=0$ or 2) and those observed in a fatal context ($\delta_2=3$), as described below.

1.3.3.1 Analysis of Incidental Tumors

Since incidental tumors are assumed to have no effect on mortality, the prevalence method proposed by Hoel and Walburg (1972) can be used to test for equal tumor incidence in all dose groups. The time period encompassed by the long-term carcinogenicity study is subdivided into smaller time periods, with the terminal sacrifice constituting its own stratum. The time periods can be chosen arbitrarily, although Peto *et al.* (1980) discuss an adaptive interval method for defining suitable subdivisions based on tumor prevalence data. The National Toxicology Program uses the strata defined in weeks as 0-52, 53-78, 79-92, 93-Terminal Sacrifice, and Terminal Sacrifice (Haseman, 1984).

Within each time-dependent stratum and dose group, x_{is} is the number of deaths for which the tumor of interest is observed at necropsy and c_{is} is the number of animals which die without the tumor of interest, computed as shown below:

$$x_{is} = \sum_{j: t_{s-1} < t_{ij} \leq t_s} \delta_{0ij} \cdot \delta_{1ij} \quad \text{and} \quad c_{is} = \sum_{j: t_{s-1} < t_{ij} \leq t_s} \delta_{0ij} (1 - \delta_{1ij})$$

The number of animals at risk, r_{is} , is defined as the total number of deaths within a time period, i.e., $r_{is} = x_{is} + c_{is}$.

An alternative method for comparison of tumor prevalence in the analysis of non-lethal tumors is the use of a logistic regression model which adjusts for differential survival by incorporating age as a covariable in the regression model (Dinse and Lagakos, 1983). This method avoids the difficulty and potential bias sometimes encountered by the Hoel-Walburg method in subdividing the length of study into smaller time intervals at the expense of using a parametric model for tumor prevalence. The logistic regression analysis models tumor prevalence as a function of both age and dose, allowing for incorporation of both discrete and continuous covariates. When mortality patterns differ across dose groups, extensive simulations have demonstrated that the logistic regression test is more powerful than the Hoel-Walburg test of dose-related trend in prevalence of non-lethal tumors (Dinse, 1985).

1.3.3.2 Analysis of Fatal Tumors

The analysis of fatal tumors, sometimes called a "death-rate" or lifetable analysis, is conducted in a manner similar to the analysis of observable tumors except that strata are formed based on the times of death for which tumors are observed, rather than the observed times of tumor onset (Tarone 1975, Haseman 1984). In terms of the generic test

statistic, x_{is} is the number of animals dying with the tumor present in the s -th stratum, c_{is} is the number of animals dying without the tumor of interest, and r_{is} is the number of animals in dose group i alive just prior to the s -th time period, as in the analysis of observable tumors.

1.3.3.3 Combination of Fatal and Incidental Tumor Analyses

The separate analyses of incidental and fatal tumors can be combined in an intuitive manner as described by Peto et al. (1980) when the tumor of interest can be observed in both contexts. This is possible if a group of animals has a high rate of mortality from causes unrelated to the tumor (termed "intercurrent mortality"), so that many potentially fatal tumors may be observed in an incidental context if tumor-bearing animals die of other causes before being killed by the tumor. The observed and expected number of events for incidental tumors are denoted in vector form by $O^I=(O^I_0, O^I_1, \dots, O^I_K)$ and $E^I=(E^I_0, E^I_1, \dots, E^I_K)$ respectively, and their corresponding variances and covariances in the matrix V^I . Similarly, the observed and expected numbers of events in the analysis of fatal tumors are denoted by O^F and E^F , and their corresponding covariance matrix by V^F . These vectors and covariance matrices are summed over analyses to obtain $O=O^I+O^F$, $E=E^I+E^F$, and $V=V^I+V^F$. The test statistic Z_G as presented in Section 1.3.1 is then based on these combined analyses.

Other authors have proposed models which estimate the joint distributions of time to tumor and time to death from tumor when individual tumors can be classified as either fatal or incidental. Kodell and Nelson (1980) describe an illness-death model for occult tumors under the assumption that the time-to-tumor, time-to-death from tumor, and time-to-death from competing risks follow independent Weibull distributions. Kodell, Shaw and Johnson (1982), Dinse and Lagakos (1982), and Turnbull and Mitchell (1984) derive nonparametric maximum likelihood estimators for the time to tumor and time to death from

tumor based on iterative procedures. All of these methods can incorporate interim sacrifice data but do not require it for estimation of the time to tumor and time to death distributions.

1.3.3.4 Potential for Bias in Analysis of Fatal or Incidental Tumors

Although the methods of analyzing long-term carcinogenicity studies appear to be straightforward when the cause of death is known, there is still the potential for bias. Both incidental and fatal tumor analyses require the assumption that tumor-bearing and tumor-free animals are equally healthy with regard to all life-shortening disorders, including toxicity, that are not caused by the presence of the tumor (Gart *et al*, 1986). It is often the case that the presence of tumors accelerates death more in exposed animals than in the control group due to some cause unrelated to the tumor of interest, especially in the early age intervals. Analyses of incidental tumors using prevalence methods actually test for equality of incidence rates only under strict assumptions (McKnight and Crowley, 1984). The choice of stratification intervals in the analysis of incidental tumors by the Hoel-Walburg method can bias comparisons of tumor prevalence rates when the intervals are forced to be too wide by the lack of natural deaths at comparable times in all dose groups (McKnight, 1988). Although prevalence tests are regarded as tests which avoid biases due to treatment-induced mortality, Bailer and Portier (1988) have shown that these test can be sensitive to effects of extreme treatment lethality. The analysis of fatal tumors can be biased by the fact that no distinction is made between rapidly lethal tumors and tumors which kill their hosts slowly. In addition, Bailer and Portier (1988) have shown that the lifetable analysis is extremely sensitive to the degree of treatment lethality.

Analyses based on the combination of fatal and incidental tumors can also suffer from a number of sources of bias. Lagakos (1982) has discussed possible biases resulting from misclassification of the context of observation. The type of analysis proposed by Kodell and Nelson (1980) is dependent upon the choice of a certain parametric model for

which the appropriateness may not be able to be validated, and also depends upon the strong assumption that the time until tumor onset and time until death from tumor are independent. A lack of interim sacrifice data may create difficulties in computation of the nonparametric maximum likelihood estimates of the time to tumor distribution using the methods proposed by Kodell *et al*, Dinse and Lagakos, and Turnbull and Mitchell.

1.3.4 Case III: Analysis of Occult Tumors, Unknown Cause of Death

In many long-term carcinogenicity studies involving non-observable tumors, it is not possible to classify deaths of tumor-bearing animals as occurring in an incidental context or fatal context. When the tumor type is occult ($\delta_0=1$) and the cause of death is unknown ($\delta_2=1$), then the analysis of the long-term carcinogenicity study must rely on additional assumptions about the nature of the tumor or additional data as provided by interim sacrifices. These additional assumptions or data are needed to allow for characterization of the tumor incidence rates while still adjusting for differential survival rates among the dose groups.

1.3.4.1 Assumed Cause of Death

In many cases in which the cause of death is unknown for an animal dying with an occult tumor, an assumption is made that the tumor type of interest is, in general, either incidental or fatal. If it is assumed that the tumor type is incidental, then the data are analyzed using the methods described in Section 1.3.3.1. If it is assumed that the tumor type is generally fatal, then the death-rate methods of analyzing fatal tumors are employed as described in Section 1.3.3.2. The assessment of the carcinogenicity of chemical compounds by the National Toxicology Program currently includes both a life table analysis under the assumption of fatal tumors and a prevalence analysis under the assumption of incidental tumors.

In some situations, it may be possible to determine the contexts of observation for some tumors but not others. In this case, Peto *et al.* (1980) recommend classification of individual occult tumors on an ordinal scale, with a score of 1 if the tumor is definitely incidental, 2 if probably incidental, 3 if probably fatal, and 4 if definitely fatal. Although the usual combined analysis would be based on grouping tumors classified as 1 or 2 together as incidental tumors and classifications 3 and 4 together as fatal tumors, other groupings can be used to assess the impact of misclassification. For instance, classifications 1, 2, and 3 can be considered incidental and classification 4 considered fatal, or classifications 2, 3, and 4 can be considered fatal and classification 1 incidental. Comparison of the interpretation of the usual combined analysis and the latter two suggested analyses can help to strengthen conclusions or indicate possible misclassification problems.

1.3.4.2 Interim Sacrifice Analyses

In the situation in which tumors are not classified as incidental or fatal and no assumption is made regarding the influence of tumor incidence on the risk of death, sacrifice data is essential to the estimability of the tumor incidence function (McKnight and Crowley, 1984). Nonparametric analyses suggested by Turnbull and Mitchell (1978), Mitchell and Turnbull (1979), McKnight and Crowley (1984), Dewanji and Kalbfleisch (1986), and Malani and Van Ryzin (1986) rely on numerous animals being sacrificed at various times during the experiment. Turnbull and Mitchell (1978) and Mitchell and Turnbull (1979) compare dose groups by fitting log-linear or logistic models to survival and sacrifice data and testing whether certain interaction terms are zero. McKnight and Crowley (1984), Dewanji and Kalbfleisch (1986), and Malani and Van Ryzin (1986) all propose comparison of tumor incidence rates by calculation of nonparametric maximum likelihood estimates based on multistate models for tumor development and death. These multistate frameworks are simple stochastic models of the transition rates between the states

of being alive and tumor free, tumor-bearing, and dead. The multistate model proposed by McKnight and Crowley is developed under a continuous time framework, while discrete multistate models are suggested by Malani and Van Ryzin and by Dewanji and Kalbfleisch.

Although the nonparametric methods for survival and sacrifice data discussed above avoid potential biases from both misclassification of contexts of observation and untestable assumptions regarding the relationship between tumor incidence and death, the extra cost associated with the additional animals necessary for interim sacrifices may prove prohibitive. In addition, intensive computational algorithms are sometimes necessary for estimating the nonparametric maximum likelihood estimators. Dinse (1988a, 1988b) has noted that the characterization of the tumor prevalence function from such nonparametric analyses can be severely limited by the number of interim sacrifice times, since estimates of the tumor incidence function can only be obtained for intervals defined by interim sacrifice times. To avoid the problem of unstable prevalence estimates sometimes produced by nonparametric methods, Dinse (1988b) suggests use of simple parametric functions of time to model a set of functions which can be transformed to obtain estimates of the tumor incidence rate. Comparisons of the results of the parametric model fitting with the results of nonparametric methods can provide an assessment of the appropriateness of the particular parametric model employed.

In order to balance the opposing desires of protection against bias and avoidance of numerous interim sacrifices, Portier (1986) considered a semi-parametric model which requires fewer interim sacrifices than the fully nonparametric analyses and fewer distributional assumptions than the fully parametric analyses. In these semi-parametric analyses, the tumor incidence function is modelled as a continuous parametric function, while no parametric restrictions are placed on the death rates with and without tumors. The semi-parametric framework developed by Portier (1986) is dependent upon the Markov

assumption that the risk of death for a tumor-bearing animal does not depend on the prior time of tumor onset. Portier and Dinse (1987) and Dinse (1988a) subsequently showed that a similar semi-parametric model could be specified in terms of hazard rates which do not require the Markov assumption. In addition, the latter formulation does not require the use of latent failure times, i.e., potential random variables which cannot always be observed. The semi-parametric models discussed above hold great promise as statistical techniques which require neither a large number of interim sacrifices nor assumptions of questionable validity; however, they do require intensive computational algorithms similar to the fully nonparametric models. In addition, knowledge of the statistical properties of the proposed estimators is still limited.

1.3.5 Case IV: Analysis of Occult Tumors by Quantal Response

In the analysis of long-term carcinogenicity experiments by quantal response methods, the entire time period encompassed by the long-term study is regarded as collapsed into a single stratum ($S=1$). With regard to the generic test statistic described in Section 1.3.1, x_{i1} is the number of animals in dose group i which developed the tumor of interest before the time of terminal sacrifice and r_{i1} is the total number of animals in dose group i which were placed on study. There are three possible reasons for using quantal response methods in the analysis of long-term animal experiments: (1) based on historical precedent and simplicity, (2) no times of death are available, or (3) no interim sacrifices were performed and the analyst is not willing to make assumptions regarding the relationship between tumor incidence and death.

Fisher's Exact Test can be used to compare the proportion of animals that developed tumors in each treated dose group, x_{i1} / r_{i1} , with that of the control group, x_{01} / r_{01} , during the course of the long-term carcinogenicity experiment. Under the assumption that the number of animals in each dose group is fixed, the number of treated animals

which develop tumors follows a hypergeometric distribution, and a one-tailed p-value for the null hypothesis of no difference in tumor incidence can be calculated as the probability of detecting at least as many tumors in the treated group as that observed. A separate pairwise comparison of each treatment group with the control group is necessary using Fisher's Exact Test.

If more than one dose group received active treatment, the Cochran-Armitage test can be used to test the hypothesis of no trend in the proportion of animals with tumors with increasing dose levels (Armitage, 1955). Letting π_i represent the probability of tumor onset before terminal sacrifice for an animal in dose group i , Chapman and Nam (1968) have noted that the Cochran-Armitage Trend test can also be expressed as a test of the hypothesis $H_0: \beta=0$ versus $H_0: \beta>0$ for which $\pi_i = \alpha + \beta z_i$.

Since the quantal response tests described above regard the entire study period as a single time interval for comparisons of tumor incidence, the tumor lethality (fatal, non-fatal, or intermediate) will have no effect on the test statistics. However, these quantal response tests do not take into account the possibility of different levels of tumor-free mortality (treatment lethality) among dose groups, and may therefore be subject to this source of bias. More specifically, if the MTD of the chemical is highly toxic, then animals in the higher dose groups may die of unrelated causes before the tumor has time to develop. Several adjustment procedures can be applied to either Fisher's Exact Test or the Cochran-Armitage Trend Test in order to control for differential effects of intercurrent mortality among dose groups. The Truncated Trend Test suggested by Gart *et al.* (1979) adjusts the number at risk by eliminating all those deaths that occurred before the first tumor was observed in any dose group. A second method of adjustment considers the number at risk in dose group i to be

$$r_{i1} = x_{i1} + \sum_{j=1}^{n_i} (t_{ij}/t_{\max})^3 (1 - \delta_{1ij})$$

where $t_{\max}$ is the maximum survival time over all dose groups. When this adjustment is applied to the Cochran-Armitage Trend Test, the resulting test has been denoted the Poly-3 Trend Test (Bailer and Portier, 1988), which reflects the observation that many tumors appear at the rate of a 3rd to a 5th order polynomial in time (Portier *et al.*, 1986). If the unavailability of death times motivates the use of a quantal response test, then the application of the Poly-3 Trend Test is clearly not feasible. However, a comparison of the Type I error rates and power of the Cochran-Armitage, Truncated and Poly-3 Trend Tests, the Hoel-Walburg and logistic regression prevalence tests, and the lifetable test revealed the Poly-3 Trend Test to be the most robust when little information was available regarding the degree of tumor lethality and treatment lethality (Bailer and Portier, 1988).

1.4 Statement of Problem and Outline

The primary objective of a long-term carcinogenicity experiment is the comparison of tumor incidence rates among treatment groups. The most common situation in the analysis of tumor incidence data is that tumor onset cannot be observed and the lethality of the tumor is unknown. The assumption that the tumor is either incidental or fatal are extreme and difficult to justify; most types of tumors are recognized to be of intermediate lethality. The recognition of the biases introduced both by tumor lethality and treatment lethality on past methods of analysis have led to the use of multistate stochastic models. Yet the need for intensive computer algorithms to obtain maximum likelihood estimators developed in the framework of these multistate methods precludes the investigation of small sample operating characteristics relevant to typical long-term animal studies. As a result, these methods have not been adopted for routine use by regulatory agencies.

In this research, attention is focused on the estimation of tumor incidence rates from

long-term animal carcinogenicity experiments which incorporate interim sacrifices. This estimation is based on a completely nonparametric model containing separate parameters for the tumor incidence rate, death rate for tumor-free animals, and overall death rate for each interval. Analytic solutions are derived for the maximum likelihood estimators of the nonparametric death hazards and tumor incidence rate. The existence of analytic solutions for these hazards reduces the need for intensive computational algorithms relied upon in current maximum likelihood methods, and provides protection against the biases of tumor lethality and treatment lethality. The availability of analytic solutions for the tumor incidence rate and death rates for tumor-free and tumor-bearing animals allows straightforward investigation of the operating characteristics of these estimators, and the forms of the estimators also provides intuitive appeal towards the understanding of the tumor incidence rate and death rates.

Chapter II focuses on a description of the assumptions and form of the multistate model upon which the nonparametric model is based. Under the framework of this model, the death hazards and tumor incidence function are described and the likelihood function is derived. Analytic solutions are first provided for the unconstrained maximum likelihood estimators of the hazards of death and tumor incidence. It is then recognized that certain boundary conditions on these estimators should be enforced, and analytic solutions for constrained MLE's are accordingly derived. Relationships are explored between the estimators derived under the framework of this nonparametric model and those of a nonparametric model which relies on potential random variables.

The asymptotic properties of the unconstrained MLE's of the tumor incidence rate and death rates are presented in Chapter III, and alternate estimates of the asymptotic variances of the unconstrained MLE's are obtained by comparison with the MLE's proposed by Malani and Van Ryzin (1986). While the asymptotic properties of the

unconstrained estimators can be obtained as a standard result for maximum likelihood estimators, the determination of the asymptotic variance-covariance matrix for the constrained MLE's is complicated by the imposition of boundary constraints. Consequently, more details are provided for the derivation of the asymptotic properties of the constrained MLE's.

Due to limitations of the proposed MLE's for study designs with more than three time intervals, additional estimators of the discrete death rates and tumor incidence rate are developed heuristically by pooling together data from adjacent time intervals. Several different algorithms for pooling data are developed for a study design consisting of three time intervals, and two generalized algorithms are described for a study design consisting of more than three intervals. These pooling methods are described in Chapter IV.

Test statistics for comparing the tumor incidence rates among treatment groups are presented in Chapter V. These testing procedures are based on similar statistical methods first suggested by McKnight and Crowley (1984) and subsequently adapted by Malani and Van Ryzin (1986) for a discrete nonparametric framework. Additional testing procedures are described for the comparison of tumor lethality among treatment groups and the comparison of death hazards in the presence or absence of a tumor among treatment groups.

The methodology developed in this research is illustrated in Chapter VI by an application of the results to data gathered from a long-term animal study. The estimators calculated by maximum likelihood methods and pooling methods are compared with those obtained by Kodell and Nelson (1980), Portier and Dinse (1987), and Malani and Van Ryzin (1988) for the same data. The small sample operating characteristics of the proposed estimators are evaluated on the basis of a Monte Carlo simulation study, with emphasis on

the comparison of the proposed estimators with some of the commonly used tests of carcinogenicity. Chapter VII provides a discussion of the results and directions for future research in the analysis of long-term animal carcinogenicity experiments.

CHAPTER II: MAXIMUM LIKELIHOOD ESTIMATION OF PARAMETERS

2.1 Description of a Multistate Model

The most common situation in the analysis of long-term animal experiments is that the tumor type of interest is occult and the cause of death is unknown. In this case, the results of an experiment can be conveniently described by a three-state stochastic model as illustrated in Figure 2.1.1. Define the random variable E_1 to be the time from initial exposure to the occurrence of the first event, which is either tumor onset or tumor-free death, let E_2 be the time to natural death for tumor-bearing animals and let D denote the time to natural death. Also define an indicator $\delta(t)$ that has value 1 if the tumor has occurred by time t and 0 otherwise. The transition rates from one state of the stochastic model to another are described by the following hazard functions:

$$\lambda(t) = \lim_{\Delta \rightarrow 0} \Pr [t \leq E_1 < t+\Delta, \delta(t+\Delta)=1 \mid E_1 \geq t] / \Delta \quad (2.1.1)$$

$$\beta(t) = \lim_{\Delta \rightarrow 0} \Pr [t \leq E_1 < t+\Delta, \delta(t+\Delta)=0 \mid E_1 \geq t] / \Delta \quad (2.1.2)$$

$$\phi(t) = \lim_{\Delta \rightarrow 0} \Pr [t \leq D < t+\Delta \mid D \geq t] / \Delta \quad (2.1.3)$$

in which $\lambda(t)$ is the tumor incidence function, $\beta(t)$ is the tumor-free death rate, and $\phi(t)$ is the overall death rate. This model has been previously discussed by Portier and Dinse (1987), and similar three-state models have been employed by McKnight and Crowley (1984), Portier (1986), Dewanji and Kalbfleisch (1986), Malani and Van Ryzin (1986,1988), Gomez and Julia (1988), and Bailer and Portier (1988). Many of the latter multistate models rely on the specific death rate for tumor-bearing animals,

$$\gamma(t, \omega) = \lim_{\Delta \rightarrow 0} \Pr [t \leq E_2 < t+\Delta \mid E_1 = \omega < t, E_2 \geq t] / \Delta ,$$

rather than the overall death rate. As written, the death rate with tumor is not identifiable since the times of tumor onset are unknown. Several authors have noted that maximum likelihood estimation of parameters based on the latter formulation requires making a certain type of assumption in order to estimate the death rate with tumor. For example, the Markov assumption that $\gamma(t, \omega_1) = \gamma(t, \omega_2)$ for all $\omega_1, \omega_2 \leq t$ is commonly relied upon. The implication of this assumption is that the risk of death for tumor-bearing animals can be estimated by the "average" risk function:

$$\gamma(t) = \lim_{\Delta \rightarrow 0} \Pr [t \leq E_2 < t + \Delta \mid \delta(t + \Delta) = 1, E_2 \geq t] / \Delta \quad (2.1.4)$$

However, McKnight and Crowley (1984) claim that a stochastic model based on the tumor-bearing death rate does not restrict a tumor-bearing animal's risk of death to being independent of the time the tumor developed, but consider $\gamma(t)$ to be a marginal function that averages the risk $\gamma(t, \omega)$ over the distribution of tumor onset times ω for animals dying at time t . An advantage of the proposed formulation of hazards given above is that it avoids reliance on the Markov or similar type of assumption.

An additional advantage of the proposed hazard formulation is that it does not require the use of potential (or latent) random variables which cannot always be observed and which force untestable assumptions to be made (Portier, 1986). In comparison, the model used by Malani and Van Ryzin (1986) and Portier (1986) is based on the random variables T , the time until tumor development, and D , the time until death. This model has an implicit assumption that every animal would get the tumor if it lived long enough. In cases where animals die before developing the tumor, only the minimum of T and D is observed. The validity of the assumption that every animal would get the tumor if it lived long enough cannot be assessed without knowing more about the actual biological system. Based on a multistate model with the random variables T and D , it is also necessary to

assume that the probability of death without a tumor does not depend on what the future time of tumor development would have been in order for the hazards to be identifiable (Portier, 1986).

Under a continuous time framework, the total hazard $h(t)$ for the random variable E_1 representing the time to the first event is the sum of the first two hazards in the proposed model:

$$\begin{aligned} h(t) &= \lim_{\Delta \rightarrow 0} \Pr [t \leq E_1 < t+\Delta \mid E_1 \geq t] / \Delta , \\ &= \lim_{\Delta \rightarrow 0} \{ \Pr [t \leq E_1 < t+\Delta, \delta(t+\Delta)=1 \mid E_1 \geq t] + \\ &\quad \Pr [t \leq E_1 < t+\Delta, \delta(t+\Delta)=0 \mid E_1 \geq t] \} / \Delta \\ &= \lambda(t) + \beta(t) . \end{aligned}$$

From the above result it can be verified that the separate functions $\lambda(t)$ and $\beta(t)$ can be added to obtain the total hazard for the event E_1 . The probability of tumor-free survival based on the total hazard is:

$$\begin{aligned} S(t) &= \exp \left\{ - \int_0^t h(u) du \right\} \\ S(t) &= \exp \left\{ - \int_0^t (\lambda(u) + \beta(u)) du \right\} . \end{aligned}$$

The hazard functions introduced in (2.1.1) - (2.1.3) can be modelled as continuous functions of time using appropriate density functions. Alternatively, the following discrete rates can be defined and estimated separately for each time interval:

$$\lambda_s = \Pr [E_1 = t_s, \delta(t_s) = 1 \mid E_1 \geq t_s] , \quad (2.1.5)$$

$$\beta_s = \Pr [E_1 = t_s, \delta(t_s) = 0 \mid E_1 \geq t_s] \quad \text{and} \quad (2.1.6)$$

$$\phi_s = \Pr [D = t_s, \mid D \geq t_s] . \quad (2.1.7)$$

Although the use of a continuous distribution to model the hazards typically reduces the number of parameters which must be estimated, the selection of an appropriate density function may be difficult and an incorrect choice is likely to introduce bias. Since most long-term animal carcinogenicity experiments are unlikely to include more than three sacrifice times, a reduction in the number of parameters would only be realized if the continuous density functions each contained two parameters or less. A model with discrete death probabilities has the practical advantage that it allows adjustment for different levels of intercurrent mortality among the dose groups with as little chance for bias as possible. Additional support for the use of discrete rates is provided by arguments of Kaplan and Meier (1958) and Johansen (1978). Based on the results published by these authors, Portier and Dinse (1987) claim that the likelihood based on the above hazards is maximized when $\beta(t)$ and $\phi(t)$ place mass only at the observed times of natural death. Thus death can be treated as a discrete process for maximization purposes.

Note that both the continuous hazards and the discrete hazards are based on the indicator variable $\delta(t)$, which has a value of 1 if the tumor is present at time t and 0 otherwise. This definition of $\delta(t)$ is used by Portier and Dinse (1987) and McKnight and Crowley (1984). An alternate framework for the definition of these hazards is to define an indicator $\delta^*(t)$ equal 1 if the first event E_1 is tumor occurrence and 0 if the first event is death without tumor. The latter designation for the indicator variable is employed by Dewanji and Kalbfleisch (1986), Portier and Bailer (1987) and Gomez and Julia (1988). Under the first framework, the events $\delta(t)=0$ and $\delta(t)=1$ are mutually exclusive, and thus the conditional probability of the first event can be written:

$$\begin{aligned}\Pr[E_1 = t_j \mid E_1 \geq t_j] &= \Pr[E_1 = t_j, \delta(t_j)=1 \mid E_1 \geq t_j] + \Pr[E_1 = t_j, \delta(t_j)=0 \mid E_1 \geq t_j] \\ &= \lambda_j + \beta_j\end{aligned}$$

and the probability of tumor-free survival through the end of the j -th interval is:

$$S(t_j) = \Pr[E_1 > t_j] = \prod_{i=1}^j (1 - \lambda_i - \beta_i) \quad (2.1.8)$$

However, it is possible that tumor-free death and tumor onset could occur simultaneously, unless we assume otherwise. If we allow for simultaneous occurrence of tumor onset and tumor-free death at a given time t_j , then the interpretations of the parameters must reflect this. Under the second framework, the events $\delta^*(t)=0$ and $\delta^*(t)=1$ may not be mutually exclusive unless the assumption is explicitly made that tumor onset and tumor-free death cannot occur during the same interval. If the simultaneous occurrence of the two events is allowed, then the hazard for the event E_1 becomes:

$$\begin{aligned} \Pr[E_1 = t_j \mid E_1 \geq t_j] &= \Pr[E_1 = t_j, \delta^*(t_j)=0 \cup \delta^*(t_j)=1 \mid E_1 \geq t_j] \\ &= \Pr[E_1 = t_j, \delta^*(t_j)=1 \mid E_1 \geq t_j] + \Pr[E_1 = t_j, \delta^*(t_j)=0 \mid E_1 \geq t_j] - \\ &\quad \Pr[E_1 = t_j, \delta^*(t_j)=1, \delta^*(t_j)=0 \mid E_1 \geq t_j] \end{aligned}$$

Under the assumption of independence, the latter probability can be written as:

$$\begin{aligned} \Pr[E_1 = t_j, \delta^*(t_j)=1, \delta^*(t_j)=0 \mid E_1 \geq t_j] &= \Pr[E_1 = t_j, \delta^*(t_j)=1 \mid E_1 \geq t_j] * \\ &\quad \Pr[E_1 = t_j, \delta^*(t_j)=0 \mid E_1 \geq t_j] \\ &= \lambda_j^* \beta_j^* \end{aligned}$$

in which λ_j^* and β_j^* are the parameters defined within the second framework corresponding to λ_j and β_j in the first framework. The hazard for the event E_1 is:

$$\Pr[E_1 = t_j \mid E_1 \geq t_j] = \lambda_j^* + \beta_j^* - \lambda_j^* \beta_j^*$$

and the probability of tumor-free survival corresponding to the second framework becomes:

$$\begin{aligned} S(t_j) = \Pr[E_1 > t_j] &= \prod_{i=1}^j (1 - \lambda_i^* - \beta_i^* + \lambda_i^* \beta_i^*) \\ &= \prod_{i=1}^j (1 - \lambda_i^*) (1 - \beta_i^*) \end{aligned} \quad (2.1.9)$$

While the interpretation of the parameters λ_j and λ^*_j are equivalent in the two frameworks, the interpretations of the parameters β_j and β^*_j are different. If the simultaneous occurrence of death without tumor and tumor incidence is allowed in the second framework, then the relationship between the death rate for tumor-free animals from the first framework and that of the second framework can be expressed as:

$$\beta = \beta^* - \beta^* \lambda^* \quad \text{or} \quad \beta = \beta^* (1 - \lambda)$$

If the simultaneous occurrence of tumor incidence and death without tumor is prohibited within a given time interval, then the death rates for tumor-free animals within the two frameworks are equal and their interpretations are equivalent.

Dewanji and Kalbfleisch (1986) use the second framework and claim that the probability of tumor-free survival is given by (2.1.8), although they do not explicitly state the assumption that tumor onset and tumor-free death cannot occur simultaneously. McKnight and Crowley (1984), Portier and Dinse (1987) and Gomez and Julia (1988) base their results on continuous tumor incidence rates, so there is no possibility of simultaneous occurrence. Manlani and Van Ryzin (1986) employ a model with a discrete tumor incidence rate and death rates, but they base the maximum likelihood estimation on the tumor prevalence function rather than tumor incidence, and on time to tumor and time to death rather than time to first event. However, Malani and Van Ryzin explicitly state the assumption that all deaths and tumor occur at the end of the interval, and within each interval, tumor onset precedes natural death.

In the present development of the likelihood function, the assumption will be made that tumor incidence and death without tumor cannot occur simultaneously. Furthermore, when the events of death and tumor occur in the same interval, it will be assumed that tumor onset precedes natural death. However, preliminary results analagous to those

derived under this set of assumptions will be presented for the situation in which simultaneous occurrence of tumor and death without tumor is permitted.

Figure 2.1.1
Stochastic Model for Tumor Incidence and Death

States:

N - Normal animal

E_1 - First Event: Tumor Onset (T) or Death (D)

E_2 - Second Event (Death with Tumor)

Transition Rates Between States:

λ - tumor incidence rate

β - death rate for tumor-free animals

γ - death rate given presence of tumors

ϕ - overall death rate

2.2 Derivation of a Likelihood Function

Maximum likelihood methods can be used to find estimators for the parameter vector $\theta = \{(\lambda_1, \dots, \lambda_S), (\beta_1, \dots, \beta_S), (\phi_1, \dots, \phi_S)\}$ in the nonparametric model described in Section 2.1. In the analysis of long-term animal studies which incorporate interim sacrifices, one possible choice for the designation of the time periods are on the basis of the prespecified times of interim sacrifice. Let the random variable S designate the time of sacrifice and assume that all sacrifices occur at the end of the time period after the death status of each animal has been ascertained. Using the framework described in Figure 1, there are four possible mutually exclusive events which could occur for each animal within time period s :

- (1) sacrifice without tumor (SWOT_s), $\{S = t_s, E_1 > t_s, \delta(t_s) = 0\}$
- (2) sacrifice with tumor (SWT_s), $\{S = t_s, D > t_s, \delta(t_s) = 1\}$
- (3) death without tumor (DWOT_s), $\{S \geq t_s, E_1 = t_s, \delta(t_s) = 0\}$
- or (4) death with tumor (DWT_s), $\{S \geq t_s, D = t_s, \delta(t_s) = 1\}$.

The collection of the four events described above for all S time intervals determines a set of $4S$ possible outcomes which are mutually exclusive and exhaustive, so that the death of an animal can be classified as falling into one and only one such category. Because the response of an individual animal is independent of the response of any other animal, the numbers of animals falling into the $4S$ possible categories follow a multinomial distribution. Let c_s^- and c_s^+ denote the respective numbers of tumor-free and tumor-bearing animals that were sacrificed in time period s , and let d_s^- and d_s^+ denote the respective numbers of tumor-free and tumor-bearing animals that died naturally in time period s . Under the multinomial model, the full likelihood is proportional to the product of the probabilities of all such events over all animals:

$$L(\lambda(t), \beta_s, \phi_s) \propto \prod_{s=1}^S \{ \Pr[\text{SWOT}_s] \}^{c_s^-} \{ \Pr[\text{SWT}_s] \}^{c_s^+} \{ \Pr[\text{DWOT}_s] \}^{d_s^-} \{ \Pr[\text{DWT}_s] \}^{d_s^+}$$

and the log-likelihood, apart from an additive constant, is

$$L = \ln L(\lambda(t), \beta_s, \phi_s) = \sum_{s=1}^S \left(c_s^- \ln \Pr[\text{SWOT}_s] + c_s^+ \ln \Pr[\text{SWT}_s] + d_s^- \ln \Pr[\text{DWOT}_s] + d_s^+ \ln \Pr[\text{DWT}_s] \right)$$

It will be assumed in this likelihood development that the probability of sacrifice for any animal is unrelated to its tumor status and its future time of natural death, i.e., sacrifice is an unbiased sampling method. At each sacrifice time during the study, a prespecified proportion of animals are selected at random from those which remain alive. Other sacrifice schemes are possible, such as the random allocation of sacrifice times to individual animals at the beginning of the study, or the designation of a fixed number of animals to be sacrificed at the end of each interval. However, it can be easily verified through examination of the likelihood function that the maximum likelihood estimators for λ , β , and ϕ are equivalent under any sacrifice scheme which is an unbiased sampling method.

Within the sacrifice scheme selected for use in this development, the conditional probability of sacrifice given that an animal is alive at time t_s is designated as:

$$\Pr[S = t_s \mid D > t_s, S \geq t_s] = \pi_s.$$

The probability of an animal escaping sacrifice through the first s time periods given that it is alive is:

$$\begin{aligned} \Pr[S > t_s \mid D > t_s] &= \Pr[S \neq t_s, S > t_{s-1} \mid D > t_s] \\ &= \Pr[S \neq t_s \mid S > t_{s-1}, D > t_s] \Pr[S > t_{s-1} \mid D > t_s] \\ &= \Pr[S \neq t_s \mid S \geq t_s, D > t_s] \Pr[S > t_{s-1} \mid D > t_s] \\ &= (1 - \Pr[S = t_s \mid S \geq t_s, D > t_s]) \Pr[S > t_{s-1} \mid D > t_s] \end{aligned}$$

$$\begin{aligned}
&= (1-\pi_s) \Pr[S > t_{s-1} | D > t_{s-1}] \\
&= (1-\pi_s) \Pr[\underset{s}{S} \neq t_{s-1}, S > t_{s-2} | D > t_{s-1}] \\
&= \prod_{j=1}^s (1 - \pi_j) \quad (\text{by recursion}). \tag{2.2.1}
\end{aligned}$$

Animals that die of natural causes during time period s and are tumor-free contribute the following component to the likelihood:

$$\begin{aligned}
 \Pr[\text{DWOT}_s] &= \Pr[S \geq t_s, E_1 = t_s, \delta(t_s)=0] \\
 &= \Pr[S \geq t_s, D = t_s, T > t_s] \\
 &= \Pr[S \geq t_s \mid D = t_s, T > t_s] \Pr[D = t_s, T > t_s] \\
 &= \Pr[S \geq t_s \mid D > t_{s-1}] \Pr[D = t_s, T > t_s] \\
 &= \Pr[S > t_{s-1} \mid D > t_{s-1}] \Pr[D = t_s, T > t_s] \\
 &\quad \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[D = t_s, T > t_s] \quad \text{by (2.2.1)} \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[E_1 = t_s, \delta(t_s) = 0] \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[E_1 = t_s, \delta(t_s)=0, E_1 \geq t_s] \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[E_1 = t_s, \delta(t_s)=0 \mid E_1 \geq t_s] \Pr[E_1 \geq t_s] \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \beta_s \Pr[E_1 \geq t_s] \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \beta_s \Pr[E_1 > t_{s-1}] \\
 &= \prod_{j=1}^{s-1} (1 - \pi_j) \beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \quad \text{by (2.1.8)}
 \end{aligned}$$

A tumor-bearing animal that is sacrificed in time period s makes the following contribution:

$$\begin{aligned}
 \Pr[\text{SWT}_s] &= \Pr[S = t_s, D > t_s, \delta(t_s)=1] \\
 &= \Pr[S = t_s \mid D > t_s, \delta(t_s)=1] \Pr[D > t_s, \delta(t_s)=1] \\
 &= \Pr[S = t_s \mid D > t_s] \Pr[D > t_s, \delta(t_s)=1]
 \end{aligned}$$

$$\begin{aligned}
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[D > t_s, \delta(t_s)=1] \quad (\text{analogous to } \Pr[\text{SWOT}]) \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D > t_s] - \Pr[D > t_s, \delta(t_s)=0] \right\} \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D > t_s] - \Pr[E_1 > t_s] \right\} \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D \neq t_s, D \geq t_s] - \Pr[E_1 > t_s] \right\} \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D \neq t_s | D \geq t_s] \Pr[D \geq t_s] - \Pr[E_1 > t_s] \right\} \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ (1 - \phi_s) \Pr[D > t_{s-1}] - \Pr[E_1 > t_s] \right\} \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \prod_{j=1}^s (1 - \phi_j) - \Pr[E_1 > t_s] \right\} \quad (\text{by recursion}) \\
&= \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \prod_{j=1}^s (1 - \phi_j) - \prod_{j=1}^s (1 - \lambda_j - \beta_j) \right\}
\end{aligned}$$

Finally, an animal that dies with one or more tumors in the s -th time period donates the following to the total likelihood:

$$\begin{aligned}
\Pr[\text{DWT}_s] &= \Pr[S \geq t_s, D = t_s, \delta(t_s)=1] \\
&= \Pr[S \geq t_s | D = t_s, \delta(t_s)=1] \Pr[D = t_s, \delta(t_s)=1] \\
&= \Pr[S \geq t_s | D > t_{s-1}] \Pr[D = t_s, \delta(t_s)=1] \\
&= \Pr[S > t_{s-1} | D > t_{s-1}] \Pr[D = t_s, \delta(t_s)=1] \\
&= \prod_{j=1}^{s-1} (1 - \pi_j) \Pr[D = t_s, \delta(t_s)=1] \quad \text{by (2.2.1)}
\end{aligned}$$

$$\begin{aligned}
&= \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D = t_s] - \Pr[D = t_s \mid \delta(t_s)=0] \right\} \\
&= \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D = t_s \mid D \geq t_s] - \Pr[E_1 = t_s \mid \delta(t_s)=0] \right\} \\
&= \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \Pr[D = t_s \mid D \geq t_s] \Pr[D \geq t_s] - \Pr[\text{DWOT}_s] \right\} \\
&= \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \phi_s \Pr[D > t_{s-1}] - \Pr[\text{DWOT}_s] \right\} \\
&= \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \phi_s \prod_{j=1}^{s-1} (1 - \phi_j) - \beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \right\}
\end{aligned}$$

To summarize, the components representing the probability of the four possible events within each time interval are:

$$\Pr[\text{SWOT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \prod_{j=1}^s (1 - \lambda_j - \beta_j) \quad (2.2.2)$$

$$\Pr[\text{DWOT}_s] = \prod_{j=1}^{s-1} (1 - \pi_j) \beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \quad (2.2.3)$$

$$\Pr[\text{SWT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \prod_{j=1}^s (1 - \phi_j) - \prod_{j=1}^s (1 - \lambda_j - \beta_j) \right\} \quad (2.2.4)$$

$$\Pr[\text{DWT}_s] = \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \phi_s \prod_{j=1}^{s-1} (1 - \phi_j) - \beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \right\} \quad (2.2.5)$$

The expressions involving the probability of sacrifice or non-sacrifice can be factored out of the likelihood and incorporated with the constant of proportionality, since they do not involve the parameters for which the likelihood is to be maximized. Substituting the separate expressions for the four possible events into the log-likelihood, we have:

$$\begin{aligned}
 L = \ln L(\lambda, \beta, \phi) = & \\
 & K + \sum_{s=1}^S \left\{ \left[c_s^- \ln \left(\prod_{j=1}^s (1 - \lambda_j - \beta_j) \right) \right] + \left[c_s^+ \ln \left(\prod_{j=1}^s (1 - \phi_j) - \prod_{j=1}^s (1 - \lambda_j - \beta_j) \right) \right] \right. \\
 & \left. + \left[d_s^- \ln \left(\beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \right) \right] + \left[d_s^+ \ln \left(\phi_s \prod_{j=1}^{s-1} (1 - \phi_j) - \beta_s \prod_{j=1}^{s-1} (1 - \lambda_j - \beta_j) \right) \right] \right\}
 \end{aligned} \tag{2.2.6}$$

in which $\lambda = (\lambda_1, \lambda_2, \dots, \lambda_S)'$, $\beta = (\beta_1, \beta_2, \dots, \beta_S)'$ and $\phi = (\phi_1, \phi_2, \dots, \phi_S)'$, and K is an additive constant which does not involve any of the parameters in λ , β , or ϕ .

It can be shown that the above likelihood is correct even under very restrictive data situations such as those resulting from fatal tumors or incidental tumors. The demonstration of the appropriateness of the likelihood under these restrictive models hinges on the reduction of the general form of the conditional death rate, ϕ_i , to a simpler form allowed by these assumptions. In order to derive the general form for ϕ_i , it is first necessary to define a discrete average death rate for tumor bearing animals corresponding to the continuous rate $\gamma(t)$ given by (2.1.4) as:

$$\gamma_i = \Pr[E_2 = t_i \mid E_2 \geq t_i, \delta(t_i)=1]$$

The average risk function is used rather than the specific rate of death given tumor onset at a particular time t_{ω} , since the latter rate is not identifiable in the absence of tumor onset times.

The general form of the conditional death rate ϕ_i can now be written in terms of the remaining parameters corresponding to the first through the i -th time intervals.

$$\begin{aligned}\phi_i &= \Pr[D = t_i \mid D \geq t_i] \\ &= \Pr[D = t_i] / \Pr[D \geq t_i]\end{aligned}$$

$$\begin{aligned}\text{so } \phi_i \Pr[D \geq t_i] &= \Pr[D = t_i, \delta(t_i)=0] + \Pr[D = t_i, \delta(t_i)=1] \\ &= \Pr[E_1 = t_i, \delta(t_i)=0] + \Pr[E_2 = t_i]\end{aligned}$$

To obtain the value of the latter term, the probability of dying with the tumor must be summed over all possible times of tumor onset. For each possible time of tumor onset, the term can be written as the probability of the first event not occurring in the first $j-1$ time periods, the first event occurring at time j (tumor onset), death with tumor not occurring during intervals ω to $i-1$, and then death with tumor occurring in time interval i , as follows:

$$\begin{aligned}\Pr[E_2 = t_i] &= \sum_{j=1}^i \left\{ \Pr[E_2 = t_i \mid E_2 \geq t_j, E_1 = t_j] \Pr[E_2 \geq t_i \mid E_2 \geq t_j, E_1 = t_j] \right. \\ &\quad \left. * \Pr[E_1 = t_j, \delta(t_j)=1 \mid E_1 \geq t_j] \Pr[E_1 \geq t_j] \right\}\end{aligned}$$

Using the above construction, it is now possible to write the general form of ϕ_i as:

$$\begin{aligned}\phi_i \Pr[D \geq t_i] &= \beta_i \prod_{k=1}^i (1 - \lambda_k - \beta_k) + \gamma_i \sum_{j=1}^i \left[\prod_{k=j}^{i-1} (1 - \gamma_k) \lambda_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k) \right] \\ \phi_i &= \frac{\beta_i \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k) + \gamma_i \sum_{j=1}^i \left[\prod_{k=j}^{i-1} (1 - \gamma_k) \lambda_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k) \right]}{\prod_{k=1}^{i-1} (1 - \phi_k)}\end{aligned}\tag{2.2.7}$$

For a rapidly fatal tumor, one would expect that $\gamma_i = 1$. The form for ϕ_i can thus be reduced to:

$$\phi_i = \frac{(\beta_i + \lambda_i) \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{\prod_{k=1}^{i-1} (1 - \phi_k)}$$

Since $\phi_1 = \beta_1 + \lambda_1$, then $1 - \phi_1$ is $(1 - \lambda_1 - \beta_1)$. Solving for ϕ_2 given this value for ϕ_1 yields $\phi_2 = \beta_2 + \lambda_2$, and continuing recursively, the fatal tumor relationship can be summarized as $\phi_i = \beta_i + \lambda_i$. The components for $\text{Pr}[\text{SWOT}]$ and $\text{Pr}[\text{DWOT}]$ remain unchanged as they do not involve ϕ . The probability of sacrifice with a tumor must be zero since the tumors are assumed to be rapidly lethal. The probability of death with tumor during the s -th time interval is:

$$\text{Pr}[\text{DWT}_s] = \prod_{j=1}^s (1 - \pi_j) \lambda_s \prod_{j=1}^{s-1} (1 - \phi_j)$$

When the three components corresponding to the case of a fatal tumor are substituted into the log-likelihood, its form and interpretation become equivalent to that employed by Tarone for lifetable analysis (1975).

For an incidental tumor, the probability of death does not depend on tumor status, so that $\gamma_i = \phi_i$. By substituting ϕ_i for γ_i in expression (2.2.7) and solving recursively, the relationship for incidental tumors can be determined to be $\phi_i = \beta_i / (1 - \lambda_i)$. Recognition of this relationship allows the four components of the likelihood to be reduced to the following expressions:

$$\text{Pr}[\text{SWOT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \prod_{j=1}^s (1 - \lambda_j) (1 - \phi_j)$$

$$\Pr[\text{DWOT}_s] = \prod_{j=1}^{s-1} (1 - \pi_j) \phi_s \prod_{j=1}^s (1 - \lambda_j) \prod_{j=1}^{s-1} (1 - \phi_j)$$

$$\Pr[\text{SWT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \prod_{j=1}^s (1 - \phi_j) \left\{ 1 - \prod_{j=1}^s (1 - \lambda_j) \right\}$$

$$\Pr[\text{DWT}_s] = \prod_{j=1}^s (1 - \pi_j) \phi_s \prod_{j=1}^{s-1} (1 - \phi_j) \left\{ 1 - \prod_{j=1}^s (1 - \lambda_j) \right\}$$

When the four components shown above for the incidental tumor case are substituted into the log-likelihood, its structure takes on the same form as the log-likelihood proposed by Hoel and Walburg (1972) for the analysis of incidental tumors. An added advantage for the case of incidental tumors is that the log-likelihood becomes factorable into two parts: one part depends only on the parameter θ and the second part involves only λ . Thus the total likelihood corresponding to an incidental tumor type can be maximized by maximizing each likelihood factor separately. In the analysis of either incidental or fatal tumors, the set of three parameter vectors (λ, β, ϕ) can be reduced to a set of two parameter vectors (λ, ϕ) .

2.3 Analytic Solutions for the Unconstrained MLE's in the Nonparametric Model

Maximum likelihood techniques were first applied to the nonparametric model described in 2.1 under the most restrictive cases: those applicable to incidental and to fatal tumor analyses. Although these assumptions are not expected to be realistic in the analysis of most tumor incidence data, the derived results illustrate the types of estimators which would be used under these circumstances, provide intuition as to what is to be expected in

the general case where no assumptions are made regarding tumor lethality or treatment lethality, and serve as a comparison for the general solutions.

Theorem 2.3.1: In the nonparametric model for death and tumor development with the discrete rates

$$\lambda_s = \Pr [E_1 = t_s, \delta(t_s) = 1 \mid E_1 \geq t_s]$$

$$\phi_s = \Pr [D = t_s \mid D \geq t_s]$$

in which E_1 is a random variable representing the time to first event (tumor onset or death), D is a random variable representing the time to death and $\delta(t)$ is an indicator of tumor presence or absence, the maximum likelihood estimators for λ_s and ϕ_s in the the case of incidental tumors are given by

$$\hat{\phi}_s = \frac{d_s^- + d_s^+}{R_s}, \quad (2.3.1)$$

$$(1 - \hat{\lambda}_s) = \frac{c_s^- + d_s^-}{n_s \hat{S}_{s-1}} \quad \text{or} \quad 1 - \hat{\Lambda}_s = \frac{c_s^+ + d_s^+}{n_s} \quad (2.3.2)$$

in which Λ_s is the cumulative probability of remaining tumor-free, n_s is the total number of animals which die naturally or are sacrificed during the s -th time period, and R_s is defined to be the risk set at time t_s as defined below:

$$\Lambda_s = \prod_{i=1}^s (1 - \lambda_i) \quad \text{and} \quad R_s = \sum_{i=s}^S (c_i^- + c_i^+ + d_i^- + d_i^+) = \sum_{i=s}^S n_i.$$

The maximum likelihood estimator of ϕ_i is the simple Kaplan-Meier estimator, and the estimator of the probability that tumor onset occurs at or before time t_s (designated by $(1 - \Lambda_s)$) is the simple prevalence of tumors in the s -th time interval. The asymptotic variances of these estimators will be discussed in Chapter III.

Proof:

The log-likelihood under the incidental tumor assumption can be factored into two components, one of which involves only the vector ϕ and the other of which involves only the vector λ . The first component to be maximized with respect to ϕ is:

$$L(\phi) = K + \sum_{s=1}^S \left(\left[(c_s^- + c_s^+) \ln \left(\prod_{j=1}^s (1 - \phi_j) \right) \right] + \left[(d_s^- + d_s^+) \ln \left(\phi_s \prod_{j=1}^{s-1} (1 - \phi_j) \right) \right] \right)$$

The partial derivative with respect to ϕ is:

$$\frac{\delta L_s}{\delta \phi_i} = \frac{d_i^- + d_i^+}{\phi_i} - \frac{R_{i+1} + c_i^- + c_i^+}{(1 - \phi_i)}$$

Setting the above result to zero and solving for ϕ_i yields the value in expression (2.3.1).

Similarly, the component of the log-likelihood which is to be maximized with respect to λ can be expressed as follows:

$$L(\lambda) = K + \sum_{s=1}^S \left(\left[(c_s^- + d_s^-) \ln \Lambda_s \right] + \left[(c_s^+ + d_s^+) \ln (1 - \Lambda_s) \right] \right)$$

and the corresponding partial derivative with respect to λ_i has the form:

$$\frac{\delta L_s}{\delta \lambda_i} = \sum_{s=i}^S \left(\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i)} + \frac{(c_s^+ + d_s^+) \Lambda_s}{(1 - \lambda_i)(1 - \Lambda_s)} \right)$$

When this partial derivative is set to zero, the expression (2.3.2) satisfies the resulting equation. A necessary and sufficient condition for the maximum to exist at this set of points is that the matrix of second partial derivatives, or Hessian, is negative definite (has all negative eigenvalues). This is equivalent to requiring each of the diagonal elements in the Hessian to be negative, and the determinant of the (2x2) matrix of second partials to be

positive (Mirsky, Intro to Linear Algebra, 1963) within each time interval. Since the log-likelihood $L(\phi, \lambda)$ can be factored into two components as described above, the partial derivatives with respect to each parameter do not depend on the other parameter. The resulting Hessian is thus a diagonal matrix with zero elements on the off-diagonal, and it is therefore sufficient to show that the diagonal elements $\delta^2 L / (\delta \phi_i)^2$ and $\delta^2 L / (\delta \lambda_i)^2$ of the Hessian are negative. The second partial derivative with respect to ϕ_i is negative, as shown below.

$$\frac{\delta^2 L_s}{\delta (\phi_i)^2} = \frac{-(d_i^- + d_i^+)}{\phi_i^2} + \frac{-(R_{i+1} + c_i^- + c_i^+)}{(1 - \phi_i)^2} < 0$$

The second partial derivative with respect to λ_i has the form below, which is also strictly negative.

$$\frac{\delta^2 L_s}{\delta (\lambda_i)^2} = \sum_{s=i}^S \left(\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i)^2} + \frac{-(c_s^+ + d_s^+) \Lambda_s^2}{(1 - \lambda_i)^2 (1 - \Lambda_s)^2} \right)$$

Theorem 2.3.2:

In the nonparametric model for death and tumor development described in Theorem 2.3.1, the maximum likelihood estimators for λ_s and ϕ_s in the case of fatal tumors are given by:

$$\hat{\phi}_s = \frac{d_s^- + d_s^+}{R_s}, \quad (2.3.3)$$

$$\hat{\lambda}_s = \frac{d_s^+}{R_s} \quad (2.3.4)$$

Proof:

Define the number of animals at risk R_s and the cumulative survival probability Φ_s as:

$$\Phi_s = \prod_{i=1}^S (1 - \phi_i) \quad \text{and} \quad R_s = \sum_{i=s}^S (c_i^- + d_i^- + d_i^+).$$

It is necessary to modify the term R_s by eliminating the data value c_s^+ from the number of animals at risk, since the probability of observing an animal with a tumor that has not died is zero in the case of fatal tumors. The log-likelihood for ϕ and λ is:

$$L(\phi, \lambda) = K + \sum_{s=1}^S \left(\left[c_s^- \ln \Phi_s \right] + \left[d_s^- \ln \left((\phi_s - \lambda_s) \Phi_{s-1} \right) \right] + \left[d_s^+ \ln \left(\lambda_s \Phi_{s-1} \right) \right] \right)$$

The partial derivatives of this log-likelihood with respect to ϕ_i and λ_i are exhibited below.

It is clear that both partial derivatives involve only parameters from the (i-th) time period:

$$\frac{\partial L_s}{\partial \phi_i} = \frac{d_i^-}{(\phi_i - \lambda_i)} - \frac{R_{i+1} + c_i^-}{(1 - \phi_i)}$$

$$\frac{\partial L_s}{\partial \lambda_i} = \frac{-d_i^-}{(\phi_i - \lambda_i)} + \frac{d_i^+}{\lambda_i}$$

Solving these two equations simultaneously yields the MLE's provided in expressions (2.3.3) and (2.3.4). The second partials in the case of fatal tumors are:

$$\frac{\partial^2 L_s}{\partial (\phi_i)^2} = \frac{-d_i^-}{(\phi_i - \lambda_i)^2} + \frac{-(R_{i+1} + c_i^-)}{(1 - \phi_i)^2}$$

$$\frac{\partial^2 L_s}{\partial (\lambda_i)^2} = \frac{-d_i^-}{(\phi_i - \lambda_i)^2} + \frac{-d_i^+}{\lambda_i^2}$$

$$\frac{\delta^2 L_s}{(\delta\lambda_i)(\delta\phi_i)} = \frac{d_i^-}{(\phi_i - \lambda_i)^2}$$

Each of the second partials $\delta^2 L_s / \delta(\lambda_i)^2$ and $\delta^2 L_s / \delta(\phi_i)^2$ are strictly negative, and the determinant of the matrix of second partials is:

$$\frac{d_i^-}{(\phi_i - \lambda_i)^2} \left\{ \frac{R_{i+1} + c_i^-}{(1 - \phi_i)^2} + \frac{d_i^+}{\lambda_i^2} \right\}$$

which is strictly positive. Thus the estimators given in expressions (2.3.3) and (2.3.4) are the maximum likelihood estimators. The asymptotic variances of these estimators will be discussed in Chapter III.

It is quite interesting that the same estimators of the overall probability of death within a given time interval arise under the fatal tumor assumption and incidental tumor assumption, although their true values are quite different. The true value of ϕ_i under the fatal tumor assumption is actually $\lambda_i (1 - \lambda_i - \beta_i) / (1 - \lambda_i)$ larger than the true value of ϕ_i under the incidental tumor assumption. Yet, in either situation, the best estimator of the overall death rate in terms of maximizing the likelihood is the simple Kaplan-Meier estimator, i.e., the ratio of the number of animals dying naturally (with or without tumors) during the time interval to the total number of animals at risk at the beginning of the time interval.

Next, maximum likelihood techniques were applied to the general case of the nonparametric model described in Section 2.1. Solutions were obtained by first solving the three partial derivative equations corresponding to the last time interval in the study. This step provided solutions for β_S and λ_S in terms of the vector of parameters from the first S-

1 intervals and a closed-form solution for $\hat{\phi}_s$ in terms of the observed data from just the last interval. Next, the three partial derivative equations corresponding to the (S-1)-th time interval were solved, taking into account the solutions derived from the last interval. This process continued from the last time interval to the first time interval, and at each step the parameters from the succeeding time interval were eliminated from the system of equations.

Theorem 2.3.3: In the nonparametric model for death and tumor development with the discrete rates

$$\lambda_s = \Pr [E_1 = t_s, \delta(t_s) = 1 \mid E_1 \geq t_s]$$

$$\beta_s = \Pr [E_1 = t_s, \delta(t_s) = 0 \mid E_1 \geq t_s]$$

$$\phi_s = \Pr [D = t_s \mid D \geq t_s]$$

in which E_1 is a random variable representing the time to first event (tumor onset or death), D is a random variable representing the time to death and $\delta(t)$ is an indicator of tumor presence or absence, the maximum likelihood estimators for λ_s , β_s , and ϕ_s are given by

$$\hat{\phi}_s = \frac{d_s^- + d_s^+}{R_s}, \quad (2.3.5)$$

$$\hat{\beta}_s = \frac{d_s^- (c_{s-1}^- + c_{s-1}^+)}{R_s c_{s-1}^-}, \quad (2.3.6)$$

$$(1 - \hat{\lambda}_s) = \frac{[c_s^- R_{s+1} + (c_s^- + c_s^+) (c_s^- + d_s^-)] (c_{s-1}^- + c_{s-1}^+)}{(c_s^- + c_s^+) R_s c_{s-1}^-}, \quad (2.3.7)$$

$$\text{or } \hat{\Lambda}_s = \prod_{i=1}^s (1 - \hat{\lambda}_i) = \frac{\prod_{i=1}^s \left[c_i^- R_{i+1} + (c_i^- + c_i^+) (c_i^- + d_i^-) \right]}{(c_s^- + c_s^+) \prod_{i=1}^s R_i \prod_{i=1}^{s-1} c_i^-} \quad (2.3.8)$$

provided they are in the parameter space (0,1). In order to apply these general formulas to the first and last interval, the following constants must be defined:

$$\frac{c_0^- + c_0^+}{c_0^-} = 1, \quad \prod_{i=1}^0 c_i^- = 1, \quad \text{and } R_{S+1} = 0.$$

Proof:

The log-likelihood based on the nonparametric model with discrete death rates ϕ_s and β_s and discrete tumor incidence rate λ_s is shown in expression (2.2.6).

$$\text{Define } A_s = \prod_{k=1}^s (1 - \phi_k) - \prod_{k=1}^s (1 - \lambda_k - \beta_k) \quad \text{and} \quad B_s = \phi_s \prod_{k=1}^{s-1} (1 - \phi_k) - \beta_s \prod_{k=1}^{s-1} (1 - \lambda_k - \beta_k).$$

Then the partial derivative with respect to the i -th overall death rate, ϕ_i for $i=1, \dots, S$ can be expressed as follows:

$$\frac{\delta L_S}{\delta \phi_i} = \frac{-c_i^+ \prod_{k=1}^{i-1} (1 - \phi_k)}{A_i} + \frac{d_i^+ \prod_{k=1}^{i-1} (1 - \phi_k)}{B_i} + \sum_{s=i+1}^S \left[\frac{-c_s^+ \prod_{k=1}^s (1 - \phi_k)}{(1 - \phi_i) A_s} + \frac{-d_s^+ \phi_k \prod_{k=1}^{s-1} (1 - \phi_k)}{(1 - \phi_i) B_s} \right]$$

This partial likelihood is evaluated at the vector of maximum likelihood estimators (MLE's) defined in (2.3.5), (2.3.6), and (2.3.7):

$$\hat{\theta} = \left\{ \left(\hat{\lambda}_1, \dots, \hat{\lambda}_S \right), \left(\hat{\beta}_1, \dots, \hat{\beta}_S \right), \left(\hat{\phi}_1, \dots, \hat{\phi}_S \right) \right\}.$$

When the values of the MLE's for θ are substituted in the partial derivative $\delta L_S / \delta \phi_i$, we obtain zero. Similarly, the partial derivative of the log-likelihood L_S with respect to the i -th death rate without tumor, β_i , can be expressed as:

$$\begin{aligned} \frac{\delta L_S}{\delta \beta_i} = & \frac{-c_i^-}{(1 - \lambda_i - \beta_i)} + \frac{c_i^+ \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{A_i} + \frac{d_i^-}{\beta_i} + \frac{-d_i^+ \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{B_i} \\ & + \sum_{s=i+1}^S \left[\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i - \beta_i)} + \frac{c_s^+ \prod_{k=1}^s (1 - \lambda_k - \beta_k)}{(1 - \lambda_i - \beta_i) A_s} + \frac{d_s^+ \beta_s \prod_{k=1}^{s-1} (1 - \lambda_k - \beta_k)}{(1 - \lambda_i - \beta_i) B_s} \right] . \end{aligned}$$

When this partial derivative is evaluated at the estimated value of θ , we obtain zero. Last of all, the partial derivative of the log-likelihood with respect to λ_i can be expressed as:

$$\begin{aligned} \frac{\delta L_S}{\delta \lambda_i} = & \frac{-c_i^-}{(1 - \lambda_i - \beta_i)} + \frac{c_i^+ \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{A_i} + \\ & + \sum_{s=i+1}^S \left[\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i - \beta_i)} + \frac{c_s^+ \prod_{k=1}^s (1 - \lambda_k - \beta_k)}{(1 - \lambda_i - \beta_i) A_s} + \frac{-d_s^+ \beta_s \prod_{k=1}^{s-1} (1 - \lambda_k - \beta_k)}{(1 - \lambda_i - \beta_i) B_s} \right] . \end{aligned}$$

and when this partial derivative is evaluated at the maximum likelihood estimator for θ , a value of zero is obtained.

A necessary condition for a continuous function $f(\mathbf{x})$ with continuous first and second partial derivatives to have an extreme point (maximum or minimum) at $\mathbf{x}^0$ is that each first partial derivative of $f(\mathbf{x})$ evaluated at $\mathbf{x}^0$ vanish (p.30, Gue and Thomas, 1968). This necessary condition is satisfied by the fact that values of zero were obtained when each of the partial derivatives were evaluated at the proposed MLE's for ϕ_i , β_i , and λ_i . In order for the likelihood to have a strict maximum at this set of values, it is also necessary and sufficient to show that the matrix of second partial derivatives, or Hessian, is negative definite (has eigenvalues strictly less than 0) when evaluated at the MLE's for θ_i .

In the case of the log-likelihood given (2.2.6), the Hessian is a symmetric $(3 \times S) \times (3 \times S)$ matrix with diagonal elements consisting of the second partials $\delta^2 L_S / (\delta \phi_i)^2$, $\delta^2 L_S / (\delta \beta_i)^2$, and $\delta^2 L_S / (\delta S_i)^2$ for each of the S time periods. It can be shown that each of these diagonal elements from the matrix of second partials is negative, as follows:

$$\begin{aligned} \frac{\delta^2 L_S}{(\delta \phi_i)^2} &= \frac{-c_i^+ \left[\prod_{k=1}^{i-1} (1 - \phi_k) \right]^2}{A_i^2} + \frac{-d_i^+ \left[\prod_{k=1}^{i-1} (1 - \phi_k) \right]^2}{B_i^2} \\ &+ \sum_{s=i+1}^S \left[\frac{-c_s^+ \left[\prod_{k=1}^s (1 - \phi_k) \right]^2}{(1 - \phi_i)^2 A_s^2} + \frac{-d_s^+ \phi_s^2 \left[\prod_{k=1}^{s-1} (1 - \phi_k) \right]^2}{(1 - \phi_i)^2 B_s^2} \right] \end{aligned}$$

$$\begin{aligned}
\frac{\delta^2 L_s}{(\delta \beta_i)^2} &= \frac{-c_i^-}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_i^+ \left[\prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k) \right]^2}{A_i^2} + \frac{-d_i^-}{\beta_i^2} + \frac{-d_i^+ \left[\prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k) \right]^2}{B_i^2} \\
&+ \sum_{s=i+1}^S \left[\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_s^+ \left[\prod_{k=1}^s (1 - \lambda_k - \beta_k) \right]^2}{(1 - \lambda_i - \beta_i)^2 A_s^2} + \frac{-d_s^+ \beta_s^2 \left[\prod_{k=1}^{s-1} (1 - \lambda_k - \beta_k) \right]^2}{(1 - \lambda_i - \beta_i)^2 B_s^2} \right] \\
\text{and } \frac{\delta^2 L_s}{(\delta \lambda_i)^2} &= \frac{-c_i^-}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_i^+ \left[\prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k) \right]^2}{A_i^2} + \\
&\sum_{s=i+1}^S \left[\frac{-(c_s^- + d_s^-)}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_s^+ \left[\prod_{k=1}^s (1 - \lambda_k - \beta_k) \right]^2}{(1 - \lambda_i - \beta_i)^2 A_s^2} + \frac{-d_s^+ \beta_s^2 \left[\prod_{k=1}^{s-1} (1 - \lambda_k - \beta_k) \right]^2}{(1 - \lambda_i - \beta_i)^2 B_s^2} \right]
\end{aligned}$$

Since the observed counts of sacrificed and dead animals with or without tumors are always non-negative and all squared terms must be non-negative, it is clear that each of the second partials must be less than or equal to 0. In the case of the death rate due to tumor, β_i , or the tumor incidence rate, λ_i , the second partials are strictly negative if any animal is sacrificed or dies naturally in any of the time periods from the i -th time period until the end of the study. In the case of the overall death rate, ϕ_i , at least one animal must die naturally or be sacrificed with the tumor present in one of the time periods from the i -th time period until the end of the study. The conditions for strictly negative second partials

for β_i and λ_i are self-enforcing since the time periods are formed based on the observed times of natural death or planned sacrifices. The tumor incidence rate is typically an increasing function of time, and so it would be expected that at least one animal would be sacrificed or die with the tumor in one of the last time periods, which would satisfy the requirement for a strictly negative second partial for ϕ_i .

Unfortunately, it is not possible to determine whether the Hessian is negative definite within each time interval as it was for the case of fatal tumors. In this general case, the first partial derivatives from a given time period depend not only on the parameters from that time period, but also on parameters from all other time intervals. Although it is possible to show that the (3x3) matrix of second partials corresponding to a single time period is negative definite, the general form for the eigenvalues of multiple-interval study designs did not appear to be tractable. Numeric evaluations of the Hessian matrix under a variety of study conditions within simulation studies have supported the premise that the Hessian is indeed negative definite.

Several important features of the MLE's given by Theorem 2.3.3 should be emphasized. First of all, note that the maximum likelihood estimator of the overall death rate ϕ_i is simply the familiar Kaplan-Meier estimator formed as the ratio of the number of deaths to the total number of animals at risk. Since ϕ_i is an overall rather than a cause-specific probability of death, its value should not depend on the tumor site of interest in any specific animal experiment.

The MLE's for β_s and $(1-\lambda_s)$ are also intuitively meaningful. If one recognizes the fact that the ratio $p_s = c_s^- / (c_1^- + c_s^-)$ provides an unbiased estimate of the probability of not having the tumor among animals which remain alive, then $R_s * p_{s-1}$ is an unbiased estimate of the number of tumor-free animals at risk. It then becomes evident that the MLE for $\beta_s =$

$d_s^- / (R_s * p_{s-1})$ is simply the number of natural deaths of tumor-free animals divided by the best estimate of the number of tumor-free animals at risk. Similarly, the probability of remaining tumor-free can be expressed as the number of animals which died or were sacrificed without tumors plus an estimate of the number of live animals which remain tumor-free, all divided by an estimate of the number of tumor-free animals at risk, as follows: $(1 - \hat{\lambda}_s) = (R_{s+1} * p_s + c_s^- + d_s^-) / (R_s * p_{s-1})$.

Several cautionary statements must also be made regarding the MLE's given by (2.3.5) through (2.3.8). There is no guarantee that the proposed MLE's will exist for all possible outcomes of a long-term animal carcinogenicity experiment. For example, if all of the animals sacrificed at a given time are discovered to have tumors ($c_s^- = 0$), then the estimate p_s of the probability of remaining tumor-free among live animals will be 0, and will result in undefined estimates for β_s and λ_s . In addition, if no animals die or are sacrificed with tumors in the last interval, then there is no information regarding ϕ_s and as a result there is no unique maximum likelihood estimator for this parameter. This claim can be easily verified by examination of the likelihood, since ϕ appears only in the contributions of $\Pr[\text{SWT}]$ and $\Pr[\text{DWT}]$. In fact, this claim can be extended to include all study outcomes in which there are no deaths or sacrifices of tumor-bearing animals in the last k intervals, in which case there exists no unique set of maximum likelihood estimators for $\phi_{s-k+1}, \dots, \phi_s$.

The stipulation that there must be at least one animal falling into each of the four categories (death with tumor, death without tumor, sacrifice with tumor, sacrifice without tumor) is sufficient to guarantee the existence of the proposed maximum likelihood estimators. However, this stipulation is more severe than the necessary conditions for the maximum likelihood estimators to exist. The necessary and sufficient conditions for the critical point $\hat{\theta} = (\hat{\lambda}, \hat{\beta}, \hat{\phi})$ to maximize the likelihood given by (2.2.6) are derived under

the assumption that the likelihood is a continuous function with continuous first and second partial derivatives. In cases where the first or second partial derivatives are discontinuous due to values of zero for c_i^- , c_i^+ , d_i^- , or d_i^+ , classical optimization theory does not apply and the existence of a maximum likelihood estimator is not assured.

2.4 Analytic Solutions for the Constrained MLE's in the Nonparametric Model

As discussed in the previous section, the existence of a vector of maximum likelihood estimators $\hat{\theta} = (\hat{\lambda}, \hat{\beta}, \hat{\phi})$ is not guaranteed for all possible outcomes of a long-term animal experiment. Results of simulation studies indicate that MLE's do exist for virtually all realistic data generated from a long-term experiment, so the concern regarding existence of MLE's is of little practical importance. However, a close examination of the form for the MLE of $(1-\lambda)$ reveals that this quantity could be estimated to be greater than one. In equivalent terms, it is possible using the MLE proposed by (2.3.7) for the estimate of λ to be negative!

Malani and Van Ryzin (1986) propose analytic solutions for a different set of probability functions in a nonparametric model of tumor incidence and death, and then express the transition rate λ_i as a function of this set of parameters. In so doing, they recognize that the resulting estimate of tumor incidence could be negative for some intervals, especially if the number of natural deaths or sacrifices within the interval is small (Malani and Van Ryzin, 1988). Using these types of estimators to estimate the cumulative probability of remaining tumor-free through the i -th time interval, denoted $\Lambda(t_i)$, can result in unexpected increases in $\hat{\Lambda}(t_i)$ over time for a function which should always be non-increasing. Whereas preliminary simulation studies based on realistic death rates and tumor incidence rates have suggested that the lack of existence of MLE's is of little practical concern, they have suggested that the unconstrained estimator of tumor incidence given by (2.3.7) often yields negative estimates. The resolution of this problem is of utmost

importance in the estimation of tumor incidence rates, as they will form the basis for any decisions made regarding safe exposure levels.

Further examination of the MLE's for ϕ and β reveals that these estimators will never be negative for any study outcome, as the counts of events are always greater than or equal to zero. The estimator of λ can yield negative values as described above, but the estimate of $(1-\lambda)$ will always be positive; thus the estimate of λ cannot be greater than one. In addition, the estimator for ϕ is always strictly less than one since it is assumed that at least one animal will be sacrificed at the end of each time interval. It is possible for the estimate of β to be greater than one, but it can be shown that this will occur only in situations when the estimate of $(1-\lambda)$ is also greater than one. The data outcome in which this latter event will occur is:

$$[R_{i+1} c_i^- + (c_i^- + c_i^+) (c_i^- + d_i^-)] (c_{i-1}^- + c_{i-1}^+) > (c_i^- + c_i^+) R_i c_{i-1}^-$$

which can be simplified to the condition that:

$$R_{i+1} [c_i^- c_{i-1}^+ - c_i^+ c_{i-1}^-] > (c_i^- + c_i^+) [c_{i-1}^- (c_i^+ + d_i^+) - c_{i-1}^+ (c_i^- + d_i^-)] \quad (2.4.1)$$

It can also be shown that if $\hat{\beta}$ is greater than one in any time interval, then the following must be true:

$$d_i^- c_{i-1}^+ > c_{i-1}^- (c_i^- + c_i^+ + d_i^+ + R_{i+1})$$

Rearrangement of the terms in (2.4.1) yields the inequality:

$$d_i^- c_{i-1}^+ > c_{i-1}^- (c_i^+ + d_i^+ + R_{i+1}) - \frac{R_{i+1} c_i^- (c_{i-1}^- + c_{i-1}^+)}{(c_i^- - c_i^+)} - c_i^- c_{i-1}^+$$

It follows that if $\hat{\beta}_i > 1$, then it must also be true that $\hat{\lambda}_i < 0$. The contra-positive must also be true: that is, if $\hat{\lambda}_i \geq 0$, then $\hat{\beta}_i \leq 1$. Therefore, if the unconstrained estimate of λ_i is

greater than or equal to zero, then the estimate of β_i will also fall into the correct parameter space.

It seems clear that the next direction to pursue is to attempt to determine maximum likelihood estimators under the constraint that all tumor incidence rates are non-negative. The method developed by Kuhn and Tucker can be used to address this maximization problem. This procedure addresses the problem of maximizing a continuous function $f(\theta)$ of the $3*S$ variables in the vector of parameters $\theta = (\lambda_1, \dots, \lambda_S, \beta_1, \dots, \beta_S, \phi_1, \dots, \phi_S)$ subject to a set of "m" inequality constraints of the form $g_i(\theta) \leq b_i$ for $i=1, \dots, m$. The inequalities are first transformed to equalities by adding a non-negative variable u_i^2 to each inequality, as follows:

$$g_i(\theta) + u_i^2 = b_i \quad \text{or} \quad g_i(\theta) + u_i^2 - b_i = 0$$

Then an equality constraint can be created as $g_i^*(\theta, u_i) = g_i(\theta) + u_i^2 - b_i = 0$, and the method of constrained equality maximization can be applied. The Lagrange function is formed as:

$$F(\theta, \alpha, u) = f(\theta) + \sum_{i=1}^m \alpha_i g_i^*(\theta, u_i)$$

The Lagrange multipliers are typically denoted by the symbol " λ "; in this presentation they will be denoted by " α " to avoid confusion with the model parameters which are to be constrained. The solutions to the constrained maximization problem are obtained by solving the equations:

$$(1) \quad \frac{\delta F(\theta, \alpha, u)}{\delta \theta_j} = \frac{\delta f(\theta)}{\delta \theta_j} + \sum_{i=1}^m \alpha_i \frac{\delta g_i(\theta)}{\delta \theta_j} \quad \text{for } j=1, \dots, (3*S)$$

$$(2) \quad \frac{\delta F(\theta, \alpha, u)}{\delta \alpha_i} = g_i^*(\theta, u_i) \quad \text{for } i=1, \dots, m$$

$$(3) \quad \frac{\delta F(\theta, \alpha, u)}{\delta u_i} = 2 \alpha_i u_i \quad \text{for } i=1, \dots, m$$

The Kuhn-Tucker conditions for a maximum of $f(\theta)$ at the vector θ^0 subject to m inequality constraints are obtained by satisfying the preceding three equations and the original inequality constraints. They can be summarized by the following four necessary conditions:

$$(i) \quad \frac{\delta F(\theta^0, \alpha^0, u^0)}{\delta \theta_j} = 0 \quad \text{for } j=1, \dots, (3*S)$$

$$(ii) \quad \alpha_i g_i(\theta^0 - b_i) = 0 \quad \text{for } i=1, \dots, m$$

$$(iii) \quad \alpha_i \leq 0 \quad \text{for } i=1, \dots, m$$

$$(iv) \quad g_i(\theta^0) \leq 0 \quad \text{for } i=1, \dots, m$$

The first equation is simply that the first partials vanish at the proposed critical point. The second condition is obtained by solving the previous equations (2) and (3) simultaneously, the third condition insures that a maximum is obtained rather than a minimum, and the last condition is simply the original inequality constraints. It can be shown that the solution x^0 is the absolute maximum rather than a local maximum if the function $f(\theta)$ is strictly concave and the constraints $g_i(\theta)$ are convex, since it follows that the Lagrange function $F(\theta, \alpha, u)$ is strictly concave, and thus its derivatives vanish at only one point. Under these circumstances, the conditions (i) - (iv) given above are sufficient as well as necessary for an absolute maximum of $f(\theta)$ at θ^0 (Gue and Thomas, 1968, p.51).

In the maximization problem presently under consideration, the function to be maximized is the log-likelihood $f(\theta) = \ln L(\lambda, \beta, \phi)$, the original constraints stated in terms of the above construction are $g_i(\theta) = -\lambda_i \leq 0$ and $b_i = 0$, and the transformed equality constraints are $g_i^*(\theta) = -\lambda_i + u_i^2 = 0$ for $i=2, \dots, S$ (no constraint is necessary for λ_1 since it cannot be negative). The Kuhn-Tucker condition (ii) actually implies a duality of requirements, since the individual cases that $\alpha_i = 0$ and $g_i(\theta^0) - b_i = 0$ must be considered separately. The situation in which $\alpha_i = 0$ reflects an inactive constraint, while the second condition results in the active constraint that $\lambda_i = 0$. In effect, the imposition of an active constraint on λ_i forces the maximum likelihood estimator of λ_i to remain on the boundary of the parameter space. In a long-term animal experiment with S time intervals, the total number of cases which must be considered is 2^{S-1} . For example, the number of cases which must be considered for a long-term animal study with one interim sacrifice and a terminal sacrifice is two: the case in which the unconstrained solutions given by (2.3.5) - (2.3.8) are within the parameter space $[0,1]$, and the case in which the constraint that $\lambda_2 \geq 0$ is employed. Similarly, four cases must be considered for an animal experiment with three sacrifice intervals: the unconstrained case, the case in which the constraint on λ_2 is active (forcing $\hat{\lambda}_2 = 0$), the case in which the constraint on λ_3 is active (forcing $\hat{\lambda}_3 = 0$), and the last case in which both constraints are active (forcing both $\hat{\lambda}_2 = 0$ and $\hat{\lambda}_3 = 0$).

It is quite clear that as the number of time intervals encompassed by the design of a long-term animal study increases, the possible number of cases which must be considered in order to determine maximum likelihood estimators increases exponentially. For each individual case under consideration, a separate Lagrange function must be formed and the partial derivative equations solved simultaneously. This difficulty was addressed by first focusing attention on the derivation of results for a study design consisting of two intervals, and next addressing the three-interval design, in the hopes of determining a

general pattern for any given number of study intervals based on the active constraints to be employed.

In the derivation of the MLE's for the general unconstrained framework described in Theorem 2.3.3, the estimators of λ , ϕ , and β were all determined for the last interval, and then the next to last interval, and so on as previously described. The substitution of these values into the remaining partial derivative equations allowed a great degree of simplification of the system of equations at each step, and the solutions for the parameters at each step depended only on parameters from earlier sacrifice intervals. In the evaluation of the systems of partial derivative equations which arise when active constraints are employed, the ability to eliminate the three equations corresponding to a single sacrifice interval is precluded. Consequently, the determination of a set of solutions to the partial derivative equations becomes much more complex. In addition, the simple intuitive forms for the maximum likelihood estimators are not always available as they were for the general unconstrained situation.

The closed-form solutions for the maximum likelihood estimators in a two-interval animal study design are presented in Table 2.4.1. Note that the MLE's for β_1 and ϕ_1 are equivalent in the unconstrained and constrained frameworks. Not surprisingly, the most significant difference between the unconstrained solutions and the constrained solutions is that the MLE of λ_2 in the constrained framework is zero. The implicit assumption made by forcing the MLE for λ_2 to be zero is that all animals which either die naturally or are sacrificed with tumors during the course of the study must develop these tumors during the first interval of the study. The recognition of this implicit assumption makes the forms for $\hat{\beta}_2$ and $(1-\hat{\lambda}_1)$ very intuitive. We are no longer required to estimate the probability of remaining tumor-free among live animals as $p_1 = c_1^- / (c_1^- + c_1^+)$ and multiply this quantity by the total number of animals at risk to obtain the best estimate of the number of tumor-

free animals at risk, since it is now known that the exact number of tumor-free animals at the beginning of the second interval is $(c_2^- + d_2^-)$. Simple substitution of $(c_1^- + d_1^-)$ for $R_i \cdot p_{i-1}$ leads directly to the constrained estimates of β_2 and λ_1 .

In contrast to the expected change in the estimator for λ_2 , the difference in the new form for ϕ_2 , the overall probability of death during the second interval, is quite unexpected. Unfortunately, recognition of the implicit assumption regarding concentration of tumor incidence in the first interval does not appear to make the form for ϕ_2 any more meaningful. If no animals were sacrificed at the end of the first interval, then the estimate of ϕ_2 under the constraint that $\lambda_2 \geq 0$ would be the same as the unconstrained MLE. However, we have assumed that at least one animal is sacrificed at the end of each interval.

The MLE for the Lagrangian multiplier α_2 is also shown in Table 2.4.1 for both the unconstrained and constrained situations. Differentiation of the 2-interval Lagrangian function $F(\theta, \alpha_2, u_2)$ in which $\theta = (\lambda_1, \lambda_2, \beta_1, \beta_2, \phi_1, \phi_2)$ with respect to λ_2 yields the following solution for the Lagrangian multiplier in terms of the remaining parameters:

$$\hat{\alpha}_2 = \frac{-c_2^-}{(1 - \hat{\lambda}_2 - \hat{\beta}_2)} + \frac{c_2^+ (1 - \hat{\lambda}_1 - \hat{\beta}_1)}{(1 - \hat{\phi}_1)(1 - \hat{\phi}_2) - (1 - \hat{\lambda}_1 - \hat{\beta}_1)(1 - \hat{\lambda}_2 - \hat{\beta}_2)}$$

Substitution of the constrained MLE's in the above formula produces the result shown in Table 2.4.1 for Case II. This expression is negative for those cases in which the observed data follows the relationship:

$$c_1^+ (c_2^- + d_2^-) > c_1^- (c_2^+ + d_2^+) .$$

This condition is equivalent to that given by expression (2.4.1) under which the unconstrained estimate of λ_2 is negative. Thus, if the observed data yields a negative unconstrained estimate of λ_2 , then the constrained MLE for α_2 will be negative, satisfying

the Kuhn-Tucker condition (iii). On the other hand, if the observed data yields a non-negative unconstrained estimate, no constraints are necessary. In this case, it can be verified that substitution of the unconstrained MLE's in the above formula for α_2 results in an estimate of zero.

The maximum likelihood estimators for ϕ , β , and λ in the three-interval design are presented in Tables 2.4.2, 2.4.3, and 2.4.4 for the case of no active constraints (Case I), the case in which $\hat{\lambda}_3 = 0$ (Case II), and the case in which both active constraints are imposed, so that $\hat{\lambda}_2 = 0$ and $\hat{\lambda}_3 = 0$ (Case IV), respectively. The case in which $\hat{\lambda}_2 = 0$ (Case III) has special characteristics which merit individual attention, and the MLE's arising from this case will be presented separately. It is apparent from examination of Tables 2.4.2 - 2.4.4 that the estimators of β_1 and ϕ_1 are equivalent under Cases I, II, and IV, just as they were equivalent in the first interval of the two-interval study design. The estimators for β_2 and ϕ_2 for Case II are also the same as the unconstrained estimators, while the estimator of β_3 is equivalent under Cases II and IV. In general, it appears that the estimator for β_i under a given set of constraints is equivalent to the unconstrained estimate when there is no active constraint imposed on λ_i . Similarly, the general form for the MLE of ϕ_i in the constrained cases II and IV appears to be equivalent to the unconstrained form when λ_i is not actively constrained.

The MLE's for the Lagrangian multipliers for Case I, II, and IV are provided in Tables 2.4.2, 2.4.3, and 2.4.4, respectively. Differentiation of the 3-interval Lagrangian function $F(\theta, \alpha, u)$ in which $\theta = (\lambda_1, \lambda_2, \lambda_3, \beta_1, \beta_2, \beta_3, \phi_1, \phi_2, \phi_3)$, $\alpha = (\alpha_2, \alpha_3)$, and $u = (u_2, u_3)$ with respect to λ_3 yields the following general solution for the Lagrangian multiplier in terms of the remaining parameters:

$$\hat{\alpha}_3 = \frac{-c_3}{(1 - \hat{\lambda}_3 - \hat{\beta}_3)} + \frac{c_3^+ (1 - \hat{\lambda}_1 - \hat{\beta}_1)(1 - \hat{\lambda}_2 - \hat{\beta}_2)}{(1 - \hat{\phi}_1)(1 - \hat{\phi}_2)(1 - \hat{\phi}_3) - (1 - \hat{\lambda}_1 - \hat{\beta}_1)(1 - \hat{\lambda}_2 - \hat{\beta}_2)(1 - \hat{\lambda}_3 - \hat{\beta}_3)} \quad (2.4.2)$$

Similarly, differentiation of the Lagrangian function $F(\theta, \alpha, u)$ with respect to λ_2 yields the following general form for the MLE of α_2 :

$$\hat{\alpha}_3 = \frac{-(c_2 + c_3 + d_3)}{(1 - \hat{\lambda}_2 - \hat{\beta}_2)} + \frac{c_2^+ (1 - \hat{\lambda}_1 - \hat{\beta}_1)}{A_2} + \frac{c_3^+ (1 - \hat{\lambda}_1 - \hat{\beta}_1)(1 - \hat{\lambda}_3 - \hat{\beta}_3)}{A_3} + \frac{d_3^+ \hat{\beta}_3 (1 - \hat{\lambda}_1 - \hat{\beta}_1)}{B_3} \quad (2.4.3)$$

in which A_i and B_i are as defined in Section 2.3.

Under Case II ($\hat{\lambda}_3=0$), it can be verified from expression (2.4.1) that the unconstrained estimate of α_3 is negative if and only if the unconstrained estimate of λ_3 is also negative. Comparison of the forms of the MLE's for α_2 and α_3 under Case IV with the corresponding expressions given by (2.4.1) when $\hat{\lambda}_2$ and $\hat{\lambda}_3$ are zero also leads to the conclusion that the MLE's for α_2 and α_3 are both negative if and only if the unconstrained estimates of λ_2 and λ_3 are both negative. In any of the constrained cases, if either or both of the unconstrained estimates of λ_2 and λ_3 are negative, then the corresponding constrained MLE's for α_2 and α_3 will also be negative, satisfying requirement (iii) of the Kuhn-Tucker conditions. When both of the unconstrained estimates of λ_2 and λ_3 are non-negative, substitution of the unconstrained MLE's given in Table 2.4.2 into the above formula produces MLE's of zero for both α_2 and α_3 .

The derivation of the constrained maximum likelihood estimators for Cases II and IV through the use of the Kuhn-Tucker conditions is relatively straightforward, and the resulting estimators are intuitively reasonable, especially for the β 's and λ 's. In fact, the form of the estimators for the β 's and λ 's becomes simpler in the constrained cases because the exact number of live tumor-free animals at risk is actually known rather than estimated.

Derivation of the MLE's under the constraint that $\lambda_2 \geq 0$ (Case III) becomes awkward. The partial derivative equations are solved one by one, and at each step the solution for one of the eight unconstrained parameters is obtained in terms of the remaining parameters. Regardless of the order in which parameter solutions are obtained, or which equations are used to solve for any given parameter, the last partial derivative equation reduces to a cubic equation in terms of the last remaining parameter. A cubic equation has three roots, and the general form of these three roots is known. However, it is possible that only one of the three roots is real, while the other two are complex conjugates of one another (Dobbs and Hanks, 1980). By graphing the value of the partial derivative as a function of the last remaining parameter, chosen arbitrarily to be β_2 , over the parameter space (0,1) for various data outcomes which were representative of a Case III situation, it was determined that there were two vertical asymptotes, one positively infinite and the other negatively infinite. The only real root resides at a value of β_2 which falls between the values of the asymptotes (see Figure 2.4.1). The formula for this root was obtained using Mathematica, a computer language which allows the manipulation of symbolic parameters. The general form of a cubic equation in β_2 can be written as:

$$a^3\beta_2 + b^2\beta_2 + c\beta_2 + d = 0$$

and the solution for β_2 corresponding to the root of the partial derivative equation is:

$$\begin{aligned} \frac{-b}{3a} - \frac{1}{2} \left[\frac{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]}{\left\{ \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right] + \sqrt{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]^3 + \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right]^2} \right\}^{\frac{1}{3}}} \right. \\ \left. + \left\{ \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right] + \sqrt{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]^3 + \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right]^2} \right\}^{\frac{1}{3}} \right] \end{aligned}$$

$$-\frac{3i}{2} \left\{ \frac{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]}{\left\{ \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right] + \sqrt{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]^3 + \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right]^2} \right\}^{\frac{1}{3}}} \right. \\ \left. + \left\{ \frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right] + \sqrt{\left[\frac{-b^2}{9a^2} + \frac{c}{3a} \right]^3 + \left[\frac{-b^3}{27a^3} + \frac{bc}{6a^2} - \frac{d}{2a} \right]^2} \right\}^{\frac{1}{3}} \right\}$$

When the data values from any outcome representative of a Case III constraint situation are substituted in the above formula, the result is a function of complex numbers whose final solution is real; i.e., the coefficient "b" of the complex number $(a + b*i)$ is zero. The coefficients in the partial derivative equation which are substituted in the above formula are:

$$a = -c_1^- (c_2^- + d_2^- + n_3) (n_2 + n_3)$$

$$b = c_1^- (c_2^-)^2 + c_1^+ (c_2^-)^2 + (c_2^-)^3 + c_1^- c_2^- c_2^+ + c_1^+ c_2^- c_2^+ + 2 (c_2^-)^2 c_2^+ + \\ c_2^- (c_2^+)^2 + 4 c_1^- c_2^- d_2^- + 2 c_1^+ c_2^- d_2^- + 2 (c_2^-)^2 d_2^- + 2 c_1^- c_2^+ d_2^- + \\ c_1^+ c_2^+ d_2^- + 3 c_1^- c_2^+ d_2^- + d_2^- (c_2^+)^2 + 3 c_1^- (d_2^-)^2 + c_1^+ (d_2^-)^2 + \\ c_2^- (d_2^-)^2 + c_2^+ (d_2^-)^2 + c_1^- c_2^- d_2^+ + d_2^+ (c_2^-)^2 + d_2^+ c_2^- c_2^+ + \\ 2 c_1^- d_2^- d_2^+ + c_2^- d_2^- d_2^+ + c_2^+ d_2^- d_2^+ + 2 c_1^- c_2^- n_3 + c_1^+ c_2^- n_3 + \\ 2 (c_2^-)^2 n_3 + c_1^- d_2^+ n_3 + 2 c_2^- c_2^+ n_3 + 4 c_1^- d_2^- n_3 + c_1^+ d_2^- n_3 + \\ 2 c_2^- d_2^- n_3 + c_2^+ d_2^- n_3 + c_1^- d_2^+ n_3 + c_1^- (n_3)^2 + c_2^- (n_3)^2$$

$$c = d_2^- \{ 2 c_1^- c_2^- + 2 c_1^+ c_2^- + 2 (c_2^-)^2 + c_1^- c_2^+ + c_1^+ c_2^+ + 3 c_2^- c_2^+ + \\ (c_2^+)^2 + 3 c_1^- d_2^- + 2 c_1^+ d_2^- + 2 c_2^- d_2^- + 2 c_2^+ d_2^- + c_1^- d_2^+ + \\ c_2^- d_2^+ + c_2^+ d_2^+ + 2 c_1^- n_3 + c_1^+ n_3 + 2 c_2^- n_3 + c_2^+ n_3 \}$$

$$d = (d_2^-)^2 (c_1^- + c_1^+ + c_2^- + c_2^+)$$

The maximum likelihood solutions for the remaining parameters as functions of β_2 are shown in Table 2.4.5. Note that the MLE's for β_1 and ϕ_1 have the same form as in the unconstrained (Case I) and other constrained cases, and are simple functions of the data outcomes which do not depend on β_2 . The maximum likelihood estimator for ϕ_3 is also equivalent to the unconstrained MLE. In fact, the estimator for the overall death rate during the last interval, ϕ_5 , will always be $(d_5^- + d_5^+) / n_5$ for a design consisting of any number of intervals provided there is no active constraint on λ_5 . This result can be easily proven by solving the system of likelihood equations $\delta L(\theta) / \delta \phi_5 = 0$, $\delta L(\theta) / \delta \beta_5 = 0$, and $\delta L(\theta) / \delta \lambda_5 = 0$ simultaneously for ϕ_5 , β_5 , and λ_5 . The MLE's for the Lagrangian multipliers can be obtained by substituting the MLE's provided in Table 2.4.5 into the general form of the multipliers α_2 and α_3 given by expressions (2.4.3) and (2.4.2), respectively.

Clearly, the maximum likelihood estimator for β_2 , as written above, does not allow intuitive interpretation of its form. In addition, substitution of the value of the estimator of β_2 in the solutions for the remaining parameters precludes their interpretation with respect to individual data outcomes. The inability to derive a simple analytic formula for $\hat{\beta}_2$ as a solution to the cubic equation is an indication of even more complex systems of equations to be solved in a four-interval sacrifice design. From examination of the partial derivative equations in a four-interval study design, it can be determined that under the active constraints $\lambda_2 \geq 0$ or $\lambda_3 \geq 0$ it is first necessary to find the roots of a cubic equation, and then determine the roots of a quartic (4-th order) equation given the solution for the correct root of the cubic equation. The general forms for the roots of a fourth-order equation have also been determined, although the solutions may be complex numbers. However, it is likely that the structure of a five-interval study will lead to a system of equations requiring

determination of the roots of fifth order equation, for which no general form is known (Dobbs and Hanks, 1980). The absence of general forms for higher order polynomial equations does not necessarily imply that solutions do not exist, yet given our experience with a three-interval study design, it is not likely that the structure of the higher order equations will lend itself towards determination of solutions.

Despite the difficulties encountered in determining the form for the last remaining estimator in a three-interval design under the constraint that $\lambda_2 \geq 0$ and the disadvantages accompanying the resulting solution for this parameter, it still must be acknowledged that the solution is a closed-form analytic expression. Any analytic solution is to be preferred over an approximation of the maximum likelihood estimator which uses iterative numeric techniques requiring extensive computer facilities and computation time.

From a practical standpoint, it is unlikely that regulatory agencies involved in the evaluation of chemical compounds for carcinogenic risk would incorporate more than two interim sacrifices and a terminal sacrifice in their standard design of long-term animal carcinogenicity experiments. This hesitancy on the part of regulatory agencies to adopt designs including interim sacrifices is based almost entirely on the prohibitive costs of including the extra animals required by interim sacrifices. It is therefore of interest to regulatory agencies to determine whether adequate estimates of the tumor incidence rate and death rates may be provided by studies which can be performed within practical cost limitations. The ability of a three interval study design to provide adequate estimates of the tumor incidence rate and death rates is evaluated via simulation studies described in Chapter VI.

Figure 2.4.1

Partial Derivative of Log-likelihood as a Function of β_2

Table 2.4.1

Constrained MLE Solutions for Two Time Intervals

Case I (unconstrained)	Case II ($\hat{\lambda}_2 = 0$)
$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2}$	$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2}$
$\hat{\beta}_2 = \frac{d_2^- (c_1^- + c_1^+)}{n_2 c_1^-}$	$\hat{\beta}_2 = \frac{d_2^-}{(c_2^- + d_2^-)}$
$(1 - \hat{\lambda}_1) = \frac{n_2 c_1^- + (c_1^- + c_1^+) (c_1^- + d_1^-)}{(n_1 + n_2) (c_1^- + c_1^+)}$	$(1 - \hat{\lambda}_1) = \frac{(c_1^- + d_1^- + c_2^- + d_2^-)}{(n_1 + n_2)}$
$(1 - \hat{\lambda}_2) = \frac{(c_2^- + d_2^-) (c_1^- + c_1^+)}{n_2 c_1^-}$	$\hat{\lambda}_2 = 0$
$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2}$	$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2}$
$\hat{\phi}_2 = \frac{d_2^- + d_2^+}{n_2}$	$\hat{\phi}_2 = \frac{\left[\begin{array}{l} d_2^+ (c_2^- + d_2^-) (c_1^+ + c_2^+ + d_2^+) \\ + d_2^- (c_2^+ + d_2^+) (c_1^- + c_2^- + d_2^-) \end{array} \right]}{(c_2^- + d_2^-) (c_2^+ + d_2^+) (n_2 + c_1^- + c_1^+)}$
$\hat{\alpha}_2 = 0$	$\hat{\alpha}_2 = \frac{-c_1^+ (c_2^- + d_2^-) + c_1^- (c_2^+ + d_2^+)}{(c_1^+ + c_2^+ + d_2^+)}$

Table 2.4.2

Case I ($\hat{\lambda}_2 \neq 0, \hat{\lambda}_3 \neq 0$)

Unconstrained MLE Solutions for Three Time Intervals

$$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2 + n_3}$$

$$\hat{\beta}_2 = \frac{d_2^- (c_1^- + c_1^+)}{(n_2 + n_3) c_1^-}$$

$$\hat{\beta}_3 = \frac{d_3^- (c_2^- + c_2^+)}{n_3 c_2^-}$$

$$(1 - \hat{\lambda}_1) = \frac{(n_2 + n_3) c_1^- + (c_1^- + c_1^+) (c_1^- + d_1^-)}{(n_1 + n_2 + n_3) (c_1^- + c_1^+)}$$

$$(1 - \hat{\lambda}_2) = \frac{[n_3 c_2^- + (c_2^- + c_2^+) (c_2^- + d_2^-)] (c_1^- + c_1^+)}{(n_2 + n_3) (c_2^- + c_2^+) c_1^-}$$

$$(1 - \hat{\lambda}_3) = \frac{(c_3^- + d_3^-) (c_2^- + c_2^+)}{n_3 c_2^-}$$

$$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2 + n_3}$$

$$\hat{\phi}_2 = \frac{d_2^- + d_2^+}{n_2 + n_3}$$

$$\hat{\phi}_3 = \frac{d_3^- + d_3^+}{n_3}$$

$$\hat{\alpha}_2 = 0 ; \hat{\alpha}_3 = 0$$

Table 2.4.3

Case II ($\hat{\lambda}_2 \neq 0, \hat{\lambda}_3 = 0$)

Constrained MLE Solutions for Three Time Intervals

$$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2 + n_3}$$

$$\hat{\beta}_2 = \frac{d_2^- (c_1^- + c_1^+)}{(n_2 + n_3) c_1^-}$$

$$\hat{\beta}_3 = \frac{d_3^-}{c_3^- + d_3^-}$$

$$(1 - \hat{\lambda}_1) = \frac{(n_2 + n_3) c_1^- + (c_1^- + c_1^+) (c_1^- + d_1^-)}{(n_1 + n_2 + n_3) (c_1^- + c_1^+)}$$

$$(1 - \hat{\lambda}_2) = \frac{(c_2^- + d_2^- + c_3^- + d_3^-) (c_1^- + c_1^+)}{(n_2 + n_3) c_1^-}$$

$$\hat{\lambda}_3 = 0$$

$$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2 + n_3}$$

$$\hat{\phi}_2 = \frac{d_2^- + d_2^+}{n_2 + n_3}$$

$$\hat{\phi}_3 = \frac{\left[\begin{array}{l} d_3^+ (c_3^- + d_3^-) (c_2^+ + c_3^+ + d_3^+) \\ + d_3^- (c_3^+ + d_3^+) (c_2^- + c_3^- + d_3^-) \end{array} \right]}{(c_3^- + d_3^-) (c_3^+ + d_3^+) (n_3 + c_2^- + c_2^+)}$$

$$\hat{\alpha}_2 = 0$$

$$\hat{\alpha}_3 = \frac{-c_2^+ (c_3^- + d_3^-) + c_2^- (c_3^+ + d_3^+)}{(c_2^+ + c_3^+ + d_3^+)}$$

Table 2.4.4

Case IV ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 = 0$)

Constrained MLE Solutions for Three Time Intervals

$$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2 + n_3}$$

$$\hat{\beta}_2 = \frac{d_2^-}{c_2^- + d_2^- + c_3^- + d_3^-}$$

$$\hat{\beta}_3 = \frac{d_3^-}{c_3^- + d_3^-}$$

$$(1 - \hat{\lambda}_1) = \frac{c_1^- + d_1^- + c_2^- + d_2^- + c_3^- + d_3^-}{n_1 + n_2 + n_3}$$

$$\hat{\lambda}_2 = 0 ; \hat{\lambda}_3 = 0$$

$$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2 + n_3}$$

$$\hat{\phi}_2 = \frac{\left[\begin{array}{l} d_2^+ (c_2^- + d_2^- + c_3^- + d_3^-) (c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+) \\ + d_2^- (c_2^+ + d_2^+ + c_3^+ + d_3^+) (c_1^- + c_2^- + d_2^- + c_3^- + d_3^-) \end{array} \right]}{(c_2^- + d_2^- + c_3^- + d_3^-) (c_2^+ + d_2^+ + c_3^+ + d_3^+) (n_2 + n_3 + c_1^- + c_1^+)}$$

$$\hat{\phi}_3 = \frac{\left[\begin{array}{l} d_3^+ (c_3^- + d_3^-) (c_2^+ + c_3^+ + d_3^+) (c_2^- + d_2^- + c_3^- + d_3^-) (c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+) \\ + d_3^- (c_3^+ + d_3^+) (c_2^- + c_3^- + d_3^-) (c_2^+ + d_2^+ + c_3^+ + d_3^+) (c_1^- + c_2^- + d_2^- + c_3^- + d_3^-) \end{array} \right]}{\left\{ (c_3^- + d_3^-) (c_3^+ + d_3^+) \left[\frac{(c_2^- + c_3^- + d_3^-) (c_2^+ + d_2^+ + c_3^+ + d_3^+) (c_1^- + c_2^- + d_2^- + c_3^- + d_3^-)}{(c_2^+ + c_3^+ + d_3^+) (c_2^- + d_2^- + c_3^- + d_3^-) (c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+)} + 1 \right] \right\}}$$

$$\hat{\alpha}_2 = \frac{\left[\begin{array}{l} - (c_3^- + d_3^-) (c_2^+ + c_3^+ + d_3^+) (c_2^- + d_2^- + c_3^- + d_3^-) (c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+) \\ + (c_3^+ + d_3^+) (c_2^- + c_3^- + d_3^-) (c_2^+ + d_2^+ + c_3^+ + d_3^+) (c_1^- + c_2^- + d_2^- + c_3^- + d_3^-) \end{array} \right]}{[(c_2^+ + c_3^+ + d_3^+) (c_2^- + d_2^- + c_3^- + d_3^-) (c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+)]}$$

$$\hat{\alpha}_3 = \frac{-c_1^+ (c_2^- + d_2^- + c_3^- + d_3^-) + c_1^- (c_2^+ + d_2^+ + c_3^+ + d_3^+)}{(c_1^+ + c_2^+ + d_2^+ + c_3^+ + d_3^+)}$$

Table 2.4.5

Case III ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 \neq 0$)

Constrained MLE Solutions for Three Time Intervals

$$\hat{\beta}_1 = \frac{d_1^-}{n_1 + n_2 + n_3}$$

 $(\hat{\beta}_2 \text{ as given in text on pages 64-66})$

$$\hat{\beta}_3 = \frac{d_3^- (1 - \hat{\lambda}_3)}{c_3^- + d_3^-}$$

$$(1 - \hat{\lambda}_1) = \frac{\left[(n_3 + c_2^- + d_2^- + c_2^+) \hat{\beta}_2 - d_2^- \right] \left[d_2^- - (c_2^- + d_2^-) \hat{\beta}_2 \right] (1 - \hat{\phi}_1)}{\hat{\beta}_2 \left\{ \frac{d_2^+ (1 - \hat{\beta}_2) \left[(n_3 + c_2^- + d_2^-) \hat{\beta}_2 - d_2^- \right]}{\left[(n_3 + c_2^- + d_2^-) \hat{\beta}_2 - (n_3 + d_2^-) \right] \left[(n_3 + c_2^- + d_2^- + c_2^+) \hat{\beta}_2 - d_2^- \right]} \right\}} + \hat{\beta}_1$$

$$\hat{\lambda}_2 = 0$$

$$(1 - \hat{\lambda}_3) = \frac{(c_3^- + d_3^-) (1 - \hat{\phi}_1) (1 - \hat{\phi}_2)}{n_3 (1 - \hat{\lambda}_1 - \hat{\beta}_1) (1 - \hat{\beta}_2)}$$

$$\hat{\phi}_1 = \frac{d_1^- + d_1^+}{n_1 + n_2 + n_3}$$

$$(1 - \hat{\phi}_2) = \frac{n_3 (1 - \hat{\lambda}_1 - \hat{\beta}_1) (1 - \hat{\beta}_2)}{(1 - \hat{\phi}_1) \left[d_2^- - (c_2^- + d_2^-) \hat{\beta}_2 \right]}$$

$$\hat{\phi}_3 = \frac{d_3^- + d_3^+}{n_3}$$

2.5 Analytic Solutions for the MLE's in the Alternate Framework

Recall that the maximum likelihood estimators which are presented in Section 2.3 and 2.4 have been derived under the framework described in Section 2.1, in which the indicator $\delta(t_i)$ is defined to equal one if the tumor is present at time t_i and zero if the tumor is absent at time t_i . An analogous collection of MLE's can be developed under the alternative framework, in which $\delta^*(t_i)$ is defined to equal one if the first event is tumor and zero if the first event is death without tumor, and the simultaneous occurrence of the two possibilities is not explicitly prohibited. The parameters to be estimated in the nonparametric model of tumor incidence and death will be denoted by β^* , λ^* , and ϕ^* in order to avoid confusion with the parameters in the original framework.

The derivation of the separate contribution of each animal based on the four possible events within each time interval follows the same pattern as the original framework, and the components representing the probability of the four possible events are:

$$\Pr[\text{SWOT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \prod_{j=1}^s (1 - \lambda_j^*) (1 - \beta_j^*) \quad (2.5.1)$$

$$\Pr[\text{DWOT}_s] = \prod_{j=1}^{s-1} (1 - \pi_j) \beta_s^* \prod_{j=1}^s (1 - \lambda_j^*) \prod_{j=1}^{s-1} (1 - \beta_j^*) \quad (2.5.2)$$

$$\Pr[\text{SWT}_s] = \pi_s \prod_{j=1}^{s-1} (1 - \pi_j) \left\{ \prod_{j=1}^s (1 - \phi_j^*) - \prod_{j=1}^s (1 - \lambda_j^*) (1 - \beta_j^*) \right\} \quad (2.5.3)$$

$$\Pr[\text{DWT}_s] = \prod_{j=1}^s (1 - \pi_j) \left\{ \phi_s^* \prod_{j=1}^{s-1} (1 - \phi_j^*) - \beta_s^* \prod_{j=1}^s (1 - \lambda_j^*) \prod_{j=1}^{s-1} (1 - \beta_j^*) \right\} \quad (2.5.4)$$

The expressions involving the probability of sacrifice or non-sacrifice can again be factored out of the likelihood and incorporated with the constant of proportionality as they were in the original framework, since they do not involve the parameters for which the likelihood is to be maximized. The log-likelihood reflecting the separate expressions for the four possible events becomes:

$$\begin{aligned}
 L = \ln L(\lambda^*, \beta^*, \phi^*) = & \\
 & K + \sum_{s=1}^S \left\{ \left[c_s^- \ln \left(\prod_{j=1}^s (1 - \lambda_j^*) (1 - \beta_j^*) \right) \right] + \left[d_s^- \ln \left(\beta_s^{*s} \prod_{j=1}^s (1 - \lambda_j^*) \prod_{j=1}^{s-1} (1 - \beta_j^*) \right) \right] \right. \\
 & \quad \left. + \left[c_s^+ \ln \left(\prod_{j=1}^s (1 - \phi_j^*) - \prod_{j=1}^s (1 - \lambda_j^*) (1 - \beta_j^*) \right) \right] \right. \\
 & \quad \left. + \left[d_s^+ \ln \left(\phi_s^{*s-1} \prod_{j=1}^s (1 - \phi_j^*) - \beta_s^{*s} \prod_{j=1}^s (1 - \lambda_j^*) \prod_{j=1}^{s-1} (1 - \beta_j^*) \right) \right] \right\}
 \end{aligned}
 \tag{2.5.5}$$

in which $\lambda^* = (\lambda_1^*, \lambda_2^*, \dots, \lambda_S^*)'$, $\beta^* = (\beta_1^*, \beta_2^*, \dots, \beta_S^*)'$ and $\phi^* = (\phi_1^*, \phi_2^*, \dots, \phi_S^*)'$, and K is an additive constant which does not involve any of the parameters in λ^* , β^* , or ϕ^* .

Theorem 2.5.1: In the nonparametric model for death and tumor development with the discrete rates

$$\lambda_s^* = \Pr [E_1 = t_s, \delta^*(t_s) = 1 \mid E_1 \geq t_s]$$

$$\beta_s^* = \Pr [E_1 = t_s, \delta^*(t_s) = 0 \mid E_1 \geq t_s]$$

$$\phi_s^* = \Pr [D = t_s \mid D \geq t_s]$$

in which E_1 is a random variable representing the time to first event (tumor onset or death), D is a random variable representing the time to death and $\delta^*(t)$ is an indicator of whether the first event E_1 is tumor onset or death without tumor, the maximum likelihood estimators for λ_s^* , β_s^* , and ϕ_s^* are given by

$$\hat{\phi}_s^* = \frac{d_s^- + d_s^+}{R_s}, \quad (2.5.6)$$

$$\hat{\beta}_s^* = \frac{d_s^- (c_s^- + c_s^+)}{R_{s+1} c_s^- + (c_s^- + c_s^+) (c_s^- + d_s^-)}, \quad (2.5.7)$$

$$\text{and } (1 - \hat{\lambda}_s^*) = \frac{[R_{s+1} c_s^- + (c_s^- + c_s^+) (c_s^- + d_s^-)] (c_{s-1}^- + c_{s-1}^+)}{(c_s^- + c_s^+) R_s c_{s-1}^-}, \quad (2.5.8)$$

$$\text{or } \hat{\Lambda}_s^* = \frac{\prod_{i=1}^s [R_{i+1} c_i^- + (c_i^- + c_i^+) (c_i^- + d_i^-)]}{(c_s^- + c_s^+) \prod_{i=1}^s R_i \prod_{i=1}^{s-1} c_i^-} \quad (2.5.9)$$

provided they are in the parameter space $(0,1)$. In order to apply these general formulas to the first and last intervals, the following definitions must be applied:

$$\frac{c_0^- + c_0^+}{c_0^-} = 1, \quad \prod_{i=1}^0 c_i^- = 1, \quad R_{S+1} = 0$$

Proof:

The log-likelihood based on the nonparametric model with discrete death rates ϕ_s^* and β_s^* and discrete tumor incidence rate λ_s^* is shown in expression (2.5.5). The partial derivatives with respect to each of these parameters at an arbitrary interval i can be shown to vanish when evaluated at the matrix of maximum likelihood estimators defined in (2.5.6), (2.5.7), and (2.5.8). Using methods identical to those used in the proof of Theorem 2.3.3, the diagonal elements of the Hessian matrix can be shown to be negative, yet the general forms for the eigenvalues of the Hessian matrix appear to be intractable.

A comparison of the MLE's in (2.5.6) and (2.5.8) for ϕ_s and λ_s respectively with those under the original definition of $\delta(t_i)$ shown in equations (2.3.5) and (2.3.7) reveals that the maximum likelihood estimators for ϕ_s and λ_s are identical under the two frameworks. This did not come as a surprise since it was expected that only the interpretation and form of the parameter representing the rate of death without tumor, β_s , would change in the alternate framework. A close examination of the forms of the MLE's for β under the original framework, exhibited in (2.3.6), and the alternate framework, shown above in (2.5.7), determined that the MLE for β in the original framework could be written as a function of parameters from the alternative framework, as follows:

$$\begin{aligned}
 \widehat{\beta}_s &= \widehat{\beta}_s^* (1 - \widehat{\lambda}_s^*) \\
 &= \frac{d_s^-(c_s^- + c_s^+)}{R_{s+1}c_s^- + (c_s^- + c_s^+)(c_s^- + d_s^-)} * \frac{[R_{s+1}c_s^- + (c_s^- + c_s^+)(c_s^- + d_s^-)](c_{s-1}^- + c_{s-1}^+)}{(c_s^- + c_s^+)R_sc_{s-1}^-} \\
 &= \frac{d_s^-(c_{s-1}^- + c_{s-1}^+)}{R_sc_{s-1}^-}
 \end{aligned}$$

Since the MLE's for λ_s are equivalent under the two frameworks, the relationship can also be expressed as

$$\hat{\beta}_s^* = \frac{\hat{\beta}_s}{(1 - \hat{\lambda}_s)}$$

This relationship holds regardless of whether a constraint is imposed on any of the λ 's, and thus the maximum likelihood estimators for β^* in the constrained cases of a two interval design or a three interval design can be determined from Tables 2.4.1 and Tables 2.4.3 - 2.4.5 respectively, simply by using the above equivalence formula. The MLE's for ϕ^* and λ^* remain equal to those in the original framework in the unconstrained case and each of the constrained cases.

2.6 Derivation of Other Functions of Interest

The choice of the parameters ϕ , β , and λ for the specification of the likelihood function is advantageous when the goal of a long-term animal study is the estimation and comparison of carcinogenic risk among dose groups, as it allows direct estimation of the tumor incidence rates. In addition, constraints on the parameter space are obvious and readily applied to the estimation problem.

Other formulations for the transition rates in a multistate model of death and tumor incidence have been suggested by McKnight and Crowley (1984), Malani and Van Ryzin (1986), and Gomez and Julia (1988). Although the parameterization chosen by Malani and Van Ryzin spans the same parameter space as (λ, β, ϕ) , it is necessary to obtain the maximum likelihood estimator of the tumor incidence rate as a non-linear function of the their parameters based on the invariance principle of MLE's. In the case of the parameterization used by McKnight and Crowley, the estimator for tumor incidence must be obtained by a complex reverse integration of prevalence estimates. As a result, the set of

constraints on their parameters which are equivalent to the constraints $\lambda_i \geq 0$ for $i=1, \dots, S$ are not obvious, and may be difficult to implement.

An additional advantage of the proposed parameterization is that it does not require the use of potential random variables, as discussed in Section 2.1. Although the models used by Dewanji and Kalbfleisch (1986) and Malani and Van Ryzin (1986) utilize this approach, it is clear from the work of Prentice et al (1978) that the resulting formulae will be equivalent. Thus, it is useful to compare the estimators of the transition rates derived by these authors with the estimators derived in Sections 2.3 - 2.4. The three-state model of tumor incidence and death used by Dewanji and Kalbfleisch (1986) and Malani and Van Ryzin (1986) is based on the random variable T , which represents the time until the development of the tumor of interest, and the random variable D , representing the time until death. The transition rates between the states in this stochastic model are:

$$\begin{aligned}\lambda^T(t_i) &= \text{the tumor incidence rate} \\ &= \Pr [T = t_i \mid D \geq t_i, T \geq t_i] \\ \lambda^{D|NT}(t_i) &= \text{the rate of death in absence of tumor} \\ &= \Pr [D = t_i \mid D \geq t_i, T > t_i] \\ \lambda^{D|T}(t_i) &= \text{the rate of death in presence of tumor} \\ &= \Pr [D = t_i \mid D \geq t_i, T \leq t_i]\end{aligned}$$

These transition rates are the discrete counterparts to the continuous transition rates described by McKnight and Crowley (1984). Also defined by McKnight and Crowley is the tumor lethality at time t_i :

$$l(t_i) = \lambda^{D|T}(t_i) / \lambda^{D|NT}(t_i) .$$

Although estimation of the functions given above is the goal in analyzing the data from a long-term animal experiment, the likelihood can be expressed in terms of the following identifiable event-specific hazard rates:

$$\begin{aligned}\lambda^{DNT}(t_i) &= \text{the rate of death without tumor} \\ &= \Pr [D = t_i, T > t_i \mid D \geq t_i]\end{aligned}$$

$$\begin{aligned}\lambda^{DT}(t_i) &= \text{the rate of death with tumor} \\ &= \Pr [D = t_i, T \leq t_i \mid D \geq t_i] \quad \text{and}\end{aligned}$$

$$\begin{aligned}\lambda^D(t_i) &= \text{the overall death rate} \\ &= \Pr [D = t_i \mid D \geq t_i] \\ &= \lambda^{DNT}(t_i) + \lambda^{DT}(t_i)\end{aligned}$$

Malani and Van Ryzin also define the conditional probabilities shown below :

$$\begin{aligned}p^A(t_i) &= \text{the tumor prevalence function} \\ &= \Pr [T \leq t_i \mid D \geq t_i]\end{aligned}$$

$$\begin{aligned}p^D(t_i) &= \text{the Hoel-Walburg prevalence function} \\ &= \Pr [T \leq t_i \mid D = t_i] \\ &= \lambda^{DT}(t_i) / \lambda^D(t_i)\end{aligned}$$

and then define the four probabilities $\Pr[\text{SWOT}]$, $\Pr[\text{SWT}]$, $\Pr[\text{DWOT}]$, and $\Pr[\text{DWT}]$ as functions of the parameters $\lambda^D(t_i)$, $p^A(t_i)$, and $p^D(t_i)$.

An examination of these definitions reveals that the overall rate of death $\lambda^D(t_i)$, as defined by Malani and Van Ryzin, is equivalent to ϕ_i . The rate of death without tumor $\lambda^{DNT}(t_i)$ can be expressed as a function of (λ, β, ϕ) as follows:

$$\begin{aligned}\lambda^{DNT}(t_i) &= \Pr[D = t_i, T > t_i \mid D \geq t_i] \\ &= \Pr[D = t_i, T > t_i, D \geq t_i] / \Pr[D \geq t_i] \\ &= \Pr[D = t_i, T > t_i, D \geq t_i, T \geq t_i] / \Pr[D \geq t_i] \\ &= \Pr[D = t_i, T > t_i \mid D \geq t_i, T \geq t_i] \Pr[D \geq t_i, T \geq t_i] / \Pr[D \geq t_i]\end{aligned}$$

$$\begin{aligned}
&= \Pr[E_1 = t_i, \delta(t_i)=0 \mid E_1 \geq t_i] \Pr[E_1 \geq t_i] / \Pr[D \geq t_i] \\
&\quad \beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j) \\
&= \frac{\beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j)}{\prod_{j=1}^{i-1} (1 - \phi_j)}
\end{aligned}$$

The average rate of death with tumor can be calculated by subtracting the rate of death without tumor $\lambda^{DNT}(t_i)$ from the overall death rate $\lambda^D(t_i)$ to yield:

$$\begin{aligned}
\lambda^{DT}(t_i) &= \lambda^D(t_i) - \lambda^{DNT}(t_i) \\
&\quad \phi_i \prod_{j=1}^{i-1} (1 - \phi_j) - \beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j) \\
&= \frac{\phi_i \prod_{j=1}^{i-1} (1 - \phi_j) - \beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j)}{\prod_{j=1}^{i-1} (1 - \phi_j)}
\end{aligned}$$

The rate of death in the presence of a tumor is simply equal to γ_i , the average risk of death given that a tumor is present. Inverting the relationship given by (2.2.7), the form for $\lambda^{DT}(t_i)$ can be expressed in terms of the parameters (λ, β, ϕ) as:

$$\lambda^{DT}(t_i) = \gamma_i = \frac{\phi_i \prod_{j=1}^{i-1} (1 - \phi_j) - \beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j)}{\sum_{j=1}^{i-1} \left[\prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k) \lambda_j \prod_{k=j}^{i-1} (1 - \gamma_k) \right]} \quad (2.6.1)$$

In the case of an incidental tumor, the risk of death is independent of the presence or absence of a tumor, and thus $\Pr[D = t_i \mid D \geq t_i] = \Pr[D = t_i \mid D \geq t_i, T \leq t_i] =$

$\Pr[D = t_i \mid D \geq t_i, T > t_i]$, or $\phi_i = \gamma_i = \lambda^{DINT}(t_i)$. The rate of death in the absence of tumors is thus equivalent to the overall death rate under the incidental tumor assumption, which was demonstrated in Section 2.3. to be:

$$\lambda^{DINT}(t_i) = \beta_i / (1 - \lambda_i).$$

The lethality of the tumor of interest at time t_i can be expressed as a function of (λ, β, ϕ) in the following manner:

$$\begin{aligned} \ell(t_i) &= \lambda^{DT}(t_i) / \lambda^{DINT}(t_i) \\ &= \gamma_i (1 - \lambda_i) / \beta_i \end{aligned}$$

in which the expression given by (2.6.1) is substituted for γ_i . In the case of a rapidly fatal tumor, the probability of death in the presence of tumors γ_i can be expected to equal one, and the resulting tumor lethality is $\ell(t_i) = (1 - \lambda_i) / \beta_i$. In the case of an incidental tumor analysis, $\phi_i = \gamma_i = \beta_i / (1 - \lambda_i)$, so the lethality in this situation is equal to one.

The derivation of the tumor prevalence function is similar to the derivation of the probability of sacrifice with tumor, since $\Pr[SWT_i]$ includes a component for the probability of surviving to time t_i given a tumor onset before or at time t_i . Recognition of this similarity allows the tumor prevalence function to be written in terms of (λ, β, ϕ) as:

$$p^A(t_i) = \frac{\prod_{j=1}^i (1 - \phi_j) - \prod_{j=1}^i (1 - \lambda_j - \beta_j)}{\prod_{j=1}^i (1 - \phi_j)}$$

Finally, the Hoel-Walburg prevalence function can be written as a function of $\lambda^{DT}(t_i)$ and $\lambda^D(t_i)$, as shown below:

$$\begin{aligned}
 p^D(t_i) &= \lambda^{DT}(t_i) / \lambda^D(t_i) \\
 &= \frac{\phi_i \prod_{j=1}^{i-1} (1 - \phi_j) - \beta_i \prod_{j=1}^{i-1} (1 - \lambda_j - \beta_j)}{\phi_i \prod_{j=1}^{i-1} (1 - \phi_j)}
 \end{aligned}$$

Substitution of the preceding forms for $p^A(t_i)$, $p^D(t_i)$, and $\lambda^D(t_i)$ into the components $\Pr[\text{SWOT}_i]$, $\Pr[\text{DWOT}_i]$, $\Pr[\text{SWT}_i]$ and $\Pr[\text{DWT}_i]$ defined by Malani and Van Ryzin (1986) yields definitions for the four probabilities identical to (2.2.2) - (2.2.5); thus the likelihood constructed by Malani and Van Ryzin is equivalent to that presented in Section 2.2. The definition of the tumor incidence rate as a function of the probabilities $\lambda^D(t_i)$, $p^A(t_i)$, and $p^D(t_i)$, is stated in Theorem 1 of Malani and Van Ryzin (1986):

$$1 - \lambda^T(t_i) = \frac{[1 - p^A(t_i)] [1 - \lambda^D(t_i)] + [1 - p^D(t_i)] \lambda^D(t_i)}{[1 - p^A(t_{i-1})]}$$

It can easily be shown that this equals $(1 - \lambda_i)$ as defined by (2.1.5). In addition, it can be demonstrated that the MLE for λ_i proposed by Malani and Van Ryzin is equivalent to the unconstrained MLE given in (2.3.7). The MLE derived by these authors, in terms of their notation, is $\hat{\lambda}^T(u) = t(u)/R(u)$, in which $t(u)$ is an estimate of the number of animals getting tumors in interval u and $R(u)$ is an estimate of the number of live tumor-free animals at the beginning of the u -th interval. The expressions for $t(u)$ and $R(u)$ written in terms of our notation are:

$$R(u) = \frac{R_u c_{u-1}^-}{c_{u-1}^- + c_{u-1}^+}$$

$$t(u) = R(u) - \left[\frac{c_u^- (R_u - (d_u^- + d_u^+))}{c_u^- + c_u^+} - d_u^- \right]$$

Simple algebraic manipulation of the above forms and substitution in $\hat{\lambda}^T(t_i)$ yields the equation given in expression (2.3.7).

The relationships presented in this section allow the comparison of parameters and estimators from a variety of possible parameterizations for a multistate model of tumor incidence and death. In particular, they allow determination of the relationship between parameters in a multistate model which incorporates the random variables E_1 , the time to the first event, and D , the time to death, with a multistate model based on the potential random variable time to tumor T . The availability of these relationships can also prove useful in the development of variance estimators.

2.7 Extension of Results to Multiple Treatment Groups

The maximum likelihood estimators developed in this Chapter are presented for a group of animals receiving a single treatment. In practice, however, the comparison of tumor incidence rates among treatment groups is of primary importance. The parameters of interest and their corresponding estimators can be extended to multiple treatment groups by incorporating an index for dose. For example, the discrete tumor incidence rate, λ_s , is replaced by λ_{zs} , the tumor incidence rate for time interval s and dose group z :

$$\lambda_{zs} = \Pr[E_1 = t_s, \delta(t_s)=1 \mid E_1 \geq t_s, Z = z] .$$

The covariate Z may be taken to be an indicator of dose group or any other suitable group of covariables. The death rate without tumor and the overall death rate are similarly defined for treatment group z as β_{zs} and ϕ_{zs} . The maximum likelihood estimation of these parameters is based on the observed number of animals dying naturally with and without tumors (d_{zs}^+ and d_{zs}^-) and those animals sacrificed with and without tumors (c_{zs}^+ and c_{zs}^-)

within the group of animals receiving treatment z. The comparison of tumor incidence rates across treatment groups will be addressed in Chapter V.

CHAPTER III: ASYMPTOTIC PROPERTIES OF THE MLE'S

3.1 Optimal Properties of the MLE's

There are several reasons why maximum likelihood estimators (MLE's) are widely used in estimation problems. First, if there exists an unbiased estimator whose variance attains the Cramér-Rao lower bound, then under certain regularity conditions, the MLE coincides with this unbiased estimator. Secondly, the MLE often has high efficiency in the sense described by Fisher (Silvey, 1975). It can also be shown directly from the Factorization theorem that the MLE is a function of the minimal sufficient statistic, since maximizing the likelihood is equivalent to maximizing the factor of the density function which involves the sufficient statistic. A further useful property of MLE's is their invariance under parameter transformation (Cox and Hinkley, 1974).

However, the main justification for the method of maximum likelihood is based on its optimal asymptotic properties in terms consistency and efficiency. Under certain regularity conditions, it can be shown that when the true value θ_0 of the parameter $\theta = (\lambda, \beta, \phi)$ resides in the interior of the parameter space Θ , then the MLE $\hat{\theta}$ is a consistent estimator of θ , and the asymptotic distribution of $\sqrt{n}(\hat{\theta} - \theta_0)$ is $N(0, \Sigma_\theta)$, in which Σ_θ is the inverse of the Fisher's information matrix, I_θ (Rao, 1973). This asymptotic normality of $\sqrt{n}(\hat{\theta} - \theta_0)$ is equivalent to convergence in law to the distribution of a multivariate normal random variable with mean 0 and variance I_θ^{-1} .

The regularity conditions necessary for consistency of the MLE's are milder than those required for asymptotic normality. The original proof of the consistency of maximum likelihood estimators, due to Wald (1949), requires that:

- (i) The parameter space Θ is finite dimensional, closed and compact, and the true parameter value θ_0 is interior to Θ .
- (ii) The probability distributions defined by any two different values of θ are distinct.

When the second derivative of the log-likelihood function with respect to θ exists in a neighborhood around the true parameter value, then an estimator which satisfies the likelihood equations $\delta L(\theta) / \delta \theta_i$ for all i is consistent and unique. The MLE of θ derived in Chapter II falls into a special category of maximum likelihood estimators in that the cell probabilities of the multinomial distribution described in Section 2.2 can be written as continuous functions of θ . In this special case, the conditions (i) and (ii) are satisfied and the MLE of θ is known to be consistent (Theorem 4.4.1A, Bickel and Doksum, 1977).

The additional regularity conditions typically required to demonstrate asymptotic normality are (Cox and Hinkley, 1974):

- (iii) The first three partial derivatives of the log-likelihood $L(\theta)$ exist almost surely in the neighborhood around the true parameter value θ_0 .
- (iv) The information matrix is positive definite in the neighborhood of the true parameter value.

However, estimation of parameters from a multinomial distribution again has special advantages. The asymptotic normality of $\hat{\theta}$ can be deduced assuming only continuity of the first partial derivatives or total differentiability of the cell probabilities as functions of θ , as demonstrated by Rao (Note 3, p.363, 1973).

The parameters λ_i , β_i , and ϕ_i represent probabilities, and thus their true values must lie in the range $[0,1]$. However, it is possible to assume without ambiguity that the true values of λ_i , β_i , and ϕ_i do not reside on the endpoints of the closed interval. The premise that the true values of the parameter vector $\theta = (\lambda, \beta, \phi)$ belong to an interior point of the parameter space may at first appear to conflict with the development of the constrained parameter estimates given in Section 2.4. In fact, simulation studies which assume true values for θ strictly within the parameter space provide evidence that negative tumor incidence estimates occur quite often under realistic conditions for a long-term animal study. However, it should be recognized that the possibility of obtaining negative unconstrained estimates of tumor incidence is directly related to the small sample sizes typically used in long-term animal studies. If we assume that the number of intervals is fixed, then as the total sample size n tends to infinity, the number of observations within each interval increases. The consistency of the ML estimate $\hat{\theta}$ under large samples not only implies that θ will be unbiased, but that individual ML estimates of θ will tend to cluster more closely around the true value θ_0 . It can therefore be assumed that the ML estimate of the parameter vector θ will reside within the parameter space for large samples when θ_0 is interior to Θ .

3.2 Estimation of the Asymptotic Variance of the Unconstrained MLE's

The asymptotic normality of the unconstrained maximum likelihood estimators will be exploited in order to derive asymptotic estimates of the variance of the parameters $\hat{\lambda}_i$, $\hat{\beta}_i$, and $\hat{\phi}_i$ for the i -th time interval. As claimed in Section 3.1, the asymptotic distribution of $\sqrt{n}(\hat{\theta} - \theta_0)$ is $N(0, \Sigma_\theta)$, where Σ_θ is the inverse of the Fisher's information matrix. Equivalently, it can be stated that the asymptotic distribution of $\hat{\theta}$ is $N(\theta_0, n\Sigma_\theta)$. Under the regularity conditions described above, the i -j element of the Fisher's Information matrix can be written as:

$$I_{\theta}(i,j) = E \left[\frac{-\delta^2 L(\theta)}{\delta\theta_i \delta\theta_j} \right]$$

Malani and Van Ryzin (1986) base their development of ML estimators for tumor incidence and death on the parameter vector $\zeta = (p^D(t_s), s=1, \dots, S, p^A(t_s), s=1, \dots, S, \lambda^D(t_s), s=1, \dots, S)$ as described in Section 2.6. The information matrix corresponding to this parametrization contains zeros for all of the off-diagonal elements, and thus the variance-covariance matrix Σ_{ζ} can be determined to be the matrix with the reciprocals of the terms $E_{\zeta} [-\delta^2 L(\zeta) / (\delta\zeta_i)^2]$ on the diagonal. Since the parameters of interest are nonlinear functions of the parameter vector ζ , the asymptotic variance must be determined as a transformation of Σ_{ζ} using a Taylor series expansion of the ML estimate about the true value.

The parameterization utilized in the present development of MLE's has many advantages, as discussed in Section 2.6. One tradeoff, however, is that the information matrix cannot be described as a diagonal matrix, and no analytic solution is available for its inverse. However, the asymptotic variance-covariance matrix can be approximated numerically by first evaluating the elements of the observed information matrix I_{θ} at the data values, and then inverting this matrix using numeric methods. The observed information matrix I_{θ} is minus the Hessian matrix with elements $\delta^2 L^2(\theta) / (\delta\theta_i)(\delta\theta_j)$, evaluated at the maximum likelihood estimates. There is evidence that this approximation to the true variance may be preferable to that obtained using the inverse of Fisher's Information matrix (Efron and Hinkley, 1978), which requires taking the expected values of the elements of the Hessian matrix. The diagonal elements of the Hessian have already been presented in Section 2.3. The off-diagonal elements are shown in Appendix A. It is customary to refer to $\sigma_{ii} / \sqrt{n}$ as the standard error of the estimator $\hat{\theta}_i$, where σ_{ii}^2 is the i -th diagonal element of the asymptotic variance matrix Σ_{θ} . For a given set of observed data

values from a long-term animal study, the standard errors of the unconstrained maximum likelihood estimates are thus calculated as the square roots of the corresponding diagonal elements of the numerically inverted observed information matrix divided by the square root of the sample size.

3.3 Alternate Method of Variance Estimation for the Unconstrained MLE's

The calculation of approximate standard errors depends on the numerical approximation of the asymptotic variance-covariance matrix. Another possible way in which asymptotic variances can be calculated is to use the relationships between the probability framework employed in this research and that used by Malani and Van Ryzin (1986), as described in Section 2.6. It was demonstrated in Section 2.6 that the tumor incidence parameter λ_i is exactly the same as the parameter $\lambda^T(t_i)$ employed by these authors, and the corresponding maximum likelihood estimators are also equivalent. It is therefore possible to take advantage of the expressions for the asymptotic variance and covariance of the tumor incidence rates derived by Malani and Van Ryzin.

Malani and Van Ryzin express the discrete tumor incidence rate $\lambda^T(t_i)$ as a non-linear function of $\zeta_i = (p^D(t_i), p^A(t_i), \lambda^D(t_i))$, as described in Section 3.2. Thus the Delta method must be applied to these parameters to obtain the asymptotic variance:

$$\begin{aligned} \text{var}[\hat{\lambda}^T(t_g)] = \text{var}[1 - \hat{\lambda}^T(t_g)] &= \left[\frac{\delta[1 - \hat{\lambda}^T(t_g)]}{\delta p^D(t_g)} \right]^2 \text{var}[\hat{p}^D(t_g)] + \left[\frac{\delta[1 - \hat{\lambda}^T(t_g)]}{\delta p^A(t_g)} \right]^2 \text{var}[\hat{p}^A(t_g)] \\ &+ \left[\frac{\delta[1 - \hat{\lambda}^T(t_g)]}{\delta \lambda^D(t_g)} \right]^2 \text{var}[\hat{\lambda}^D(t_g)] + \left[\frac{\delta[1 - \hat{\lambda}^T(t_g)]}{\delta p^A(t_{g-1})} \right]^2 \text{var}[\hat{p}^A(t_{g-1})] \end{aligned}$$

Similarly, the Delta method can be applied to obtain an expression for the covariance of the tumor incidence rates:

$$\text{cov}[\hat{\lambda}^T(t_s), \hat{\lambda}^T(t_{s'})] = \begin{cases} \left[\frac{\delta[1 - \hat{\lambda}^T(t_s)]}{\delta p^A(t_s)} \right] \left[\frac{\delta[1 - \hat{\lambda}^T(t_{s'})]}{\delta p^A(t_{s'})} \right] \text{var}[\hat{p}^A(t_s)] & \text{for } s'=s+1, i=1, \dots, S \\ 0 & \text{otherwise} \end{cases}$$

After substituting the expressions for the partial derivatives given by Malani and Van Ryzin (1986), the forms for the asymptotic variance and covariance of the tumor incidence rates become:

$$\text{var}[\hat{\lambda}^T(t_s)] = \frac{1}{n \alpha(t_s) [1 - p^A(t_{s-1})]} \left\{ \lambda^D(t_s) * p^D(t_s) * [1 - p^D(t_s)] + \lambda^D(t_s) * [1 - \lambda^D(t_s)] * [p^A(t_s) - p^D(t_s)]^2 + \frac{p^A(t_s) * [1 - p^A(t_s)] * [1 - \lambda^D(t_s)]}{\lambda^S(t_s)} + \frac{[1 - \lambda^T(t_s)]^2 * p^A(t_{s-1}) * [1 - p^A(t_{s-1})] * [1 - \lambda^S(t_{s-1})]}{\lambda^S(t_{s-1})} \right\}$$

$$\text{cov}[\hat{\lambda}^T(t_i), \hat{\lambda}^T(t_{i+1})] = \frac{p^A(t_i) [1 - \lambda^T(t_{i+1})]}{n [1 - p^A(t_{i-1})] \alpha(t_i) \lambda^S(t_i)}$$

in which $\alpha(t_i) = \prod_{j=1}^{i-1} [1 - \lambda^D(t_j)] [1 - \lambda^S(t_j)]$ and $\lambda^S(t_i)$ is $\Pr[S=t_i] = \pi_i$.

By setting $\text{var}(\hat{\lambda}_s) = \text{var}[\hat{\lambda}^T(t_s)]$ and substituting the forms for $p^A(t_s)$, $p^D(t_s)$, and $\lambda^D(t_s)$ in terms of $\theta = (\lambda, \beta, \phi)$ given in Section 2.6, analytic forms for the asymptotic variance and covariance of the tumor incidence rates can be obtained. Minor discrepancies in the formula for $\text{var}[\hat{\lambda}^T(t_s)]$ and that given in Appendix B of Malani and Van Ryzin (1988) are due to errors discovered in their formulae.

An analytic form for the asymptotic variance of the overall death rate $\hat{\phi}_i$ can be obtained directly from the results of Malani and Van Ryzin (1986), since the parameter $\lambda^D(t_s)$ is equivalent to ϕ_s and the information matrix I_ζ is a diagonal matrix. The asymptotic variance of $\hat{\phi}_s$ is thus:

$$\text{var}[\hat{\phi}_s] = \text{var}[\hat{\lambda}^D(t_s)] = \frac{\lambda^D(t_s) [1 - \lambda^D(t_s)]}{n \alpha(t_s)}$$

or in terms of the parameter $\theta = (\lambda, \beta, \phi)$:

$$\text{var}[\hat{\phi}_s] = \frac{\phi_s (1 - \phi_s)}{s-1} \frac{1}{n \prod_{j=1}^{s-1} [(1 - \pi_j) (1 - \phi_j)]}$$

Note that the forms for the asymptotic variances and covariances derived by Malani and Van Ryzin depend on the sacrifice probabilities π_i , which are stipulated by the design of the long-term animal study or computed as maximum likelihood estimators which do not depend on the parameters $p^A(t_s)$, $p^D(t_s)$, and $\lambda^D(t_s)$. The reason these known or estimated probabilities appear in the asymptotic expressions shown above is that the tumor incidence rate λ_s , when written as a function of the parameters $p^A(t_s)$, $p^D(t_s)$, and $\lambda^D(t_s)$, has an implicit dependence on the sacrifice probabilities. It is evident from the form of $\text{var}[\hat{\lambda}(t_s)]$ that as the proportion of animals sacrificed at time t_s is increased, the asymptotic variability of the estimate of the tumor incidence rate decreases. This relationship is logically consistent, since the estimation of the tumor incidence rates is made possible by the incorporation of interim sacrifices into the study design.

3.4 Estimation of the Variance of the Constrained MLE's

The asymptotic results presented in the Section 3.2 are applicable to the unconstrained MLE's under large samples, but are not appropriate for determining

approximate standard errors for the constrained MLE's derived in Section 2.4. However, analogous asymptotic properties can be attributed to the constrained MLE's with slight modification of the regularity conditions stated in Section 3.2.

Under mild regularity conditions given by Silvey (1975), the restricted ML estimate $\hat{\theta}$ can be shown to be consistent for θ . However, the derivation of an asymptotic distribution for the constrained estimator is more complex than for the unconstrained case. It is more convenient at this point to write the set of restricted likelihood equations in matrix form as:

$$D_{\theta} L(\theta) + \alpha' G(\theta) = 0$$

$$g^*(\theta) = 0$$

in which $D_{\theta} L(\theta)$ represents the vector of partials $\delta L(\theta) / \delta \theta_j$, $g^*(\theta)$ is the vector of equality constraints, $G(\theta)$ is the matrix of partial derivatives $\delta g^*(\theta) / \delta \theta_j$, and α is the vector of Lagrangian multipliers defined in Section 2.4. Application of the Taylor Series expansion to linearize about the true parameter θ_0 , and slight manipulation of the resulting equations yields:

$$\begin{bmatrix} \frac{1}{n} D^2 L(\theta) & G(\theta) \\ G'(\theta) & 0 \end{bmatrix} \begin{bmatrix} \sqrt{n} (\hat{\theta} - \theta) \\ \frac{1}{n} \hat{\alpha} \end{bmatrix} = \begin{bmatrix} \frac{1}{\sqrt{n}} D^2 L(\theta) \\ 0 \end{bmatrix}$$

Now the Law of Large numbers is applied to $-n^{-1} D^2 L(\theta)$ to obtain the information matrix, and the Central Limit Theorem can be applied to $n^{-1/2} D_{\theta} L(\theta)$ to show that it is approximately distributed as $N(0, I_{\theta})$. Substitution of these results in the above matrix equation yields:

$$\begin{bmatrix} I_{\theta} & G(\theta) \\ G'(\theta) & 0 \end{bmatrix} \begin{bmatrix} \sqrt{n} (\hat{\theta} - \theta_0) \\ \frac{1}{n} \hat{\alpha} \end{bmatrix} = \begin{bmatrix} Z \\ 0 \end{bmatrix}$$

in which $\mathbf{Z}$ is distributed as $N(\mathbf{0}, \mathbf{I}_\theta)$. When the matrix $\mathbf{I}_\theta$ has full rank then the parameter vector

$$\begin{bmatrix} \sqrt{n} (\hat{\theta} - \theta_0) \\ \frac{1}{n} \hat{\alpha} \end{bmatrix}$$

is approximately normally distributed with zero mean and variance matrix equal to:

$$\begin{bmatrix} \mathbf{P}_\theta & 0 \\ 0 & -\mathbf{R}_\theta \end{bmatrix} \quad \text{for} \quad \begin{bmatrix} \mathbf{I}_\theta & \mathbf{G}(\theta) \\ \mathbf{G}'(\theta) & 0 \end{bmatrix}^{-1} = \begin{bmatrix} \mathbf{P}_\theta & \mathbf{Q}_\theta \\ \mathbf{Q}_\theta & \mathbf{R}_\theta \end{bmatrix}$$

The reader is referred to Silvey (1975) for a more rigorous development of the above results.

An analytic form for the inverse of the expanded information matrix given above is not available, and so numerical methods can again be used to estimate the asymptotic standard errors of the constrained ML estimates. As described for the estimation of the variance of the unconstrained ML estimators, the information matrix $\mathbf{I}_\theta$ and matrix of partial derivatives of the constraints $\mathbf{G}_\theta$ are evaluated at the observed counts of dead and sacrificed animals with and without tumors at each interval, and the resulting matrix is inverted numerically. Again, the estimated standard errors are obtained as the square roots of the corresponding diagonal elements of the asymptotic variance-covariance matrix.

While the use of numerical methods to obtain solutions to the maximum likelihood equations can represent a substantial burden on computer resources, the use of numerical methods to obtain asymptotic standard errors is not a problem in this respect. The difference between these two numerical applications is that the latter does not require iterative calculations, while the former procedure does.

CHAPTER IV: ESTIMATION OF MODEL PARAMETERS USING POOLING ALGORITHMS

4.1 Motivation for Use of Pooling Algorithms

The closed-form expressions for the maximum likelihood estimators of the tumor incidence rate and death rates developed in Chapter II represent a clear improvement over the methods which have typically been applied to such multistate models of tumor incidence and death. The methods suggested by Turnbull and Mitchell (1978), Mitchell and Turnbull (1979), Dewanji and Kalbfleisch (1986), and Portier and Dinse (1987) all require iterative algorithms in order to arrive at approximate numeric values for the MLE's; thus the application of such methods is often time-consuming. The availability of closed-form solutions provides a dramatic reduction in computer time necessary to determine the maximum likelihood estimators for any given set of data.

The additional availability of closed-form expressions for the MLE's of the tumor incidence rate and death rate in cases in which constraints must be applied to the parameter set allows an even broader applicability of results than those suggested by Malani and Van Ryzin (1986), since they provide only closed-form expressions for the MLE's in the specific case in which no constraints need be applied.

Despite the obvious advantages of the method which was presented in Chapter II for the estimation of tumor incidence rates and death rates in a long-term animal study, closed-form expressions for the MLE's are available only for a study design consisting of three intervals or less. This limitation on the present methodology led to the search for

alternative methods of estimating the tumor incidence rate and death rates which would compare favorably to the constrained maximum likelihood estimators and also yield estimators which were in the parameter space $[0,1]$.

One possible option is to pool data from adjacent intervals when the unconstrained MLE's given by (2.3.5) - (2.3.7) fail to lie in the parameter space. After two adjacent intervals of data have been pooled, the unconstrained MLE's corresponding to a study design with one less interval than the original number are applied, and the estimates are again checked to determine whether they fall into the parameter space. If not, the process of pooling adjacent intervals can be continued until valid estimates are obtained.

The parameters λ , β , and ϕ represent probabilities, and must therefore lie in the range $[0,1]$. The estimation of negative tumor incidence rates and rates of death without tumor greater than one has been found to occur quite often in simulation studies which assume true values of the parameter which lie strictly within the parameter space. The frequency with which invalid estimates of these probabilities are obtained is a reflection of the fact that long-term animal studies typically include only a small number of animals (e.g., 50 animals per dose/sex/species group in NTP studies). As this small number of animals is further divided into intervals of time, and four events within each interval are tabulated, the probability of obtaining counts of zero in one or more of the cells increases. Once it is recognized that the existence of estimates outside the acceptable range is a reflection of inadequate data within intervals, pooling data from adjacent intervals becomes a natural response towards obtaining valid estimates.

Other examples of pooling algorithms can be found in the literature. Hoel and Walburg (1972) employ a pooling strategy in developing estimators of disease incidence for the special case in which the disease is considered to be nonlethal. The quantities S_i ,

$i=1, \dots, k$ are formed on the basis of the observed data for each of the k time intervals, and if $S_{j+1} < S_j$ for some interval j , then the observed data for interval j and $j+1$ are pooled, S_{j+1} is recomputed, and S_j is set equal to S_{j+1} . This process continues until $S_1 \leq \dots \leq S_k$, and then the resulting estimators of the cumulative tumor incidence rates are $R_i = S_i$ for $i=1, \dots, k$. Peto *et al.* (1980) describe a method for subdividing the duration of a long-term animal study into intervals by forcing the prevalence of an incidental tumor to be increasing over the time intervals. The data from all adjacent intervals for which the prevalence estimates are non-increasing are pooled together, and new prevalence estimates are computed. This process, designated as subdivision into "ad hoc runs", is continued until the prevalence estimates are strictly increasing over time intervals.

Additional support for the method of pooling is derived from the theory of isotonic regression. (Barlow *et al.*, 1972) The pool-adjacent-violators algorithm discussed by Barlow *et al.* refers to a method of approximating the greatest convex minorant (GCM) of a cumulative sum diagram (CSD) in the graphical interpretation of regression analysis under order constraints, or isotonic regression. The GCM is of practical interest because the isotonic regression is given by the slope of GCM for a particular CSD. The procedure dictates that the graphical points for any of the pairs of adjacent violators be pooled together and a new slope computed. However, no rule is prescribed for selecting the order in which pairs of adjacent violators should be pooled.

Although the general concept of pooling intervals of data together is readily understandable, the actual algorithms for pooling data increase in complexity as the number of time intervals increases. A decision must be made for each interval which yields unconstrained parameter estimates outside the acceptable range whether this interval should be pooled with the preceding interval or the following interval, or whether other intervals of data should be pooled. Factors which influence this decision are the existence of other

intervals for which the unconstrained estimates are invalid and the extent to which the parameter estimates differ between intervals. The algorithms for pooling are divided into two sections: those algorithms specific for dealing with pooling three intervals of data, and those algorithms for pooling a general number of intervals of data.

4.2 Pooling Algorithms for Three Interval Study Designs

The possible pooling algorithms in a long-term animal study with three time intervals are limited, yet the elucidation of algorithms for this limited case illustrates the types of pooling algorithms which can arise in general. In addition, the definition of pooling algorithms for a three-interval design allows for the comparison of the estimators so derived with both the unconstrained and the constrained MLE's presented in Chapter II.

The pooling of two or more adjacent intervals will be performed when the unconstrained maximum likelihood estimate of the cumulative tumor survival rate, $\hat{\Lambda}_s$, is determined to be greater than the cumulative survival rate $\hat{\Lambda}_{s-1}$ for the previous interval. This condition is equivalent to that of a negative unconstrained estimate of the tumor incidence rate λ_s . For a three-interval design, there are six possible rankings among the estimated cumulative tumor survival rates, as follows:

- (1) $\hat{\Lambda}_1 \geq \hat{\Lambda}_2 \geq \hat{\Lambda}_3$
- (2) $\hat{\Lambda}_1 \geq \hat{\Lambda}_3 > \hat{\Lambda}_2$
- (3) $\hat{\Lambda}_3 > \hat{\Lambda}_1 \geq \hat{\Lambda}_2$
- (4) $\hat{\Lambda}_2 > \hat{\Lambda}_1 \geq \hat{\Lambda}_3$
- (5) $\hat{\Lambda}_2 \geq \hat{\Lambda}_3 > \hat{\Lambda}_1$
- (6) $\hat{\Lambda}_1 < \hat{\Lambda}_2 < \hat{\Lambda}_3$

The first possible ranking is reflective of unconstrained estimates which fall into the valid parameter space, and thus the unconstrained estimators are the MLE's and no pooling is

required. The second and third rankings are very similar in that both represent cases in which the unconstrained estimate of λ_3 is negative. The fourth and fifth ranking both represent cases in which the unconstrained estimate of λ_2 is negative. The last case reflects a situation in which the unconstrained estimates of both λ_2 and λ_3 are negative.

Three separate pooling algorithms were developed for estimating λ , β , and ϕ from long-term animal studies with three intervals. The first method, which will be denoted as "early pooling", combines the first two intervals of data together for rankings (2) through (5) above. The two intervals of data are combined by defining the following new observed counts for the four possible events within each interval:

$$\begin{aligned} c_{1p}^- &= c_1^- + c_2^- & d_{1p}^- &= d_1^- + d_2^- \\ c_{1p}^+ &= c_1^+ + c_2^+ & d_{1p}^+ &= d_1^+ + d_2^+ \end{aligned}$$

and then defining the counts for the second pooled interval, originally the third interval, to be:

$$c_{2p}^- = c_3^- \quad c_{2p}^+ = c_3^+ \quad d_{2p}^- = d_3^- \quad d_{2p}^+ = d_3^+$$

The new data outcomes are substituted into the analytic solutions for the unconstrained MLE's given in Table 2.4.1, and the estimate of the cumulative tumor survival rate Λ_{ip} for the i -th interval of pooled data is computed. If the new unconstrained estimates result in $\hat{\Lambda}_{1p} < \hat{\Lambda}_{2p}$ then all three intervals of data are combined together, so that a single estimate of λ , β , and ϕ are produced for this set of data.

The second method of pooling, denoted "late pooling", is similar to the preceding method except that in this method the last two intervals of data are pooled together. The data outcomes falling into each of the four categories remain unchanged for the first interval, and for the second pooled interval we define:

$$\begin{aligned} c_{2p}^- &= c_2^- + c_3^- & d_{2p}^- &= d_2^- + d_3^- \\ c_{2p}^+ &= c_2^+ + c_3^+ & d_{2p}^+ &= d_2^+ + d_3^+ \end{aligned}$$

As in the method of early pooling, the unconstrained estimates given in Table 2.4.1 are computed based on the two interval data. By substituting the above data counts into the formula in Table 2.4.1, it can be shown that the estimates of λ_1 , λ_2 , and λ_3 are equivalent to those exhibited for Case II in Table 2.4.3. Again, if $\hat{\Lambda}_{1p} < \hat{\Lambda}_{2p}$, then all three intervals of data are pooled together.

The last algorithm for pooling data from a three-interval study design has more intuitive appeal, since it takes into account the actual interval at which a violation of the constraint $\lambda \geq 0$ occurs. In the constrained maximum likelihood solutions presented in Section 2.4, it was noted that imposition of an active constraint $\lambda_s \geq 0$ in effect led to a parameter estimate which was forced to the boundary of the parameter space, i.e., the MLE for λ_s equals zero. When this constraint is enforced, the cumulative tumor survival rates from this interval and the previous interval are forced to be equal, since

$$\hat{\Lambda}_s = \prod_{j=1}^s (1 - \hat{\lambda}_j) = (1 - \hat{\lambda}_s) \hat{\Lambda}_{s-1} = \hat{\Lambda}_{s-1} \quad \text{when } \hat{\lambda}_s = 0$$

This last algorithm, denoted as "intuitive pooling", pools the first two intervals of data when the unconstrained MLE of λ_2 is negative, pools the second and third intervals of data when the unconstrained MLE of λ_3 is negative, and pools all three intervals of data together when both the unconstrained MLE's of λ_2 and λ_3 are negative.

The intuitive method of pooling the data derives credence from the fact that the pooled estimates of the tumor incidence rates λ_1 , λ_2 , and λ_3 are equivalent to those of the Case II constrained MLE's when the last two intervals of data are pooled and the pooled estimates of λ_1 , λ_2 , and λ_3 are equivalent to those of the Case IV constrained MLE's when all three intervals of data are pooled. In other words, when the unconstrained estimate of λ_3 is negative, the estimators of tumor incidence for all three intervals are based

on the pooled data are identical to the constrained MLE's. Similarly, when the unconstrained estimates of λ_2 and λ_3 are both negative, the pooled estimates are identical to the constrained MLE's for this situation. This is a desirable characteristic for estimators derived from pooled data, since our primary interest is in the estimation of the tumor incidence rates, and the constrained MLE's are known to have some optimal properties as discussed in Section 3.4.

It should be recognized that the intuitive pooling scheme discussed above reflects only one possible choice of an algorithm which could be described as "intuitive". For example, another possible choice would be to pool the data from the i -th interval with that from the $(i+1)$ -th interval whenever the unconstrained estimate of λ_i is negative. The latter method was deemed a less appropriate choice for three reasons: (1) it does not prescribe a rule for pooling data from the last interval when $\hat{\lambda}_3$ is negative, as there is no fourth time interval, (2) it does not allow pooling of data from the first two intervals, since $\hat{\lambda}_1$ will never be negative, and (3) it does not ensure that the order of the estimated cumulative tumor survival functions will be corrected between interval $i-1$ and interval i . The intuitive pooling method discussed earlier is applicable to all intervals for which the unconstrained estimate of λ_i could be negative, and ensures that the invalid ordering between Λ_i and Λ_{i-1} which inspired the pooling of data will be corrected. The pooling methods described by Hoel and Walburg (1972), Peto et al (1980), and Barlow et al (1972) are all intuitive pooling algorithms which pool "down", i.e., if $\hat{\lambda}_i < 0$, then intervals i and $i-1$ are pooled together.

If the unconstrained estimates of the cumulative tumor survival rate follow rankings (2) or (4), there is no possibility that the new unconstrained estimates based on intuitive pooling as described above will be outside of the parameter space $[0,1]$. If the unconstrained MLE's of the parameters based on three intervals yield rankings (3) or (5)

for Λ_1 , Λ_2 , and Λ_3 , then it may be necessary to pool all the data together into a single interval if the unconstrained estimates based on the intuitive pooling do not follow the correct ordering.

The six possible rankings of the cumulative tumor survival rates and the application of the three pooling algorithms are illustrated in Figure 4.1.1. Note that all three pooling algorithms combine the data which yield ranking (6) into a single interval. Also note that the intuitive pooling algorithm sometimes calls for the same intervals to be pooled as the early pooling algorithm, and sometimes calls for the same intervals to be pooled as the late pooling algorithm. The small sample operating characteristics of the three pooling algorithms described in this section will be evaluated through the use of Monte Carlo Simulation studies presented in Chapter VI.

Figure 4.1.1

Pooling Algorithms for Three-Interval Study Designs

* - may require additional pooling

4.3 Pooling Algorithms for General Study Designs

As the number of time intervals is increased in the analysis of long-term animal studies, the number of possible poolings of data from adjacent intervals increases. The number of possible poolings for a study design with S time intervals is 2^{S-1} , which is equal to the number of constraint cases which must be considered. Since it may not be practical to evaluate all possible poolings for a given study design, general algorithms are developed here for the pooling of data from study designs with more than three intervals.

The intuitive pooling algorithm described in Section 4.2 for the estimation of (λ, β, ϕ) from three interval studies can be easily extended to studies with more than three intervals. Just as in the three interval intuitive pooling algorithm, data from the s -th time interval are combined with the data from the previous interval whenever the unconstrained estimate of λ_s is negative. If the unconstrained estimate of λ from this combined data is still negative, then the data from these two intervals is combined with that of time interval $s-2$. The evaluation of the signs of the unconstrained tumor incidence rates begins with the first time interval and continues chronologically through the time intervals until a negative unconstrained estimate of λ is encountered. After pooling the appropriate intervals and recomputing pooled estimates of λ , β , and ϕ , the next evaluation of the signs of the tumor incidence rates again begins with the first interval. This pooling process continues until the cumulative tumor survival probabilities follow the correct ordering. The algorithm developed here is similar to that proposed by Hoel and Walburg (1972), since the evaluation of the ordering of the estimates begins at the first interval, and only a single pooling is performed at each step. In contrast, the algorithm for subdivision of the duration of a long-term animal study into "ad hoc runs", as described by Peto et al. (1980), pools all adjacent violators simultaneously before recomputing pooled prevalence estimates.

The second pooling algorithm for study designs with more than three time intervals is a method designated as "sequential pooling". In the pooling algorithms specific for three interval studies, it was possible to develop separate algorithms for early pooling and late pooling because there were only two possible ways to group data from two adjacent intervals together. In studies consisting of a larger number of intervals, this approach becomes impractical. Instead, the sequential pooling algorithm first evaluates the original unconstrained estimates of tumor incidence from the S-interval study design. If these estimates are all non-negative, no pooling is required. If any estimates are negative, then all possible poolings which result in (S-1) intervals are evaluated. Among those poolings for which the unconstrained estimates of λ are all non-negative, if any, the one pooling scheme is chosen which is judged to be the "best". Ideally, the "best" pooling scheme is the one resulting in estimates of tumor incidence which are closest to the true values. Since the true values of the tumor incidence rate are unknown, an ad hoc measure of the "best" pooling scheme must be constructed. One possible criterion is that the chosen pooling scheme should represent the least overall difference between the unconstrained estimates and the new estimates obtained by pooling. This criterion can be evaluated by comparing the quantity:

$$\sum_{s=1}^S n_s (\hat{\lambda}_{ps} - \hat{\lambda}_s)^2$$

in which $\hat{\lambda}_{ps}$ is the estimate of the tumor incidence rate derived from the pooled data.

The pooling scheme which minimizes this weighted sum of squared differences, which will be designated the Mean Squared Pooling Difference (MSPD), is then selected.

If none of the previous data poolings produce the correct ordering among the estimated Λ_s 's, then all possible poolings which yield (S-2) time intervals are evaluated. Again, the pooling scheme which minimizes the MSPD for a study design consisting of S-2 intervals is chosen from those which yield the correct ordering of the estimated Λ_s 's.

The sequential pooling algorithm continues to pool data from adjacent intervals until a pooling scheme is determined which (1) provides non-negative unconstrained estimates of the tumor incidence rate for every interval and (2) minimizes the MSPD among all pooled designs with the same number of intervals. The worst possible case is that all S intervals must be pooled together in order to obtain estimates of λ which are in the range $[0,1]$.

An example of the sequential pooling algorithm is given in Figure 4.3.1 for a six-interval study design which requires some level of pooling. In this example, the earliest time interval is denoted by a "1", the second interval is denoted by a "2", and so on. The pooling of adjacent intervals is indicated by enclosing the digits which represent these intervals in parentheses. The sequential pooling algorithm continues to the next level of pooling only if the current level fails to provide any pooling scheme with all its estimates of λ in the valid range. It is evident from this example that a pooled study design consisting of S^* intervals may be the result of a single pooling of several intervals of data, or of multiple poolings each consisting of a fewer number of intervals.

The pooling methods discussed in this Chapter are a natural response to the need to obtain valid estimates of the tumor incidence rates when the observed data from a long-term animal study are sparse. Using the algorithms described in this section, pooled estimates of $\theta = (\lambda, \beta, \phi)$ are assured of being within the valid parameter space. There are, however, some disadvantages to the pooled estimates. Since the development of the estimators is almost purely heuristic, forms for the variance of these estimators are not available. An empirical method such as bootstrapping must be used to estimate standard errors of the estimators derived from pooled data. The constrained maximum likelihood estimators force the estimate of the cumulative tumor survival function $\hat{\Lambda}_s$ to be equal to that of the previous interval when $\hat{\lambda}_s$ is negative, and allow the overall death rate and death rate without tumor to vary between intervals $s-1$ and s . The estimators from the intuitive pooling algorithm not

only force $\hat{\Lambda}_s$ to be equal to $\hat{\Lambda}_{s-1}$ when $\hat{\lambda}_s$ is negative, but also force $\hat{\phi}_s$ to be equal to $\hat{\phi}_{s-1}$ and $\hat{\beta}_s$ to be equal to $\hat{\beta}_{s-1}$. In this respect, the pooled estimators are unable to make the best use of the observed data from a long-term animal study. Yet the primary interest in the analysis of carcinogenicity data is the estimation and comparison of the tumor incidence rates, and the pooling algorithms often provide estimates of the tumor incidence rate which are equivalent to the constrained maximum likelihood estimators. While analytic expressions for the constrained maximum likelihood estimators are only available for a study design with three intervals or less, the pooled estimators can be easily calculated for a long-term animal study which has been subdivided into any number of time intervals.

Figure 4.3.1

Example of the MSPD Pooling Method for a Six-Interval Study

(If necessary, continue sequentially by evaluating all poolings which result in 3 intervals of data, all poolings which result in 2 intervals of data, and all data pooled into a single interval, until a pooling choice is determined which maximizes the likelihood for a design with the specified number of intervals among those poolings which ensure the correct ordering of tumor incidence rates)

CHAPTER V: TESTING HYPOTHESES

5.1 Comparison of Tumor Incidence Rates

McKnight and Crowley (1984) argued that the comparison of the carcinogenic risk from varying doses of a treatment should be based on the tumor incidence rates, since they are unbiased measures of corresponding increases in the production of tumors. The null hypothesis of no carcinogenic effect at time t_s can thus be stated in terms of the tumor incidence rates as $H_0: \lambda_{0s} = \lambda_{1s} = \dots = \lambda_{Ks}$. If the times of tumor onset are observable, then the tumor onset test described in Section 1.3.2 directly addresses this hypothesis and is an unbiased test of tumor incidence. In contrast, tests based on the crude proportions of animals with tumors within each treatment group, such as the Cochran-Armitage Linear Trend Test and Fisher's Exact Test, can be biased by differences in mortality across treatment groups (Gart, 1979). Tests based on the death rate from tumors (fatal tumor analyses) or with tumors (incidental tumor analyses) coincide with a test of the equality of tumor incidence rates only under certain restrictive conditions of treatment lethality and tumor lethality, as described by McKnight and Crowley (1984) and Bailer (1988).

Tests statistics for the comparison of tumor incidence rates will be derived using two different methods. The first test exploits the asymptotic normality of the maximum likelihood estimator of λ_s in the construction of a normal approximation test statistic. The second relies on specific applications of a Pearson chi-square test to a multinomial distribution as described in pages 391-398 of Rao (1973). Both of the derived tests are directed at testing the hypothesis of equal tumor incidence rates for two treatment groups;

the more general hypothesis of equal tumor incidence rates among K treatment groups can be addressed by pairwise comparisons.

The normal approximation test is motivated by a desire to simultaneously compare the differences between tumor incidence rates between two treatments over all time intervals. The difference between estimated tumor incidence rates for two treatments during the s-th time interval, $\hat{\lambda}_{2s} - \hat{\lambda}_{1s}$, is asymptotically distributed as a normal random variable with mean 0 and variance $\sigma_{2ss}^2 + \sigma_{1ss}^2$ under the null hypothesis of equal tumor incidence rates, with $\sigma_{zss}^2 = \text{var}[\hat{\lambda}_{zs}]$. This result is obtained by first noting that the expanded parameter vector $\hat{\theta} = (\hat{\theta}_1, \hat{\theta}_2)$ representing the maximum likelihood estimators for both dose groups has a block diagonal covariance matrix, reflecting the independence of the treatment groups, as shown below:

$$\text{asymptotic Var}(\hat{\theta}_1, \hat{\theta}_2) = \begin{bmatrix} \Sigma_1 & 0 \\ 0 & \Sigma_2 \end{bmatrix}$$

Rao (1973, p.387) stated that if T_n is a statistic for estimating θ , such that the asymptotic distribution of $\sqrt{n}(T_n - \theta)$ is normal with mean 0 and variance matrix $\Sigma = \{\sigma_{ij}^2\}$, and g is a continuous function of T_n which is totally differentiable, then the asymptotic distribution of $\sqrt{n}[g(T_n) - g(\theta)]$ is normal with mean zero and variance:

$$\text{Var}(g(T_n)) = \sum_j \sum_j \sigma_{ij}^2 \frac{\delta g}{\delta \theta_i} \frac{\delta g}{\delta \theta_j}$$

Since the maximum likelihood estimator of θ is such a statistic, the result stated above can be proven by designating the function $g(\hat{\theta}_1, \hat{\theta}_2) = \hat{\lambda}_{2s} - \hat{\lambda}_{1s}$. The results for each interval can be combined by summing the observed differences in maximum likelihood estimates over the S time intervals to obtain:

$$\hat{\Lambda}_2^T - \hat{\Lambda}_1^T = \sum_{s=1}^S [\hat{\lambda}_{2s} - \hat{\lambda}_{1s}] = \sum_{s=1}^S \hat{\lambda}_{2s} - \sum_{s=1}^S \hat{\lambda}_{1s}$$

Note that Λ_z^T is the cumulative tumor incidence rate defined as the sum of the tumor incidence rates over all intervals, and should be distinguished from the cumulative tumor survival function Λ_z defined in Chapter II as the product of $(1-\lambda_s)$ over all time intervals. The variance of the estimated difference between the cumulative tumor incidence rates for two treatments is:

$$\text{var} \left[\hat{\Lambda}_2^T - \hat{\Lambda}_1^T \right] = \frac{\sigma_{T1}^2}{N_1} + \frac{\sigma_{T2}^2}{N_2}$$

Rao's result can be applied to the estimate of Λ_z^T to obtain the asymptotic variance of this estimator, which has the resulting form:

$$\sigma_{Tz}^2 = \text{var} \left(\hat{\Lambda}_z^T \right) = \sum_{s=1}^S \sigma_{zss}^2 + 2 * \sum_{s \neq s'} \sigma_{zss'} \text{ in which } \sigma_{zss'} = \text{cov} [\hat{\lambda}_{zs}, \hat{\lambda}_{zs'}]$$

A consistent estimator, $\hat{\sigma}_z^2$, for σ_z^2 is obtained by replacing the unknown parameters with their corresponding maximum likelihood estimates. A normal approximation test can now be constructed based on the statistic $\hat{\Lambda}_2^T - \hat{\Lambda}_1^T$ and its asymptotic variance, as displayed below:

$$Z_T = \frac{\hat{\Lambda}_2^T - \hat{\Lambda}_1^T}{\left(\frac{\hat{\sigma}_{T1}^2}{N_1} + \frac{\hat{\sigma}_{T2}^2}{N_2} \right)^{1/2}} \quad (5.1.1)$$

Under the null hypothesis, this statistic converges in distribution to a normal random variable with mean 0 and variance 1. Approximate significance probabilities can thus be determined by comparing Z_T to the standard normal distribution.

McKnight and Crowley (1984) also proposed a statistic for testing the hypothesis $H_0: \lambda_{1s} = \lambda_{2s}$, and claimed that this statistic is asymptotically normal under the null hypothesis. However, they did not present the variance component of this asymptotic distribution. The form of their statistic is similar to the statistic Z_T shown above in that both sum the differences between consistent estimates of the tumor incidence rate for each of two treatments over all time intervals. The test statistic derived by Malani and Van Ryzin (1988) has the same form as Z_T , but different estimates of the variance of the MLE for Λ_z^T are used. These authors used a first-order Taylor Series approximation to the variance of the individual tumor incidence estimates $\hat{\lambda}_s$. Under this approximation the covariance between estimates of the tumor incidence rates for two different time intervals is zero unless the time intervals are adjacent. Since the Hessian matrix is evaluated numerically and inverted to obtain the observed information matrix in the testing procedure described above, the estimates of covariances between any two time intervals must be included in the total variance.

A second test statistic for the comparison of tumor incidence rates is derived using Rao's results for specific applications of the chi-square distribution to a multinomial distribution in which the "k" cell probabilities can be written in terms of an unknown parameter $\theta = (\theta_1, \dots, \theta_q)$. In the special case in which an efficient estimator for θ exists and the first partial derivatives of the cell probabilities with respect to θ_i are continuous for $i=1, \dots, q$, Rao presents a statistic for testing whether the parameter θ belongs to a subset of admissible values specified by the locus

$$\theta_i = g_i(\tau_1, \dots, \tau_r), \quad i=1, \dots, q; \quad r < q \quad (5.1.2)$$

in which the functions g_i are continuous and $\tau_1, \dots, \tau_r$ are the new parameters. Under the null hypothesis specified by (5.1.2), the asymptotic distribution of the statistic

$$\chi^2 = \sum_{i=1}^k \frac{[E_{1i} - E_{2i}]^2}{E_{2i}} \quad (5.1.3)$$

is $\chi^2(q-r)$, in which E_{1i} is the i -th cell probability of the multinomial distribution evaluated at the efficient estimator for θ , and E_{2i} is the same cell probability evaluated at an efficient estimator for τ under the restrictions of (5.1.2).

The maximum likelihood estimator of $\theta = (\lambda, \beta, \phi)$ is an efficient estimator in the sense that the asymptotic variance of this estimator is equivalent to the Cramér-Rao lower bound, as described in Section 3.2. In addition, the cell probabilities $\text{Pr}[\text{SWOT}]$, $\text{Pr}[\text{SWT}]$, $\text{Pr}[\text{DWOT}]$, and $\text{Pr}[\text{DWT}]$ in the multinomial model derived in Section 2.2 are functions of the parameter θ . However, in order for Rao's results as stated above to provide a test of equality of tumor incidence rates, it is necessary to derive a maximum likelihood estimator for the restricted parameter τ , i.e., for θ under the restriction that $\lambda_{1s} = \lambda_{2s}$ for all time intervals. Unfortunately, an analytic solution for the MLE of θ under this set of restrictions does not appear to be tractable. Malani and Van Ryzin also noted that they were unable to determine an analytic solution for their set of parameters in this situation. A number of different iterative procedures can be used to obtain the MLE of θ under the null hypothesis. Evaluation of the cell probabilities $\text{Pr}[\text{SWOT}]$, $\text{Pr}[\text{SWT}]$, $\text{Pr}[\text{DWOT}]$, and $\text{Pr}[\text{DWT}]$ at the unrestricted MLE's for θ given in Theorem 2.3.3 yield the values of E_1 in expression (5.1.3), and the corresponding restricted MLE's for θ obtained from an iterative computer algorithm can be substituted in the cell probabilities to yield the values of E_2 . The test statistic given by (5.1.3) can then be computed and compared with the χ^2 distribution.

By making two additional assumptions, Malani and Van Ryzin are able to derive an analytic solution for the MLE of their set of parameters. These assumptions are that: (1) the rate of death given that tumor is absent is equal for the two treatment groups, and (2) the

probability of sacrifice at the end of each time interval is the same for the two treatment groups. The second assumption is claimed to be of little concern, since the long-term animal study can be designed to fulfill this requirement. The first assumption, however, limits the applicability of this test in comparison to the former test based on Z_T as given in expression (5.1.1). Malani and Van Ryzin argued that the rates of death given that tumor is absent should be approximately equal if all treatment doses are at or below the MTD.

These authors also emphasize that these assumptions are less restrictive than those required by the score test proposed by Dewanji and Kalbfleisch (1986), for which it is necessary to make the additional assumption that the rate of death given tumor is present is equal for the two treatment groups.

When the two assumptions discussed above are satisfied, the multinomial likelihood can be reduced to a simpler form. This reduction allows the derivation of an analytic solution for the maximum likelihood estimator under the null hypothesis that the tumor incidence rates for the two treatments are equal. The statistic proposed by Malani and Van Ryzin is a direct application of Rao's result as stated in (5.1.3). In terms of the notation used in this research, the chi-square statistic is:

$$\chi_T^2 = \frac{\sum_{s=1}^S \left[N_2 (c_{1s}^+ + d_{1s}^+) - N_1 (c_{2s}^+ + d_{2s}^+) \right]^2}{\sum_{s=1}^S \left[N_1 N_2 (c_{1s}^+ + d_{1s}^+ + c_{2s}^+ + d_{2s}^+) \right]} + \sum_{s=1}^S \left[\frac{N_2 (c_{1s}^+ + d_{1s}^+) - N_1 (c_{2s}^+ + d_{2s}^+)}{N_1 N_2 (c_{1s}^+ + d_{1s}^+ + c_{2s}^+ + d_{2s}^+)} \right]^2 \quad (5.1.3)$$

in which N_1 is the total number of animals in treatment group 1 and N_2 is the total number of animals in treatment group 2. This test-statistic converges in distribution to a chi-square random variable with S degrees of freedom as $(N_1 + N_2) \rightarrow \infty$, provided that the number

of intervals (S) is fixed and the proportion of animals in each treatment group, $N_z/(N_1+N_2)$, is fixed.

Malani and Van Ryzin claim that selection of doses at or below the MTD should yield death rates for tumor-free animals that are equal over treatment groups, since the MTD is defined to be the highest dose that can be given that does not cause a significant decrease in survival from effects other than carcinogenicity. However, it has been shown that even differences in tumor-free survival that are not statistically significant can have an appreciable impact on the estimated tumor incidence rates (Bailer and Portier, 1988). In addition, accurate determination of the MTD from subchronic toxicity studies has historically been difficult. Consequently, treatment toxicity has often resulted in a higher death rate for tumor-free animals in upper dose groups than for control-group animals, challenging the validity of the latter test. For this reason, the test based on the statistic given by (5.1.1) is to be preferred over that based on the statistic given by (5.1.3).

5.2 Tests for Nonlethality of Tumors

A statistical procedure for testing the hypothesis of tumor nonlethality can also be developed as a direct application of the results given by Rao. Recall that the lethality of a tumor type is defined as $l(t_s) = \lambda^{DIT}(t_s) / \lambda^{DINT}(t_s)$. A test for nonlethality of tumor is thus equivalent to testing whether the tumor lethality is one, or $\lambda^{DIT}(t_s) = \lambda^{DINT}(t_s)$. Based on the relationships for tumor lethality derived in Section 2.6, the hypothesis for tumor nonlethality can be shown to be equivalent to the hypothesis of $H_0: \phi_s = \beta_s / (1-\lambda_s)$. This is not surprising, since the latter relationship is exactly the form for ϕ_s derived in Section 2.2 under the condition of incidental tumors. The hypothesis of nonlethal tumors can also be stated in terms of the cell probabilities of the multinomial distribution described in Section 2.2, in that $H_0: \phi_s = \beta_s / (1-\lambda_s)$ is equivalent to the hypothesis:

$$H_0: \frac{\Pr[\text{DWT}_j]}{(\Pr[\text{DWT}_j] + \Pr[\text{DWOT}_j])} = \frac{\Pr[\text{SWT}_j]}{(\Pr[\text{SWT}_j] + \Pr[\text{SWOT}_j])}$$

Regardless of which of the above ways the hypothesis of tumor nonlethality is expressed, the maximum likelihood estimators for θ under this null hypothesis are simply the MLE's for θ in the restricted case of an incidental tumor type, which were presented in Theorem 2.3.1. Evaluation of the cell probabilities $\Pr[\text{SWOT}]$, $\Pr[\text{SWT}]$, $\Pr[\text{DWOT}]$, and $\Pr[\text{DWT}]$ at the restricted maximum likelihood estimates for θ yields the values of E_2 in expression (5.1.3), and evaluation of these cell probabilities at the general MLE for θ given by Theorem 2.3.3 provides the values of E_1 . Substitution of the values of E_1 and E_2 in the chi-square statistic given by (5.1.3) results in the following statistic for testing the null hypothesis of tumor nonlethality:

$$\chi_{NL}^2 = \sum_{s=1}^S \left[\frac{n_s \left[(c_s^- d_s^+) - (c_s^+ d_s^-) \right]^2}{(c_s^+ + d_s^+) (c_s^- + d_s^-) (d_s^- + d_s^+) (c_s^- + c_s^+)} \right] \quad (5.2.1)$$

It can be shown that χ_{NL}^2 converges in distribution to a chi-square random variable with S degrees of freedom as $N \rightarrow \infty$ such that the number of time intervals is fixed.

The result given by (5.3.1) can immediately be recognized as the sum of the S individual chi-square statistics resulting from the S contingency tables of the form below:

Type of Death	with tumor	without tumor	Total
Natural death	d_s^+	d_s^-	$(d_s^- + d_s^+)$
Sacrifice	c_s^+	c_s^-	$(c_s^- + c_s^+)$
<hr/>			
Total	$(c_s^+ + d_s^+)$	$(c_s^- + d_s^-)$	$(c_s^+ + d_s^+ + c_s^- + d_s^-)$

Lagakos and Mosteller (1981) give heuristic arguments supporting the combination of chi-square statistics computed from contingency tables like the one above as a test of tumor nonlethality, although they present no formal derivation of a test statistic. Malani and Van Ryzin derive a test statistic for the evaluation of tumor lethality based on the same result due to Rao. It can be demonstrated that their test statistic is equivalent to that given by expression (5.2.1).

CHAPTER VI: NUMERICAL EVALUATION OF RESULTS

6.1 Application of Results to a Long-Term Animal Study

To illustrate the methodology developed in the previous chapters, the estimators obtained by unconstrained and constrained maximum likelihood methods and pooling algorithms are applied to survival/sacrifice data generated by a long-term animal study. An experiment conducted at the National Center for Toxicological Research (NCTR) to study sex and strain differences in mice with respect to the incidence of liver tumors induced by benzidine dihydrochloride provided the data analyzed originally by Kodell and Nelson (1980) and more recently by Portier and Dinse (1987) and Malani and Van Ryzin (1988). In the NCTR experiment, male and female mice from each of two strains (F1 and F2) were administered one of six doses of benzidine ranging from 0 ppm to 400 ppm. The animals received the chemical treatment in their drinking water from the time they were weaned until they died or were sacrificed. The analyses presented in this section are limited to the F2-strain mice from the NCTR data.

Kodell and Nelson (1980) calculated parameter estimates for the NCTR data based on a parametric stochastic model of illness and death using maximum likelihood methods. However, they were unable to obtain analytic solutions to the likelihood equations, and were forced to rely on a computerized maximum likelihood estimation routine. They discovered that their model was not applicable to data from the control (0 ppm) and 30 ppm dose groups due to an insufficient number of liver tumors among the mice in these groups. Consequently, data were reported only for animals receiving benzidine doses of 60 ppm, 120 ppm, 200 ppm, and 400 ppm.

Interim sacrifices were performed at various times throughout the study, but tended to cluster tightly around three points in time. Accordingly, sacrifices performed at 278 to 281 days are considered to have occurred at 40+ weeks, sacrifices performed at 420 to 422 days are considered to have occurred at 60+ weeks, and terminal sacrifices performed at 559-561 days are considered to have occurred at 80+ weeks. The above grouping includes all recorded sacrifice times for both male and female F2 strain mice. The small dispersion in sacrifice times was likely the result of a practical constraint on the personnel needed to sacrifice the animals and perform histopathologic examinations, and grouping the sacrifice times in the manner described should not introduce bias into the results. The original data published by Kodell and Nelson (1980) are in terms of months (days/30); it should therefore be noted that 40+ weeks corresponds to 9.37 months, 60+ weeks is 14.07 months, and 80+ weeks is equivalent to 18.70 months.

In addition to the number of sacrificed animals with and without liver tumors at each sacrifice time, the reported data included the time of death and liver tumor status for animals that died naturally during the course of the experiment. For some animals, it was possible to attribute the death as due to a cause other than liver tumors, i.e., to a competing risk. For example, Kodell and Nelson note that reticulum cell sarcomas and lymphomas were two prominent competing causes of death in the NCTR experiment. Clearly, all deaths in absence of liver tumors fall into the category of deaths due to competing risks. In addition, competing risks were determined to be the cause of death for some mice that died with liver tumors. In the present analysis, no distinction is made between animals with liver tumors that died of a competing risk and those whose deaths were directly attributed to the presence of the tumors.

The actual times of death for animals dying naturally were condensed into three intervals by simply counting the number of deaths with and without liver tumors occurring before 40+ weeks, 60+ weeks, and 80+ weeks for the first, second and third intervals, respectively. Similarly, the number of sacrificed animals with and without liver tumors were totalled for the three approximate sacrifice times. The resulting raw counts for fitting the discrete multinomial likelihood are presented in Table 6.1.1 for F2 strain female mice and Table 6.1.2 for F2 strain male mice. A similar representation of the raw counts for F2 female mice is provided in Table 4 of Malani and Van Ryzin (1988). It should be noted, however, that the 16 sacrifices without tumor at 60 weeks in the 120 ppm group reported by Malani and Van Ryzin is a typographical error, and should be replaced by a count of 26. In addition, an erroneous value of 3 deaths without tumor at 80 weeks in the 60 ppm dose group is reported by Malani and Van Ryzin. The correct value of 4 deaths can be verified from the original data published by Kodell and Nelson. The parameter estimates calculated by Malani and Van Ryzin reflect the correct data value for the first case, but the incorrect data value for the latter situation. However, this error results in only minor discrepancies between the estimators derived in this research with those presented by Malani and Van Ryzin.

The unconstrained maximum likelihood estimates of the tumor incidence rate λ , death rate without tumor β , and overall death rate ϕ are presented in Table 6.1.3 for F2 strain female mice. The accompanying asymptotic standard errors were computed numerically using the observed information matrix as described in Section 3.2. In general, the tumor incidence rate appears to be an increasing function of both time and dose. Exceptions to this standard pattern are seen in the 200 ppm and 400 ppm dose groups, for which the tumor incidence increases from the first interval to the second, but then decreases during the third interval. In fact, it is evident from Table 6.1.3 that the unconstrained estimate of tumor incidence during the third interval for the 400 ppm dose group is

negative. In addition, the estimate of the rate of death without tumor for the same interval and dose group is greater than one. With the exception of the latter estimate, the estimates for the rate of death without tumor are consistently lower than the overall death rate.

The asymptotic standard errors presented in Table 6.1.3 were computed by inverting the Hessian matrix from Appendix A at the data values for F2-strain female mice to obtain the observed information matrix. In the 400 ppm dose group, the Hessian matrix evaluated at the observed data for the third interval is semi-definite, and negative diagonal entries in the inverted matrix precluded the estimation of standard errors for λ and ϕ in this time interval. The lack of a negative definite Hessian matrix implies that the proposed estimators may not maximize the likelihood for this particular set of observed data, as discussed in Section 2.3. The large estimated standard error for the last interval in the 200 ppm dose group should also be noted. The instability of this estimate is a consequence of the fact that only three animals were sacrificed during the third interval and none of these animals were found to be tumor-free, yielding an observed count of zero for c_3^- . Insufficiency of the data for a given interval is thus reflected in large standard errors for the parameter estimates, and this is felt to be a desirable feature of the model.

The unconstrained maximum likelihood estimates of the tumor incidence rate and death rates for F2 strain male mice are displayed in Table 6.1.4. Although some decreases in tumor incidence rates are observed for female mice in the upper dose groups, the estimated tumor incidence rates for male mice are strictly increasing functions of both dose level and time interval. A comparison of the parameter estimates presented in Tables 6.1.3 and 6.1.4 reveals that the estimates of the tumor incidence rate are consistently lower for male mice than for female mice, with the exception of the single negative estimate for female mice in the 400 ppm dose group. This finding is consistent with the results of Kodell and Nelson, who note that the estimated mean time to tumor for females is less than

that for males, indicating higher tumor incidence rates. A similar, although weaker, relationship is observed among the death rates, for which the F2 male mice typically have lower estimates than the female mice.

Using the relationships derived in Section 2.7, it is possible to calculate estimates of the rate of death given that tumor is present, $\lambda^{DT}(t_s)$, and the rate of death given that tumor is absent, $\lambda^{DNT}(t_s)$, as functions of the estimated values of ϕ , β , and λ . These estimates are presented in Table 6.1.5, along with the corresponding estimate of tumor lethality computed as the ratio of $\hat{\lambda}^{DT}(t_s)$ to $\hat{\lambda}^{DNT}(t_s)$. The conditional death rates displayed in Table 6.1.5 are identical to those published by Malani and Van Ryzin. It is interesting to note that the rate of death given that tumor is absent is often greater than the rate of death given that tumor is present, resulting in a tumor lethality estimate which is less than one. The instability of these estimates is primarily due to the small number of liver tumors observed during the first 40 weeks of exposure to benzidine dihydrochloride. Table 6.1.6 contains the maximum likelihood estimates of the conditional death rates $\lambda^{DT}(t_s)$ and $\lambda^{DNT}(t_s)$ and the tumor lethality rate $\ell(t_s)$ for F2 strain male mice. A comparison with those rates resulting from the method of Malani and Ryzin is not possible in this case, as these authors present estimates for female F2 mice only.

A comparison of the estimated cumulative tumor survival function $\hat{\Lambda}_s$ defined by:

$$\hat{\Lambda}_s = \prod_{i=1}^s (1 - \hat{\lambda}_i)$$

as based on the tumor incidence rates presented in Table 6.1.3 and on those estimated by Malani and Van Ryzin (1988) is provided in Table 6.1.7 for F2 female mice. Malani and Van Ryzin present estimates of a different function $\Lambda^T(s)$, calculated as the sum of the individual tumor incidence rates through the s -th interval. However, the corresponding

estimate of λ_s can be easily calculated as the difference between the estimates of $\Lambda^T(s)$ and $\Lambda^T(s-1)$. In addition, parametric estimates of the tumor survival function are calculated based on the MLE's of parameters from a Weibull model of tumor incidence as presented by Kodell and Nelson (1980). This estimate of the cumulative tumor survival rate for the time interval ending at t_s is computed by the relationship:

$$\hat{\Lambda}_s = \exp \left[- \int_0^{t_s} \beta_1 t^{\gamma_1} dt \right] = \exp \left[- \left(\frac{t_s}{b_1} \right)^{c_1} \right]$$

where β_1 and γ_1 refer to the parameters in the Weibull model of tumor incidence described by Kodell and Nelson, and c_1 and b_1 are the transformed parameters for which MLE's are presented, with the following relationships:

$$b_1 = \left[\frac{\gamma_1 + 1}{\beta_1} \right]^{\frac{1}{\gamma_1 + 1}} \quad \text{and} \quad c_1 = \gamma_1 + 1$$

For convenience, the methodology developed in the present research is denoted by "Williams" in Table 6.1.7 and later tables involving comparisons with estimators suggested by other researchers.

From Table 6.1.7, it is clear that the estimates of the cumulative tumor survival function Λ_s calculated using the MLE's derived in Chapter II are identical to those calculated using the estimators suggested by Malani and Van Ryzin, with the exception of the single estimate for the 60 ppm dose group which reflects the incorrect data value discussed previously. The estimates of Λ_s based on the MLE's presented by Kodell and Nelson are similar in magnitude to those of the methodology developed here, and the differences between these estimates can be attributed to the use of a parametric model rather than a nonparametric model. Note that the cumulative tumor survival function estimates for the 400 ppm dose group increase from the second to the third interval for the two

nonparametric methods, while a consistent decrease is seen over all three intervals in the same dose group for the Weibull model used by Kodell and Nelson. The increase observed in the nonparametric models is due to the negative estimate of tumor incidence shown in Table 6.1.3, and reflects the sparseness of the observed data in the last time interval, especially for sacrificed animals.

The cumulative tumor survival function estimates for F2 strain male mice are exhibited in Table 6.1.8 along with those computed using the semi-parametric framework proposed by Portier and Dinse (1987) and the estimates derived from the MLE's presented by Kodell and Nelson (1980). The semi-parametric model employed by Portier and Dinse specifies a Weibull model for the distribution of tumor incidence rates; thus the estimates of Λ_s can be computed in a manner similar to that of the Kodell and Nelson estimates described above. The Weibull hazard for tumor incidence proposed by Portier and Dinse has the form:

$$\lambda(t) = \mu \gamma \left(\frac{t}{t_{\max}} \right)^{\gamma-1}$$

in which $t_{\max}$ is the maximum observed time of death or sacrifice. The estimates of the cumulative tumor survival function can thus be calculated as:

$$\Lambda_s = \exp \left[- \int_0^t \mu \gamma \left(\frac{t}{t_{\max}} \right)^{\gamma-1} dt \right] = \exp \left[- \mu \left(\frac{t_s}{t_{\max}} \right)^{\gamma} \right]$$

Examination of the estimated cumulative tumor survival functions for the three different methods presented in Table 6.1.8 reveals very close agreement between those based on the parametric model of Kodell and Nelson and those based on the semi-parametric model proposed by Portier and Dinse. This agreement is not surprising, since Portier and Dinse note that the shape and scale parameters of the Weibull hazard for tumor

incidence estimated by Kodell and Nelson are very similar to theirs when transformed to the same time scale. The estimates based on the nonparametric model are also close to those of the parametric and semi-parametric analyses, although the estimates of Λ_s are generally lower than those for the two Weibull models during the second interval, and higher for the last interval. All three methods exhibit a decrease in tumor survival rates over both time intervals and dose levels, so that the lowest estimated probability of not developing liver tumors is experienced from 60+ to 80+ weeks by animals receiving 400 ppm of benzidine.

In the previous discussion of the estimated tumor survival functions presented in Table 6.1.7 for female F2 mice, it was noted that the increase observed during the third interval for mice receiving 400 ppm of benzidine reflects a negative estimate of tumor incidence for this interval for the two nonparametric models. This is a legitimate cause for concern regarding these models, since the discrete rate of tumor incidence is a probability, and should only take on values between 0 and 1. The parametric model assumed by Kodell and Nelson forces the cumulative tumor survival rate to be a decreasing function of time due to the use of a Weibull hazard, yet this choice of a parametric model may not always be appropriate.

While Malani and Van Ryzin make no suggestions for improving their invalid estimate of tumor incidence in this situation, the analytic expressions for the constrained MLE's derived in Chapter II can be applied to the observed data to yield asymptotically unbiased estimates of the tumor incidence rates which are contained in the valid parameter space. The constrained MLE's of λ , β , and ϕ for F2-strain females in the 400 ppm dose group are shown in Table 6.1.9, along with their estimated standard errors computed as described in Section 3.4. For the sake of comparison and validation, constrained MLE's computed numerically using an iterative gradient search algorithm which maximizes a

function subject to boundary constraints are also presented in Table 6.1.9. The analytic and numeric solutions for the constrained maximum likelihood estimators are essentially identical, with the exception of differences of 0.001 in the estimates of β and λ in the last interval. These differences are due to the necessity of setting upper and lower boundaries for the parameters which are in the interior of the parameter space in the iterative computer algorithm.

A comparison of the constrained MLE's presented in Table 6.1.9 with the unconstrained MLE's shown in Table 6.1.3 reveals that the estimates of β and ϕ remain unchanged during the first two intervals, and the constrained estimate of the overall death rate during the last interval is almost identical to the unconstrained estimate. The estimate of tumor incidence in the last interval is zero, reflecting the imposition of an active constraint on λ_3 , and the corresponding estimate of β_3 is forced to the upper boundary of the parameter space. The estimated tumor incidence rate during the second interval falls between the unconstrained estimates of λ_2 and λ_3 , but is closer to the unconstrained estimate of λ_2 . The estimates of the cumulative tumor survival function based on the constrained MLE's are 0.652 for 0 to 40+ weeks, 0.134 for 40+ to 60+ weeks, and 0.134 for 60+ to 80+ weeks.

Another method of obtaining estimates of the tumor incidence rate which are in the range $[0,1]$ is through the use of pooling algorithms, as described in Chapter IV. The estimates of the tumor incidence rates and death rates resulting from the intuitive pooling, early pooling, and late pooling algorithms are provided in Table 6.1.10 for female F2 mice receiving 400 ppm of benzidine. The unconstrained estimates of tumor incidence within all remaining dose groups for female mice and all dose levels for male mice are positive, and thus no pooling is required. It is evident from Table 6.1.10 that the late pooling algorithm and the intuitive pooling algorithm yield identical parameter estimates, since both of these

algorithms pool the second and third intervals of data together. An examination of the estimates of tumor incidence resulting from these two pooling algorithms reveals that they are identical to the constrained maximum likelihood estimates presented in Table 6.1.9. This is to be expected, since it was noted in Section 4.2 that the analytic expressions for the estimators obtained by intuitive or late pooling and maximum likelihood methods are equivalent in this situation. The estimates of ϕ_1 and β_1 for the late pooling and intuitive pooling methods are also equivalent to the corresponding constrained maximum likelihood estimates of the death rates for the first interval. For the second and third intervals, the late and intuitive pooled estimates of ϕ and β are between the values of the constrained MLE's for these two intervals. The early pooling algorithm does not appear to provide reliable estimates of the tumor incidence and death rate for this particular set of data.

The maximum likelihood estimates presented in Tables 6.1.9 can be used to compute statistics for testing the hypothesis of equal tumor incidence for female F2 mice from the NCTR animal study, as described in Section 5.1. Ideally, a test of the carcinogenicity of benzidine would involve a comparison of the tumor incidence rates for each of the treated groups with those of the control (0 ppm) group. Since control group data is not available, comparisons of the tumor incidence rate will be performed among the active treatment groups. The normal approximation test statistics Z_T are calculated by subtracting an estimate of the cumulative tumor incidence rate for the lower treatment group from that of the higher treatment group and dividing by an estimate of the variance under the null hypothesis. Positive values for Z_T are thus an indication of overall increases in tumor incidence rates with corresponding increases in dose. The results displayed in Table 6.1.11 show that the cumulative tumor incidence rate for the 60 ppm treatment group is significantly different than each of the higher-level treatment groups, with an estimate that is less than half the value of the estimates for mice receiving 120 ppm, 200 ppm, and 400 ppm. The comparison between the 120 ppm and 400 ppm dose groups also yields a test

statistic which is highly significant, but in this case the value of Z_T is negative, indicating an overall decrease in tumor incidence rates.

The normal approximation test statistics presented in Table 6.1.11 for female mice are qualitatively the same for comparisons among the 60 ppm, 120 ppm, and 200 ppm dose groups as those presented by Malani and Van Ryzin. Although the form of their statistic is equivalent to the normal approximation test statistic derived in Chapter V, they compute estimates of the asymptotic standard errors using a first-order Taylor series approximation to the Fisher information matrix rather than using the observed information matrix. Thus the actual values of the statistics differ. Malani and Van Ryzin found no significant difference between the tumor incidence rates for the 400 ppm dose group and any of the lower dose groups due to their large estimated standard error for the negative MLE of λ during the third time interval, while comparisons based on the constrained MLE's revealed significant differences between the 400 ppm dose group and each of the 60 ppm and 120 ppm dose groups. Kodell and Nelson did not provide any test statistics for the comparison of tumor incidence rates, but did note that a dose-response relationship with respect to time to tumor was demonstrated by their estimates.

A second type of test statistic for the comparison of tumor incidence rates among treatment groups is applicable under the assumptions that (1) the same sacrifice scheme is employed for each treatment group and (2) the rate of death in the absence of tumor is equal among treatment groups. The chi-square statistics generated by this testing procedure, as described in Section 5.1, are given in Table 6.1.12, along with their associated probabilities in comparison with the χ^2 distribution with 3 degrees of freedom. Although the same sacrifice schemes were not used for all four treatment groups, and the rate of death in the absence of tumors appears to increase steadily with increasing dose levels (see Table 6.1.5), the chi-square statistics give qualitatively the same results as the normal

approximation test regarding statistical significance of observed differences. However, one disadvantage of the chi-square test statistic in comparison with the normal approximation test is that it indicates only the magnitude of the difference in treatments with respect to tumor incidence rates, and not the direction. The results obtained from this chi-square testing procedure are consistent with the analogous values reported by Malani and Van Ryzin.

The normal approximation test statistics for comparison of tumor incidence rates among male mice are shown in Table 6.1.13. The results indicate significant differences between estimates of the cumulative tumor incidence rate for all pairwise comparisons at individual significance levels of $\alpha=0.05$. Application of a Bonferoni correction to ensure an overall significance level of $\alpha=0.05$ dictates that the significance level for each individual test be 0.008, in which case the comparison between male mice receiving 120 ppm and 200 ppm of benzidine is no longer significant. In contrast to the estimates of the cumulative tumor incidence rate for female mice, the estimates for male mice show a steady increase with increasing levels of dose. This finding agrees with that of Portier and Dinse, who noted a significant linear trend in dose for this data. The test statistics presented in Table 6.1.13 test for an overall difference in tumor incidence rates between two treatment groups only, yet the consistent trend in dose provides additional support for the rejection of the broader null hypothesis of no carcinogenic effect of benzidine dihydrochloride in male F2-strain mice.

The corresponding chi-square statistics for the comparison of tumor incidence rates among male mice are given in Table 6.1.14. The qualitative agreement between the normal approximation tests and chi-square tests for differences in tumor incidence rates observed for female F2 mice is not repeated for male mice. Examination of the death rates given absence of tumor for male mice (Table 6.1.5) indicates that the second assumption

necessary for the application of this test is not violated to the same degree as it was for female mice. The same sacrifice scheme was employed for male mice as for female mice; however, the probabilities of sacrifice at each time interval vary over dose groups, violating the first assumption discussed previously. Since this test is not appropriate for comparison of tumor incidence rates when the two stated assumptions are not met, the concurrence between the normal approximation test and the chi-square test for female mice was likely coincidental, and the normal approximation test should clearly serve as the basis of our statistical decisions under the circumstances of the NCTR animal study.

The final analysis to be presented for the NCTR long-term animal carcinogenicity experiment is the evaluation of tumor lethality for each of the treatment groups among male and female F2 mice. The statistic for testing the null hypothesis of tumor nonlethality was described in Section 5.2, and is a summation of chi-square test statistics over the intervals of the animal study. The chi-square statistics χ_{NL}^2 are compared with the χ^2 distribution with 3 degrees of freedom, corresponding to the three intervals in the study. These results presented in Table 6.1.15 for both female and male mice indicate that the data are consistent with the null hypothesis of tumor nonlethality for all but female mice in the 200 ppm dose group, for which there is a marginally significant estimate of tumor lethality.

Table 6.1.1

Observed Data for F2-strain Female Mice
from the NCTR Benzidine Dihydrochloride Experiment *

Dose (in ppm)	Time interval in weeks†	<u>deaths</u>		<u>sacrifices</u>		Total
		<u>with tumors</u> d-	<u>without tumors</u> d+	<u>with tumors</u> c-	<u>without tumors</u> c+	
60	0 - 40+	2	0	70	0	72
	40+ - 60+	1	0	38	10	49
	60+ - 80+	4	7	20	15	45
120	0 - 40+	7	0	44	2	53
	40+ - 60+	4	6	26	15	51
	60+ - 80+	2	16	1	20	39
200	0 - 40+	2	1	43	4	50
	40+ - 60+	1	17	12	23	53
	60+ - 80+	2	11	0	3	16
400	0 - 40+	3	0	14	8	25
	40+ - 60+	4	17	1	13	35
	60+ - 80+	1	9	0	1	11

* - original data published by Kodell and Nelson (1980)

† - 40+ weeks = 281 days or 9.37 months (days/30)

60+ weeks = 422 days or 14.07 months

80+ weeks = 561 days or 18.70 months

Table 6.1.2

Observed Data for F2-strain Male Mice
from the NCTR Benzidine Dihydrochloride Experiment*

Dose (in ppm)	Time interval in weeks†	<u>deaths</u>		<u>sacrifices</u>		Total
		<u>with</u>	<u>without</u>	<u>with</u>	<u>without</u>	
		<u>tumors</u>	<u>tumors</u>	<u>tumors</u>	<u>tumors</u>	
		d ⁻	d ⁺	c ⁻	c ⁺	
60	0 - 40 ⁺	8	0	68	0	76
	40 ⁺ - 60 ⁺	3	0	41	3	47
	60 ⁺ - 80 ⁺	3	3	34	4	44
120	0 - 40 ⁺	3	0	46	0	49
	40 ⁺ - 60 ⁺	1	1	39	8	49
	60 ⁺ - 80 ⁺	2	3	27	12	44
200	0 - 40 ⁺	8	0	47	0	55
	40 ⁺ - 60 ⁺	4	1	31	6	42
	60 ⁺ - 80 ⁺	3	1	10	7	21
400	0 - 40 ⁺	3	0	23	1	27
	40 ⁺ - 60 ⁺	3	2	17	4	26
	60 ⁺ - 80 ⁺	0	6	6	6	18

* - original data published by Kodell and Nelson (1980)

† - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

Table 6.1.3

Unconstrained Maximum Likelihood Estimates
of the Tumor Incidence Rates and Death Rates
For F2-strain Female Mice

Dose (in ppm)	Time interval in weeks [†]	$\hat{\lambda}$	$se(\hat{\lambda})$	$\hat{\beta}$	$se(\hat{\beta})$	$\hat{\phi}$	$se(\hat{\phi})$
60	0 - 40 ⁺	0.000	0.008	0.012	0.001	0.012	0.007
	40 ⁺ - 60 ⁺	0.206	0.009	0.011	0.001	0.011	0.007
	60 ⁺ - 80 ⁺	0.341	0.008	0.110	0.004	0.239	0.005
120	0 - 40 ⁺	0.041	0.007	0.049	0.002	0.049	0.007
	40 ⁺ - 60 ⁺	0.364	0.006	0.046	0.002	0.111	0.003
	60 ⁺ - 80 ⁺	0.879	0.006	0.081	0.005	0.462	0.007
200	0 - 40 ⁺	0.091	0.004	0.017	0.001	0.025	0.001
	40 ⁺ - 60 ⁺	0.707	0.015	0.015	0.001	0.261	0.005
	60 ⁺ - 80 ⁺	0.635	0.169	0.365	0.024	0.812	0.048
400	0 - 40 ⁺	0.348	0.015	0.042	0.003	0.042	0.014
	40 ⁺ - 60 ⁺	0.802	0.008	0.137	0.008	0.457	0.009
	60 ⁺ - 80 ⁺	-0.273	-----*	1.273	0.151	0.909	-----*

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

* - Estimates of the asymptotic standard errors could not be obtained
due to a semi-definite observed information matrix

Table 6.1.4

Unconstrained Maximum Likelihood Estimates
of the Tumor Incidence Rates and Death Rates
For F2-strain Male Mice

Dose (in ppm)	Time interval in weeks [†]	$\hat{\lambda}$	$se(\hat{\lambda})$	$\hat{\beta}$	$se(\hat{\beta})$	$\hat{\phi}$	$se(\hat{\phi})$
60	0 - 40 ⁺	0.000	0.008	0.048	0.001	0.048	0.007
	40 ⁺ - 60 ⁺	0.066	0.009	0.033	0.001	0.033	0.007
	60 ⁺ - 80 ⁺	0.098	0.005	0.073	0.003	0.136	0.004
120	0 - 40 ⁺	0.000	0.010	0.021	0.001	0.021	0.008
	40 ⁺ - 60 ⁺	0.177	0.010	0.011	0.001	0.022	0.001
	60 ⁺ - 80 ⁺	0.206	0.008	0.055	0.003	0.114	0.004
200	0 - 40 ⁺	0.000	0.010	0.068	0.002	0.068	0.009
	40 ⁺ - 60 ⁺	0.165	0.009	0.063	0.003	0.079	0.003
	60 ⁺ - 80 ⁺	0.261	0.013	0.171	0.008	0.190	0.008
400	0 - 40 ⁺	0.040	0.014	0.042	0.003	0.042	0.014
	40 ⁺ - 60 ⁺	0.180	0.012	0.071	0.005	0.114	0.006
	60 ⁺ - 80 ⁺	0.588	0.033	0.000	0.039	0.333	0.025

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days of 18.70 months

Table 6.1.5

Maximum Likelihood Estimates of Conditional Death Rates
Given the Presence or Absence of Liver Tumors
For F2-strain Female Mice

Dose (in ppm)	Time interval in weeks [†]	$\hat{\lambda}^{DIT}(t_s)$	$\hat{\lambda}^{DINT}(t_s)$	$\hat{a}(t_s)$
60	0 - 40 ⁺	0.000	0.012	0.000
	40 ⁺ - 60 ⁺	0.000	0.013	0.000
	60 ⁺ - 80 ⁺	0.318	0.167	1.909
120	0 - 40 ⁺	0.000	0.051	0.000
	40 ⁺ - 60 ⁺	0.170	0.073	2.328
	60 ⁺ - 80 ⁺	0.444	0.667	0.667
200	0 - 40 ⁺	0.092	0.018	4.973
	40 ⁺ - 60 ⁺	0.337	0.054	6.221
	60 ⁺ - 80 ⁺	0.786	1.000	0.786
400	0 - 40 ⁺	0.000	0.065	0.000
	40 ⁺ - 60 ⁺	0.423	0.691	0.611
	60 ⁺ - 80 ⁺	0.900	1.000	0.900

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

Table 6.1.6

Maximum Likelihood Estimates of Conditional Death Rates
Given the Presence or Absence of Liver Tumors
For F2-strain Male Mice

Dose (in ppm)	Time interval in weeks [†]	$\hat{\lambda}^{DIT}(t_s)$	$\hat{\lambda}^{DINT}(t_s)$	$\hat{a}(t_s)$
60	0 - 40 ⁺	0.000	0.048	0.000
	40 ⁺ - 60 ⁺	0.000	0.035	0.000
	60 ⁺ - 80 ⁺	0.429	0.081	5.286
120	0 - 40 ⁺	0.000	0.021	0.000
	40 ⁺ - 60 ⁺	0.061	0.013	4.640
	60 ⁺ - 80 ⁺	0.200	0.069	2.900
200	0 - 40 ⁺	0.000	0.068	0.000
	40 ⁺ - 60 ⁺	0.096	0.076	1.264
	60 ⁺ - 80 ⁺	0.125	0.231	0.542
400	0 - 40 ⁺	0.000	0.044	0.000
	40 ⁺ - 60 ⁺	0.212	0.087	2.444
	60 ⁺ - 80 ⁺	0.500	0.000	∞

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

Table 6.1.7
Estimates of the Cumulative Tumor Survival Function
For F2-strain Female Mice

Dose (in ppm)	Time interval in weeks [†]	Estimate of Λ_s		
		Williams	Malani & Van Ryzin	Kodell & Nelson
60	0 - 40 ⁺	1.000	1.000	0.987
	40 ⁺ - 60 ⁺	0.794	0.794	0.864
	60 ⁺ - 80 ⁺	0.523	0.513*	0.444
120	0 - 40 ⁺	0.959	0.959	0.956
	40 ⁺ - 60 ⁺	0.609	0.609	0.558
	60 ⁺ - 80 ⁺	0.074	0.074	0.031
200	0 - 40 ⁺	0.909	0.909	0.910
	40 ⁺ - 60 ⁺	0.266	0.266	0.309
	60 ⁺ - 80 ⁺	0.097	0.097	0.007
400	0 - 40 ⁺	0.652	0.652	0.686
	40 ⁺ - 60 ⁺	0.129	0.128	0.115
	60 ⁺ - 80 ⁺	0.164	0.164	0.001

* - This estimate is calculated based on $d_{13^-} = 3$ rather than $d_{13^-} = 4$. Recalculation of $\hat{\Lambda}_s$ based on the correct value yields $\hat{\Lambda}_3 = 0.523$ for the 60 ppm dose group.

† - 40⁺ weeks = 281 days or 9.37 months (days/30)
 60⁺ weeks = 422 days or 14.07 months
 80⁺ weeks = 561 days or 18.70 months

Table 6.1.8
Estimates of the Cumulative Tumor Survival Function
For F2-strain Male Mice

Dose (in ppm)	Time interval in weeks [†]	Estimate of Λ_s		
		Williams	Portier & Dinse	Kodell & Nelson
60	0 - 40 ⁺	1.000	0.994	0.997
	40 ⁺ - 60 ⁺	0.934	0.954	0.965
	60 ⁺ - 80 ⁺	0.843	0.823	0.819
120	0 - 40 ⁺	1.000	0.976	0.985
	40 ⁺ - 60 ⁺	0.823	0.869	0.889
	60 ⁺ - 80 ⁺	0.653	0.621	0.613
200	0 - 40 ⁺	1.000	0.984	0.985
	40 ⁺ - 60 ⁺	0.835	0.871	0.860
	60 ⁺ - 80 ⁺	0.617	0.536	0.463
400	0 - 40 ⁺	0.960	0.965	0.966
	40 ⁺ - 60 ⁺	0.787	0.764	0.759
	60 ⁺ - 80 ⁺	0.324	0.329	0.303

† - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

Table 6.1.9

Constrained Maximum Likelihood Estimates
of the Tumor Incidence Rates and Death Rates
For F2-strain Female Mice Receiving 400 ppm of Benzidine

Method	Time interval in weeks [†]	$\hat{\lambda}$	$se(\hat{\lambda})$	$\hat{\beta}$	$se(\hat{\beta})$	$\hat{\phi}$	$se(\hat{\phi})$
Analytic Solutions	0 - 40 ⁺	0.348	0.014	0.042	0.003	0.042	0.014
	40 ⁺ - 60 ⁺	0.795	0.000	0.137	0.005	0.457	0.009
	60 ⁺ - 80 ⁺	0.000	0.000	1.000	0.119	0.908	0.014
Numeric Solutions	0 - 40 ⁺	0.348	0.014	0.042	0.003	0.042	0.014
	40 ⁺ - 60 ⁺	0.795	0.000	0.137	0.005	0.457	0.009
	60 ⁺ - 80 ⁺	0.001	0.000	0.999	0.119	0.908	0.014

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days or 18.70 months

Table 6.1.10

Estimates of the Tumor Incidence Rates and Death Rates
 Derived From Pooled Data
 For F2-strain Female Mice Receiving 400 ppm of Benzidine

Method	Time interval in weeks [†]	$\hat{\lambda}$	$\hat{\beta}$	$\hat{\phi}$
Early Pooling	0 - 60 ⁺	0.626	0.099	0.338
	60 ⁺ - 80 ⁺	0.781	0.218	0.909
Late Pooling	0 - 40 ⁺	0.348	0.042	0.042
	40 ⁺ - 80 ⁺	0.795	0.171	0.674
Intuitive Pooling	0 - 40 ⁺	0.348	0.042	0.042
	40 ⁺ - 80 ⁺	0.795	0.171	0.674

[†] - 40⁺ weeks = 281 days or 9.37 months (days/30)

60⁺ weeks = 422 days or 14.07 months

80⁺ weeks = 561 days of 18.70 months

Table 6.1.11

Normal Approximation Tests for Comparison of Treatment Groups
with Respect to Tumor Incidence Rates
For F2-strain Female Mice

				<u>Comparison with Treatment Group Receiving:</u>		
Dose (in ppm)	N	$\Sigma \hat{\lambda}_s$	$\text{var}(\Sigma \hat{\lambda}_s)$	<u>120 ppm</u> Z_T (p-value)	<u>200 ppm</u> Z_T (p-value)	<u>400 ppm</u> Z_T (p-value)
60	167	0.547	0.029	43.85 (< 0.001)	5.43 (< 0.001)	30.91 (< 0.001)
120	143	1.284	0.016		0.92 (0.359)	-7.99 (< 0.001)
200	119	1.434	3.157			-1.78 (0.075)
400	71	1.143	0.014			

Table 6.1.12

Chi-Square Tests for Comparison of Treatment Groups
with Respect to Tumor Incidence Rates
For F2-strain Female Mice

		<u>Comparison with Treatment Group Receiving:</u>		
Dose (in ppm)	N	<u>120 ppm</u> χ_T^2 (p-value)	<u>200 ppm</u> χ_T^2 (p-value)	<u>400 ppm</u> χ_T^2 (p-value)
60	167	27.41 (< 0.001)	38.90 (< 0.001)	54.82 (< 0.001)
120	143		5.14 (0.162)	14.39 (< 0.001)
200	119			5.92 (0.116)
400	71			

Table 6.1.13

Normal Approximation Tests for Comparison of Treatment Groups
with Respect to Tumor Incidence Rates
For F2-strain Male Mice

Dose (in ppm)	N	$\Sigma \hat{\lambda}_s$	$\text{var}(\Sigma \hat{\lambda}_s)$	<u>Comparison with Treatment Group Receiving:</u>					
				<u>120 ppm</u>		<u>200 ppm</u>		<u>400 ppm</u>	
				Z_T	(p-value)	Z_T	(p-value)	Z_T	(p-value)
60	167	0.164	0.022	12.24	(< 0.001)	12.91	(< 0.001)	17.41	(< 0.001)
120	142	0.383	0.027			2.00	(0.046)	11.25	(< 0.001)
200	118	0.426	0.033					9.80	(< 0.001)
400	71	0.808	0.088						

Table 6.1.14

Chi-Square Tests for Comparison of Treatment Groups
with Respect to Tumor Incidence Rates
For F2-strain Male Mice

		<u>Comparison with Treatment Group Receiving:</u>					
Dose (in ppm)	N	<u>120 ppm</u>		<u>200 ppm</u>		<u>400 ppm</u>	
		χ_T^2	(p-value)	χ_T^2	(p-value)	χ_T^2	(p-value)
60	167	10.81	(0.013)	8.75	(0.033)	23.80	(< 0.001)
120	143			6.69	(0.082)	6.47	(0.091)
200	119					6.22	(0.101)
400	71						

Table 6.1.15
Evaluation of Tumor Lethality

Dose (in ppm)	Female Mice		Male Mice	
	χ_{NL}^2	p-value	χ_{NL}^2	p-value
60	1.71	0.635	6.25	0.100
120	2.69	0.443	3.08	0.380
200	7.76	0.051	0.40	0.939
400	2.69	0.443	5.63	0.131

6.2 Evaluation of Estimators for Three-Interval Study Designs

The analysis of the Kodell and Nelson data presented in the previous section demonstrated that the current methodology agrees closely with other methods proposed for the estimation and comparison of tumor incidence rates. Yet this agreement cannot be interpreted as implying that any of these methods produce optimal estimates in the sense of being unbiased and having minimum variance. The unconstrained and constrained MLE's are known to have some optimal asymptotic properties; under certain regularity conditions discussed in Chapter III, they are asymptotically consistent and efficient. However, long-term animal studies are rarely conducted with what could be regarded as large samples, and the operating characteristics remain to be determined for the MLE's and estimates derived from pooling methods in small samples.

In Chapter II, it was noted that certain data outcomes could hypothetically result in negative unconstrained estimates of tumor incidence. Constrained estimators of the entire set of parameters were therefore derived under the restriction of non-negative tumor incidence rates. The availability of these constrained MLE's proved useful in the analysis of the Kodell and Nelson data. However, the issue of whether the existence of constrained estimators is of practical benefit or only theoretical interest cannot be judged on the basis of a single study, since it is unclear how often it would be necessary to employ the constrained estimators in analyzing many different types of long-term animal studies.

In an attempt to evaluate the operating characteristics and applicability of the estimators proposed in this research, a series of Monte Carlo simulation studies were designed and implemented. Data for these simulation studies were generated under the conditions which were felt *a priori* to have the greatest impact on the accuracy and variability of the estimators of the tumor incidence rate. These conditions were: (1) the

background death rates without tumor, (2) the shape of the tumor survival curve, (3) the cumulative tumor survival rate, and (4) the lethality of the tumor.

Death rates without tumor were chosen to represent low and high background mortality rates. Levels for the low background death rates were obtained by computing age-specific estimates of survival rates at the designated sacrifice times based on a Weibull hazard for death proposed for historical control animals in the National Toxicology Program's carcinogenicity experiments (Portier, Hedges and Hoel, 1986). These low levels were felt to be typical of those exhibited by male and female rats and mice in the control group of a long-term animal study. The high levels of background death rates without tumor were computed by doubling the low background rates. The high background rates are intended to reflect the mortality rates of animals in upper dose groups, or perhaps a high spontaneous death rate for a particular species.

Data were simulated for three possible shapes of the tumor survival curve: (1) concave, (2) linear, and (3) convex. A concave curve is one which curves upward, and reflects a tumor type which has a very high incidence early in the duration of a long-term animal study, which then tapers off quickly so that the tumor incidence is close to zero by the end of the study. This type of curve is exhibited for the incidence of thyroid tumors in male mice. A linear survival curve reflects a tumor type resulting in a decrease in the proportion of tumor-free animals which is directly proportional to time. The incidence of lung tumors in male rats typically produces a linear survival curve. A convex tumor survival curve reflects a tumor type with an incidence which is very low early in the lifespan of the animal but begins to increase rapidly in the later part of the animal's lifespan. An example of this pattern is given by pituitary tumors in female rats. This latter level for the shape of the tumor survival curve is probably most typical of the tumor types which

have been studied in the past, yet the inclusion all three levels broadens the applicability of the simulation studies.

Three levels of the cumulative tumor survival rate were also designated for the purposes of the simulation studies, since it was expected that the ability to calculate accurate estimates of the tumor incidence rates would depend to some extent on the total proportion of animals which develop tumors over the course of the carcinogenicity experiment. The cumulative tumor survival rate levels of 20%, 50%, and 80% reflect respective probabilities of 0.2, 0.5, and 0.8 of remaining tumor-free through the end of the long-term animal study. The three levels for the shape of the cumulative tumor survival curve and three levels of cumulative tumor survival together determine nine possible combinations under which true values of the tumor incidence rates are calculated for the purposes of the simulation studies.

The last factor taken into consideration in the simulation studies was the lethality of the tumor, which is reflected by the value of γ_s . Recall that γ_s is defined to be the average rate of death during the s -th interval given the previous onset of a tumor. In the case of an incidental tumor type, it was shown in Chapter II that the relationship between this rate of death, the death rate without tumor, and the tumor incidence rate can be expressed as $\gamma_s = \beta_s / (1 - \lambda_s)$. The rate of death given previous tumor onset in the case of fatal tumors is $\gamma_s = 1$. Thus, these two levels for γ_s are used to generate data under incidental and fatal tumor scenarios. An intermediate level of tumor lethality was also designated by setting the true value of γ_s at some level between the values applicable to the incidental and fatal tumor situations. Given the true background mortality rates as defined by the first factor, the true tumor incidence rates as defined by the combination of the factors (2) and (3), and the true rates of death given tumor onset as defined by the tumor lethality levels, the true value of

the overall death rate, ϕ_s , can be calculated for each interval using expression (2.2.7) of Chapter II, Section 2.2.

The combination of the four conditions described above determines a $2 \times 3 \times 3 \times 3$ factorial design with 54 cross-classifications. For each of the simulation studies to be discussed in the following sections, a total of 10,000 datasets were generated for each of the 54 cross-classifications determined by the factorial design. Each of the 10,000 datasets for a particular cross-classification of background mortality rates, shape of tumor survival curve, cumulative tumor survival, and tumor lethality represents the results of a single long-term animal study. The simulation programs were written in FORTRAN and executed on a SUN 4 workstation. Summary statistics for each of the 54 cross-classifications were written to an output file, along with the starting seeds for random number generators so that the results of any individual long-term animal study could easily be recreated.

The first simulation study was designed to evaluate the bias and variance of the estimators derived in Chapters II and IV for a 2-year animal study with three sacrifice intervals based on a discrete nonparametric model of tumor incidence and death. The bias was measured by comparing the mean estimate for the 10,000 simulated datasets with the true value, and the variance was measured by computing the mean squared error (MSE), or sum of squared differences between the estimated and true values over the 10,000 simulated datasets divided by the number of simulations. It was assumed that 70 animals would be included in each 2-year study, interim sacrifices would be performed at 12 and 18 months, and a terminal sacrifice would be performed at 24 months (2 years). Since the number of animals which die naturally and are sacrificed with and without tumors is generated on the basis of a discrete model, the actual times of interim sacrifices are irrelevant except in that they allow selection of reasonable true values under which the

Monte Carlo simulation is performed. The low background rates for the death rates without tumor were chosen to be 0.010 for the first interval, 0.030 for the second interval, and 0.150 for the third interval based on the experience of historical control animals in the NTP studies as described previously. The intermediate level of tumor lethality for this simulation study was based on setting γ_s equal to the incidental level $\beta_s / (1 - \lambda_s)$ plus a constant factor of 0.3 for all time intervals. For the one case in which this formulation resulted in a true value for γ_s greater than one, the death rate given tumor onset was set equal to one.

For each animal, the time interval during which the first event E_1 occurs is determined by comparison of a uniform (0,1) random number, U_1 , with the cumulative survival probability for the event E_1 , calculated as $\Pi(1 - \lambda_s - \beta_s)$. If U_1 falls between the cumulative survival probability for the s -th and $(s-1)$ -th time interval, then the event E_1 is said to occur during the s -th time interval. Next, a second uniform (0,1) random number, U_2 , is generated and used to determine whether the first event is tumor onset or death without tumor by comparison with the ratio $\beta_s / (\lambda_s + \beta_s)$ (see Portier and Bailer, 1987). If the first event is tumor onset, then a third uniform (0,1) random number, U_3 , is generated to determine the interval during which death with tumor occurs by comparison with the cumulative probability of remaining alive given previous tumor onset in the s -th interval, calculated as the product $\Pi(1 - \gamma_i)$ beginning with the s -th interval. In this manner, the data counts d_s^+ and d_s^- for animals dying naturally with and without tumors can be generated for each interval.

For any of the 70 animals, if U_1 is determined to be larger than the cumulative survival probability for the last interval, then this animal is noted as being sacrificed without the tumor at the end of the last interval. Similarly, if the first event for a particular animal is tumor onset and U_3 is larger than the product $\Pi(1 - \gamma_i)$ from interval s through the

end of the study, then this animal is noted as being sacrificed with the tumor at the end of the last interval. After the death times for all 70 animals in the 2-year study are calculated, interim sacrifices are incorporated into the study design by randomly selecting 15% of the animals which remain alive at 12 months and 17% of the animals which remain alive at 18 months and noting their tumor status. These percentages were chosen in order to mimic the standard NTP long-term animal experiment which includes interim sacrifices, since these designs typically begin with 50 animals within each dose-sex-species group and add 10 animals for each proposed sacrifice time. In this manner, the data counts c_s^- and c_s^+ are generated for each of the three intervals.

In the simulation study described above, estimates of the tumor incidence rate, death rate without tumor, and the overall death rate were first calculated using the unconstrained MLE's derived in Chapter II, Section 2.3. Based on the classification of the unconstrained estimates of the tumor incidence rates λ_2 and λ_3 as negative or non-negative, the constraint case was determined as I, II, III, or IV as described in Chapter II, Section 4. One question of interest addressed by this simulation study was the frequency with which the unconstrained MLE's yield parameter estimates within the range $[0,1]$ for a typical long-term animal study. Under the levels of the four conditions studied by this simulation study, it was found that the unconstrained parameter estimates often failed to provide valid estimates of the tumor incidence rate.

Of the 10,000 datasets generated for each combination of simulation factors, the number which were classified in each constraint case is shown in Table 6.2.1 for low background rates and Table 6.2.2 for high background rates. Examination of these two tables reveals that the datasets simulated under the conditions of a fatal tumor, i.e., with $\gamma_s = 1$ for all intervals, always provide estimates which are in the correct parameter space. This result is explained by the fact that no animals will ever be sacrificed with tumors when

the data is simulated under the conditions of a fatal tumor type, so that $c_s^+ = 0$ for all intervals. Application of this restriction in the data outcomes to the general forms for the unconstrained MLE's given in (2.3.5) - (2.3.7) yields an unconstrained estimator for the death rate without tumor of $\hat{\beta}_s = d_s^- / R_s$, the simple prevalence of deaths without tumor, and an unconstrained estimator for the tumor incidence rate of $\hat{\lambda}_s = d_s^+ / R_s$, the simple prevalence of deaths with tumor. Estimates derived from either of these MLE's will always lie in the range $[0,1]$. The number of simulated datasets falling into a Case I classification is consistently lowest for incidental tumor types within each category of cumulative tumor survival and shape of cumulative tumor survival curve, but the number of datasets simulated based on intermediate tumor lethality which require no constraints is only slightly higher than that of incidental tumors.

For the incidental and intermediate tumor types, the number of simulations falling into the "no constraint" case increases as the tumor survival rate decreases from 0.8 to 0.2. This pattern is intuitively consistent, since a lower cumulative tumor survival rate implies that a higher percentage of the animals will get the tumor by the end of the study, increasing our ability to accurately estimate the tumor incidence rate. The shape of the cumulative tumor survival curve also affects the number of simulated datasets which yield valid estimates of tumor incidence, with convex tumor survival patterns yielding the highest rate of valid estimates and concave tumor survival patterns yielding the lowest rate of non-negative tumor incidence rate estimates. Comparison of the results displayed in Table 6.2.1 and 6.2.2 reveals little difference between low and high background mortality rates with regard to the number of simulated datasets falling into the four constraint cases.

When the unconstrained MLE for tumor incidence given by expression (2.3.7) yields a negative value, alternative estimates of the tumor incidence rate and death rates can be calculated by the analytic expressions for the constrained MLE's displayed in Tables

2.4.3 - 2.4.5 or by using the intuitive pooling, early pooling, and late pooling algorithms described in Section 4.2 of Chapter IV. The constrained estimators are clearly to be preferred over those derived from pooled data based on their optimal asymptotic properties, yet the 3-interval study design allows a comparison of the operating characteristics of both types of estimators. If the pooling algorithms compare favorably to the constrained MLE's in a 3-interval design, it would be expected that they would compare at least as favorably in a general study design with more than three intervals for which analytic solutions for the constrained MLE's are unavailable.

One minor disadvantage of the analytic solutions for the constrained MLE's which became evident during the implementation of the simulation studies is that they could not always be computed, especially for the Case III constraint. In the latter situation, an analytic solution is provided for $\hat{\beta}_2$ as a function of the observed data counts and the remaining estimators are defined in terms of $\hat{\beta}_2$. When the number of animals dying naturally without tumors, d_2^- , is equal to zero, then the resulting estimate of β_2 is zero. Under this circumstance, $\hat{\lambda}_1$ cannot be calculated as it involves division by β_2 . The inability to compute the constrained maximum likelihood estimates of the parameters when the unconstrained estimate of λ_2 is negative and $d_2^- = 0$ does not imply that such an estimator does not exist, but only that it cannot be computed using the analytic expression derived in Chapter II.

The constrained MLE's of the tumor incidence rate and death rates were also computed numerically using an iterative gradient search algorithm which maximizes a function subject to boundary constraints. The constrained MLE's computed numerically appeared to be equal to the constrained MLE's computed using the analytic expressions for all cases in which both methods could be applied. The ability to compute constrained MLE's numerically when the analytic solution could not be computed is an indication that

the analytic expressions given in Chapter II for the constrained MLE's do not completely describe the possible set of estimators. This disadvantage is discussed only for the sake of completeness; analytic solutions could be used to compute the estimators of tumor incidence rates and death rates for over 95% of the simulated datasets. The number of simulations within each constraint case for which no analytic or numeric solution is available is reported in the tables of Appendix B for each cross-classification of background death rates, cumulative tumor survival rate, shape of tumor survival curve, and tumor lethality. Convergence to a set of constrained MLE's using the gradient search algorithm appeared to be very sensitive to the upper and lower boundary conditions, which were set just within the parameter space. However, the total number of simulated datasets for which no convergence was obtained was less than 0.05% of the total simulated datasets.

A secondary goal of the three-interval simulation study was to determine a set of estimators for the tumor incidence rates and death rates which would not require constrained MLE's to be computed, but which would compare favorably with the constrained estimators in terms of being close to the true values and having small variances. Although the constrained MLE's are the basis for comparison in the sense described above, a set of estimators was desired which could be easily calculated for a study design with any number of time intervals without relying on iterative computer algorithms.

On the basis of preliminary simulation studies, such a set of estimators were defined to be the unconstrained MLE's for the overall death rate, the unconstrained MLE's for the death rate without tumor for all intervals except the last, for which the intuitive pooling estimate is substituted, and the intuitive pooling estimates of tumor incidence. Although the unconstrained estimators of the tumor incidence rate were often observed to have smaller mean biases than the intuitive pooling estimates, the possibility of invalid (negative) tumor incidence rate estimates excluded the unconstrained parameter estimates

from consideration. In addition, both the intuitively pooled estimates and the constrained MLE's were observed to have smaller variances than the unconstrained MLE's as measured by the mean squared error. The set of estimators described above is simply a subset of the unconstrained and intuitively pooled estimates chosen in an attempt to obtain minimum-variance unbiased estimators. For the sake of completeness, however, this set of estimators will be displayed in tables of parameter estimates and mean squared error values and designated as Williams method.

The mean values of the estimated tumor incidence rates computed for the three-interval discrete model simulated data based on low background death rates are provided in Tables 6.2.3, 6.2.4, and 6.2.5 for cumulative tumor survival rates of 20%, 50% and 80%, respectively. Tables 6.2.6, 6.2.7, and 6.2.8 display the corresponding mean estimates for simulations based on high background death rates. It should be noted that the estimates of the tumor incidence rates for all methods of estimation are equal to the unconstrained estimates when no constraints are enforced, so that the mean estimates displayed in these tables for any given method of estimation are mixtures of the unconstrained estimates with the estimates computed using the given method. Examination of Tables 6.2.2 through 6.2.8 reveals that the mean values for the unconstrained estimates are typically closest to the true values, demonstrating the least average bias. However, it will be emphasized as discussed above that the unconstrained estimators of tumor incidence are not considered legitimate estimators of the tumor incidence rates since they allow the possibility of negative tumor incidence rate estimates.

The mean estimated values for the analytically constrained and numerically constrained methods are fairly close to the true values. However, it is evident from these mean estimated values that the effect of enforcing non-negative estimates of tumor incidence is to dilute some of the differences between the true tumor incidence rates. The

constrained estimates computed analytically were observed to agree with the numerically constrained estimates for individual datasets; thus the differences in the mean values for these two methods can be attributed to the cases for which the analytically constrained estimates could not be computed but the numeric constrained MLE's were determined. This situation occurred primarily for simulated datasets classified as Case III, so that the numerically constrained estimate of tumor incidence for the second interval is constrained to be zero and the constrained estimate of tumor incidence for the first interval would be somewhat higher than the unconstrained estimate. Recognition of this situation provides an explanation for the fact that the analytically constrained estimates of tumor incidence are lower than the numerically constrained estimates for the first interval and higher for the second.

The estimates of tumor incidence for all methods appear to get somewhat closer to the true values as the shape of the tumor survival curve changes from concave to linear to convex and as the cumulative tumor survival rate changes from 80% to 50% to 20%. However, this observation can be explained by the fact that the conditions for the simulated datasets with mean estimates closer to the true values are the same conditions which were seen to require fewer constrained estimates, as shown in Tables 6.2.1 and 6.2.2. The true values of the tumor incidence rates for a cumulative tumor survival rate of 80% are often very close to zero; thus it is not surprising that a larger number of constrained cases are observed for this simulation condition.

There appears to be little difference between the estimates of tumor incidence rates for datasets simulated under low and high background death rates for any of the methods of estimation. In many cases the estimated values for high background death rates which appear in Tables 6.2.6 - 6.2.8 are identical to those for low background rates displayed in Tables 6.2.3. - 6.2.5 within a given level of tumor survival curve shape, tumor lethality,

and cumulative tumor survival rate. The mean estimates derived from intuitively pooled data are very close to the numerically constrained estimates of tumor incidence, especially for the datasets simulated based on the condition of incidental tumor lethality. The largest mean biases among the methods of estimation are observed for the method of early pooling and late pooling, although in some cases in which the shape of the tumor incidence curve is concave, the late pooling method appears to have smaller mean biases than the intuitive pooling estimates.

The mean estimated values for the death rate without tumor and overall death rate are provided in Appendix C for the three-interval discrete model simulation study. Although the emphasis of this simulation study is the evaluation of estimators of the tumor incidence rate, the estimates of the death rates deserve some attention, as their unbiased estimation allows proper adjustment for differences in intercurrent mortality. Note that the mean estimates of the death rate without tumor are very close to the true values in the datasets simulated under the fatal tumor condition, just as they were for the tumor incidence rate. For the incidental and intermediate levels of tumor lethality, both the unconstrained and constrained MLE's tend to be slight overestimates of the true death rates without tumor, especially in the last time interval. It was for this reason that the collection of estimators designated as "Williams" method substituted the intuitive pooling estimator of the death rate without tumor for the last interval.

The mean estimates of the overall death rate, also displayed in Appendix C, are almost identical for the unconstrained and constrained MLE's, and both the unconstrained and constrained MLE's exhibit essentially zero bias in comparison with the true values. Relative to the MLE's, the estimators of the overall death rate obtained from pooled data can be observed to show greater levels of average bias. Recall that the analytic solutions for the overall death rates under the imposition of constraints often had complex and

lengthy forms. The finding that the overall death rates can be estimated in an unbiased manner by the simple Kaplan-Meier estimator under the conditions of this simulation study is an important discovery, since a joint set of valid estimators of the tumor incidence rate, death rate without tumor and overall death rate can now be described in terms of simple, intuitive formulas. In addition, for study designs with more than three intervals, numeric iterative algorithms can focus attention on determining MLE's for the death rate without tumor and tumor incidence rate under the assumption that the overall death rates are the Kaplan-Meier estimates, reducing the computer time required for such calculations by one-third.

The second criterium in the evaluation of the methods of estimating tumor incidence rates and death rates is that of minimum variance, as measured by the mean square error (MSE). The MSE values for the estimates of the tumor incidence rate are shown in Tables 6.2.9, 6.2.10, and 6.2.11 for simulated datasets based on low background death rates and cumulative tumor survival rates of 20%, 50%, and 80%, respectively. Tables 6.2.12, 6.2.13, and 6.2.14 display the corresponding MSE values for datasets simulated with high background rates of death without tumor. It is evident from these tables that, while the analytically and numerically constrained maximum likelihood methods of obtaining estimates of the tumor incidence have slightly greater bias than the unconstrained estimates, they gain the advantage of having smaller values for the MSE. In fact, all of the methods of estimation considered in this simulation study fare better than the unconstrained MLE's with respect to MSE levels, with the exception of some of the estimates derived from the early pooling algorithm for convex tumor survival curves. This finding can be explained by the fact that all of these methods eliminate the negative estimates of tumor incidence which are clearly outliers in comparison with the true values.

The MSE values for the method of intuitive pooling, or equivalently for Williams method, compare remarkably well with those of the numerically constrained estimates. In fact, the intuitive pooling MSE values are less than or equal to those of the numerically constrained estimates for the majority of simulation conditions and time intervals. The MSE values for the numerically constrained estimates are used as the basis for this comparison because there were very few cases in which these estimates could not be computed relative to the analytically constrained estimates. The MSE values for the early pooling method appear to be inflated relative to both the constrained MLE's and the intuitive pooling method. This increased variability is especially evident during the second interval for convex-shaped tumor survival curves. While the late pooling algorithm appears to provide MSE values which are sometimes smaller than the constrained MLE's or intuitive pooling methods, in certain cases the MSE values are even larger than those of the unconstrained MLE's (for example see Table 6.2.9, linear survival curve, intermediate tumor lethality).

The effect of the simulation conditions can also be evaluated through examination of the MSE values displayed in Tables 6.2.9 - 6.2.14. Comparison of Tables 6.2.9, 6.2.10, and 6.2.11 reveals decreasing values of MSE with increasing levels of the cumulative tumor survival rate among datasets simulated with low background death rates. MSE values appear to be unaffected by the level of background mortality rates, as verified by a comparison of the latter three tables with Tables 6.2.12, 6.2.13, and 6.2.14, which contain the same information for simulated datasets based on high background death rates. In contrast, the shape of the tumor survival curve does seem to have an impact on the variability of tumor incidence estimates. From Table 6.2.9, it is apparent that the MSE values increase during the second time interval as the shape changes from concave to linear to convex, while the MSE values for the first interval are consistently lowest among datasets simulated with convex tumor survival curves. The MSE values for the last interval

appear to remain constant for the three different shapes. These patterns, also observed in Tables 6.2.10 - 6.2.14, are consistent with the degree of change in the tumor incidence rates associated with each survival curve shape. The convex-shaped curve is almost flat during the first interval since the tumor incidence rates associated with a late tumor onset time are very small in the early portion of the animals' lifetime. Similarly, the concave-shaped curve is almost flat during the second interval, reflecting the fact that the tumor incidence rates associated with an early tumor onset time have already tapered off. Thus, the variability in estimates is smaller for the concave-shaped curve than for the linear or convex-shaped tumor survival curves, which have greater decreases between 12 and 18 months.

Appendix D contains the mean squared error values for the estimates of the death rate without tumor and the overall death rate. The analytically and numerically constrained estimates and estimates derived from intuitively and late pooled data typically exhibit the same MSE values as the unconstrained estimates during the first two intervals, and lower MSE values than the unconstrained estimates during the last interval for the death rate without tumor. While the MSE values were typically lowest during the last interval for tumor incidence rates, the MSE values increase over time intervals for the estimates of death rate without tumor. Thus the MSE values for Williams method, based on the unconstrained estimates of death rate during the first two intervals and the intuitively pooled estimates for the last interval, are generally the lowest overall. There appears to be no difference between the MSE values for the constrained and unconstrained estimates of the overall death rate. However, the MSE values for the three pooling methods are consistently larger than those of the unconstrained or constrained maximum likelihood estimates of the overall death rates for all conditions addressed in this simulation study.

To summarize the results of the three-interval discrete model simulation study, the analytic solutions for the constrained maximum likelihood estimators appear to offer an estimation method which is easily applied, and provides valid estimates of the tumor incidence rate which have smaller variability than the unconstrained MLE's at the expense of a slight increase in bias. The estimates of the tumor incidence rate obtained from intuitively pooled data are very close to those of the constrained MLE's, and have MSE values which compare remarkably well to those of the constrained estimates. The estimates of the tumor incidence rate obtained from the early or late pooling methods appear to be unreliable.

As a final note regarding the three-interval discrete model simulation study, it should be mentioned that a preliminary simulation study was conducted under conditions of tumor lethality, background death rates, and cumulative tumor survival rates similar to those previously described with the goal of determining the savings in computation time afforded by the existence of analytic solutions over iterative numeric algorithms. In this preliminary study, the unconstrained, constrained, and intuitive pooling estimators were computed and compared with the true values by computing the mean bias and mean squared error. The computation time for a total of 72,000 simulated datasets representing 36 different cross-classifications of tumor lethality, background death rates, and cumulative tumor survival rates was approximately 8 times faster when the constrained MLE's were computed analytically for the cases with active constraints as opposed to using the gradient search algorithm.

Table 6.2.1
Number of Simulations Within Each Constraint Case
Based on Low Background Death Rates

tumor survival ¹	tumor shape ²	tumor lethality ³	Constraint Case			
			No Constraints ($\hat{\lambda}_2 > 0, \hat{\lambda}_3 > 0$)	Case II ($\hat{\lambda}_2 > 0, \hat{\lambda}_3 = 0$)	Case III ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 > 0$)	Case IV ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 = 0$)
0.8	CV	I	2656	2812	2597	1935
		M	2967	2691	2715	1627
		F	10000	0	0	0
	LN	I	3885	2623	2540	952
		M	4531	2301	2551	617
		F	10000	0	0	0
	CX	I	6054	1550	2162	234
		M	6775	1140	1949	136
		F	10000	0	0	0
0.5	CV	I	2784	2613	3106	1497
		M	3010	2572	3138	1280
		F	10000	0	0	0
	LN	I	5249	2160	2335	256
		M	5766	1809	2286	139
		F	10000	0	0	0
	CX	I	7736	19	2245	0
		M	7990	5	2005	0
		F	10000	0	0	0
0.2	CV	I	4615	2065	2927	393
		M	4832	1934	2917	317
		F	10000	0	0	0
	LN	I	7226	1085	1669	20
		M	7620	832	1535	13
		F	10000	0	0	0
	CX	I	8470	108	1422	0
		M	8763	53	1184	0
		F	10000	0	0	0

1 - probability of remaining tumor free through end of study

2 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

3 - I=incidental, M=intermediate, F=fatal

Table 6.2.2
Number of Simulations Within Each Constraint Case
Based on High Background Death Rates

tumor survival ¹	tumor shape ²	tumor lethality ³	Constraint Case			
			No Constraints ($\hat{\lambda}_2 > 0, \hat{\lambda}_3 > 0$)	Case II ($\hat{\lambda}_2 > 0, \hat{\lambda}_3 = 0$)	Case III ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 > 0$)	Case IV ($\hat{\lambda}_2 = 0, \hat{\lambda}_3 = 0$)
0.8	CV	I	2756	2783	2425	2036
		M	2977	2653	2632	1738
		F	10000	0	0	0
	LN	I	3896	2684	2379	1041
		M	4469	2363	2496	672
		F	10000	0	0	0
	CX	I	6008	1610	2118	264
		M	6737	1132	1961	170
		F	10000	0	0	0
0.5	CV	I	2758	2657	2922	1663
		M	3011	2543	3003	1443
		F	10000	0	0	0
	LN	I	5140	2201	2343	316
		M	5782	1803	2236	179
		F	10000	0	0	0
	CX	I	7709	22	2269	0
		M	7983	8	2009	0
		F	10000	0	0	0
0.2	CV	I	4532	2072	2919	477
		M	4890	1917	2794	399
		F	10000	0	0	0
	LN	I	7121	1138	1712	29
		M	7621	884	1481	14
		F	10000	0	0	0
	CX	I	8421	109	1470	0
		M	8820	52	1128	0
		F	10000	0	0	0

1 - probability of remaining tumor free through end of study

2 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

3 - I=incidental, M=intermediate, F=fatal

Table 6.2.3
Mean Values for Estimates of Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.600	0.600	0.567	0.578	0.578	0.593	0.605	0.578
		18	0.250	0.248	0.346	0.306	0.306	0.369	0.213	0.306
		24	0.333	0.349	0.329	0.348	0.348	0.210	0.379	0.348
	M	12	0.600	0.600	0.568	0.580	0.589	0.594	0.617	0.589
		18	0.250	0.250	0.338	0.298	0.304	0.362	0.217	0.304
		24	0.333	0.343	0.332	0.349	0.322	0.216	0.343	0.322
	F	12	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
		18	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
		24	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333
LN	I	12	0.400	0.399	0.382	0.389	0.389	0.398	0.412	0.389
		18	0.333	0.336	0.371	0.355	0.355	0.409	0.299	0.355
		24	0.500	0.496	0.488	0.495	0.495	0.407	0.515	0.495
	M	12	0.400	0.400	0.385	0.391	0.397	0.400	0.416	0.397
		18	0.333	0.330	0.359	0.345	0.348	0.393	0.308	0.348
		24	0.500	0.500	0.492	0.501	0.489	0.423	0.500	0.489
	F	12	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
		18	0.333	0.332	0.332	0.332	0.332	0.332	0.332	0.332
		24	0.500	0.499	0.499	0.499	0.499	0.499	0.499	0.499
CX	I	12	0.200	0.199	0.189	0.191	0.192	0.199	0.195	0.192
		18	0.250	0.248	0.271	0.266	0.265	0.330	0.260	0.265
		24	0.667	0.666	0.660	0.662	0.662	0.596	0.663	0.662
	M	12	0.200	0.201	0.192	0.194	0.199	0.201	0.200	0.199
		18	0.250	0.250	0.267	0.261	0.262	0.314	0.260	0.262
		24	0.667	0.666	0.662	0.664	0.660	0.611	0.660	0.660
	F	12	0.200	0.199	0.199	0.199	0.199	0.199	0.199	0.199
		18	0.250	0.251	0.251	0.251	0.251	0.251	0.251	0.251
		24	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.4
Mean Values for Estimates of Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.400	0.402	0.355	0.360	0.361	0.375	0.391	0.361
		18	0.083	0.072	0.138	0.123	0.124	0.150	0.057	0.124
		24	0.091	0.094	0.119	0.127	0.125	0.056	0.139	0.125
	M	12	0.400	0.402	0.359	0.365	0.375	0.380	0.406	0.375
		18	0.083	0.082	0.137	0.121	0.125	0.145	0.062	0.125
		24	0.091	0.086	0.112	0.122	0.097	0.057	0.104	0.097
	F	12	0.400	0.401	0.401	0.401	0.401	0.401	0.401	0.401
		18	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
		24	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091
LN	I	12	0.250	0.250	0.228	0.232	0.233	0.245	0.266	0.233
		18	0.167	0.167	0.192	0.182	0.183	0.217	0.119	0.183
		24	0.200	0.198	0.207	0.212	0.211	0.142	0.230	0.211
	M	12	0.250	0.251	0.231	0.235	0.243	0.248	0.273	0.243
		18	0.167	0.166	0.187	0.178	0.178	0.210	0.127	0.178
		24	0.200	0.197	0.205	0.210	0.196	0.145	0.206	0.196
	F	12	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
		18	0.167	0.165	0.165	0.165	0.165	0.165	0.165	0.165
		24	0.200	0.201	0.201	0.201	0.201	0.201	0.201	0.201
CX	I	12	0.050	0.050	0.037	0.039	0.039	0.050	0.039	0.039
		18	0.051	0.049	0.074	0.072	0.072	0.137	0.071	0.072
		24	0.446	0.446	0.439	0.439	0.439	0.366	0.440	0.439
	M	12	0.050	0.051	0.039	0.041	0.044	0.051	0.044	0.044
		18	0.051	0.048	0.069	0.066	0.066	0.124	0.066	0.066
		24	0.446	0.446	0.441	0.442	0.439	0.377	0.439	0.439
	F	12	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
		18	0.051	0.049	0.049	0.049	0.049	0.049	0.049	0.049
		24	0.446	0.445	0.445	0.445	0.445	0.445	0.445	0.445

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.5
Mean Values for Estimates of Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.150	0.149	0.113	0.115	0.115	0.124	0.138	0.115
		18	0.035	0.036	0.061	0.058	0.058	0.067	0.022	0.058
		24	0.024	0.024	0.054	0.055	0.055	0.028	0.062	0.055
	M	12	0.150	0.151	0.119	0.121	0.127	0.130	0.147	0.127
		18	0.035	0.035	0.058	0.053	0.055	0.062	0.025	0.055
		24	0.024	0.023	0.049	0.050	0.040	0.026	0.044	0.040
	F	12	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
		18	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
		24	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024
LN	I	12	0.100	0.101	0.078	0.080	0.080	0.089	0.103	0.080
		18	0.056	0.057	0.070	0.067	0.067	0.079	0.032	0.067
		24	0.060	0.057	0.076	0.077	0.076	0.048	0.084	0.076
	M	12	0.100	0.100	0.080	0.082	0.087	0.092	0.108	0.087
		18	0.056	0.056	0.068	0.065	0.066	0.076	0.036	0.066
		24	0.060	0.060	0.075	0.076	0.068	0.048	0.072	0.068
	F	12	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
		18	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056
		24	0.060	0.059	0.059	0.059	0.059	0.059	0.059	0.059
CX	I	12	0.050	0.050	0.037	0.039	0.039	0.047	0.053	0.039
		18	0.051	0.049	0.063	0.061	0.061	0.073	0.039	0.061
		24	0.113	0.114	0.116	0.115	0.115	0.089	0.122	0.115
	M	12	0.050	0.051	0.039	0.040	0.043	0.048	0.054	0.043
		18	0.051	0.048	0.061	0.059	0.060	0.070	0.044	0.060
		24	0.113	0.114	0.115	0.116	0.112	0.092	0.115	0.112
	F	12	0.050	0.049	0.049	0.049	0.049	0.049	0.049	0.049
		18	0.051	0.050	0.050	0.050	0.050	0.050	0.050	0.050
		24	0.113	0.114	0.114	0.114	0.114	0.114	0.114	0.114

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.6
Mean Values for Estimates of Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.600	0.601	0.572	0.578	0.579	0.593	0.605	0.579
		18	0.250	0.246	0.329	0.308	0.309	0.371	0.218	0.309
		24	0.333	0.346	0.341	0.353	0.351	0.207	0.382	0.351
	M	12	0.600	0.600	0.575	0.580	0.590	0.594	0.616	0.590
		18	0.250	0.250	0.315	0.298	0.302	0.357	0.221	0.302
		24	0.333	0.340	0.344	0.353	0.322	0.218	0.341	0.322
	F	12	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
		18	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
		24	0.333	0.333	0.333	0.333	0.333	0.333	0.333	0.333
LN	I	12	0.400	0.399	0.387	0.389	0.390	0.399	0.413	0.390
		18	0.333	0.338	0.361	0.355	0.356	0.408	0.298	0.356
		24	0.500	0.490	0.492	0.495	0.494	0.402	0.517	0.494
	M	12	0.400	0.400	0.389	0.391	0.397	0.400	0.417	0.397
		18	0.333	0.330	0.350	0.345	0.348	0.388	0.305	0.348
		24	0.500	0.498	0.496	0.499	0.489	0.425	0.501	0.489
	F	12	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
		18	0.333	0.332	0.332	0.332	0.332	0.332	0.332	0.332
		24	0.500	0.499	0.499	0.499	0.499	0.499	0.499	0.499
CX	I	12	0.200	0.200	0.191	0.191	0.192	0.200	0.195	0.192
		18	0.250	0.248	0.266	0.266	0.266	0.329	0.260	0.266
		24	0.667	0.666	0.662	0.662	0.661	0.590	0.663	0.661
	M	12	0.200	0.200	0.193	0.194	0.198	0.200	0.200	0.198
		18	0.250	0.250	0.263	0.261	0.262	0.309	0.259	0.262
		24	0.667	0.665	0.662	0.663	0.659	0.611	0.659	0.659
	F	12	0.200	0.199	0.199	0.199	0.199	0.199	0.199	0.199
		18	0.250	0.251	0.251	0.251	0.251	0.251	0.251	0.251
		24	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.7
Mean Values for Estimates of Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.400	0.401	0.358	0.359	0.360	0.374	0.390	0.360
		18	0.083	0.073	0.129	0.125	0.125	0.150	0.059	0.125
		24	0.091	0.088	0.125	0.130	0.127	0.060	0.143	0.127
	M	12	0.400	0.402	0.363	0.365	0.376	0.380	0.406	0.376
		18	0.083	0.080	0.126	0.121	0.123	0.144	0.061	0.123
		24	0.091	0.084	0.120	0.124	0.097	0.058	0.106	0.097
	F	12	0.400	0.401	0.401	0.401	0.401	0.401	0.401	0.401
		18	0.083	0.082	0.082	0.082	0.082	0.082	0.082	0.082
		24	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.091
LN	I	12	0.250	0.250	0.231	0.232	0.233	0.245	0.267	0.233
		18	0.167	0.165	0.183	0.181	0.181	0.216	0.116	0.181
		24	0.200	0.195	0.210	0.213	0.210	0.140	0.230	0.210
	M	12	0.250	0.251	0.235	0.236	0.244	0.247	0.273	0.244
		18	0.167	0.166	0.180	0.177	0.179	0.210	0.128	0.179
		24	0.200	0.195	0.209	0.210	0.195	0.145	0.205	0.195
	F	12	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
		18	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
		24	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
CX	I	12	0.050	0.050	0.038	0.039	0.039	0.050	0.040	0.039
		18	0.051	0.049	0.073	0.072	0.073	0.136	0.071	0.073
		24	0.446	0.446	0.439	0.439	0.439	0.363	0.439	0.439
	M	12	0.050	0.051	0.041	0.041	0.045	0.051	0.045	0.045
		18	0.051	0.050	0.067	0.067	0.066	0.123	0.066	0.066
		24	0.446	0.447	0.444	0.444	0.439	0.376	0.439	0.439
	F	12	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
		18	0.051	0.049	0.049	0.049	0.049	0.049	0.049	0.049
		24	0.446	0.445	0.445	0.445	0.445	0.445	0.445	0.445

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.8
Mean Values for Estimates of Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.150	0.149	0.114	0.114	0.115	0.123	0.138	0.115
		18	0.035	0.038	0.059	0.059	0.059	0.067	0.023	0.059
		24	0.024	0.022	0.056	0.056	0.055	0.029	0.062	0.055
	M	12	0.150	0.151	0.121	0.121	0.128	0.130	0.147	0.128
		18	0.035	0.035	0.055	0.055	0.054	0.062	0.026	0.054
		24	0.024	0.022	0.052	0.051	0.040	0.026	0.043	0.040
	F	12	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
		18	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035
		24	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024
LN	I	12	0.100	0.101	0.079	0.079	0.080	0.088	0.103	0.080
		18	0.056	0.056	0.068	0.067	0.067	0.079	0.031	0.067
		24	0.060	0.057	0.077	0.078	0.077	0.048	0.085	0.077
	M	12	0.100	0.100	0.082	0.082	0.088	0.091	0.108	0.088
		18	0.056	0.056	0.066	0.065	0.066	0.076	0.037	0.066
		24	0.060	0.060	0.077	0.077	0.067	0.049	0.071	0.067
	F	12	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
		18	0.056	0.056	0.056	0.056	0.056	0.056	0.056	0.056
		24	0.060	0.059	0.059	0.059	0.059	0.059	0.059	0.059
CX	I	12	0.050	0.050	0.038	0.038	0.039	0.046	0.053	0.039
		18	0.051	0.049	0.062	0.061	0.061	0.074	0.039	0.061
		24	0.113	0.114	0.116	0.117	0.116	0.089	0.122	0.116
	M	12	0.050	0.051	0.040	0.040	0.044	0.049	0.054	0.044
		18	0.051	0.047	0.059	0.059	0.059	0.068	0.044	0.059
		24	0.113	0.115	0.117	0.117	0.112	0.091	0.115	0.112
	F	12	0.050	0.049	0.049	0.049	0.049	0.049	0.049	0.049
		18	0.051	0.050	0.050	0.050	0.050	0.050	0.050	0.050
		24	0.113	0.114	0.114	0.114	0.114	0.114	0.114	0.114

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to intuitive pooling estimates of the tumor incidence rate

Table 6.2.9
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.022	0.018	0.017	0.017	0.019	0.017	0.017
		18	0.021	0.012	0.012	0.012	0.011	0.011	0.012
		24	0.005	0.005	0.004	0.004	0.005	0.005	0.004
	M	12	0.020	0.017	0.016	0.016	0.017	0.017	0.016
		18	0.018	0.011	0.011	0.010	0.010	0.009	0.010
		24	0.005	0.004	0.004	0.004	0.004	0.004	0.004
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.022	0.019	0.019	0.019	0.021	0.022	0.019
		18	0.023	0.017	0.017	0.017	0.017	0.015	0.017
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
	M	12	0.018	0.015	0.016	0.017	0.018	0.020	0.017
		18	0.019	0.015	0.015	0.014	0.015	0.012	0.014
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.015	0.013	0.013	0.013	0.015	0.014	0.013
		18	0.024	0.021	0.021	0.021	0.029	0.020	0.021
		24	0.004	0.004	0.004	0.004	0.005	0.004	0.004
	M	12	0.012	0.010	0.010	0.011	0.012	0.011	0.011
		18	0.018	0.016	0.017	0.016	0.024	0.015	0.016
		24	0.003	0.003	0.003	0.003	0.005	0.003	0.003
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Table 6.2.10
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.022	0.015	0.014	0.014	0.014	0.012	0.014
		18	0.024	0.008	0.009	0.009	0.006	0.008	0.009
		24	0.006	0.005	0.005	0.005	0.004	0.005	0.005
	M	12	0.019	0.013	0.012	0.012	0.012	0.011	0.012
		18	0.017	0.007	0.007	0.006	0.006	0.006	0.006
		24	0.005	0.004	0.004	0.004	0.004	0.004	0.004
	F	12	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
LN	I	12	0.018	0.013	0.013	0.013	0.015	0.016	0.013
		18	0.023	0.012	0.012	0.012	0.011	0.010	0.012
		24	0.006	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.014	0.011	0.011	0.011	0.013	0.014	0.011
		18	0.017	0.010	0.011	0.009	0.010	0.008	0.009
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
CX	I	12	0.004	0.003	0.003	0.003	0.004	0.003	0.003
		18	0.008	0.007	0.007	0.007	0.029	0.007	0.007
		24	0.005	0.005	0.005	0.005	0.007	0.005	0.005
	M	12	0.004	0.002	0.002	0.002	0.004	0.002	0.002
		18	0.005	0.005	0.005	0.005	0.025	0.005	0.005
		24	0.005	0.005	0.005	0.005	0.007	0.005	0.005
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months

Table 6.2.11
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.012	0.007	0.007	0.007	0.007	0.005	0.007
		18	0.014	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.009	0.005	0.005	0.005	0.005	0.004	0.005
		18	0.009	0.004	0.004	0.003	0.003	0.003	0.003
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.008	0.005	0.005	0.005	0.005	0.005	0.005
		18	0.012	0.005	0.005	0.005	0.004	0.004	0.005
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.006	0.004	0.004	0.004	0.004	0.005	0.004
		18	0.008	0.004	0.004	0.004	0.004	0.003	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.004	0.003	0.003	0.003	0.004	0.004	0.003
		18	0.008	0.005	0.005	0.005	0.006	0.004	0.005
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.003	0.002	0.002	0.002	0.003	0.003	0.002
		18	0.006	0.004	0.004	0.004	0.005	0.003	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Table 6.2.12
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.021	0.017	0.017	0.017	0.018	0.016	0.017
		18	0.021	0.012	0.012	0.012	0.011	0.011	0.012
		24	0.006	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.019	0.016	0.015	0.016	0.017	0.016	0.016
		18	0.018	0.011	0.011	0.010	0.010	0.009	0.010
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.022	0.019	0.019	0.019	0.021	0.021	0.019
		18	0.023	0.017	0.017	0.017	0.017	0.015	0.017
		24	0.005	0.004	0.004	0.004	0.005	0.004	0.004
	M	12	0.018	0.015	0.015	0.017	0.018	0.020	0.017
		18	0.018	0.015	0.015	0.014	0.015	0.012	0.014
		24	0.004	0.004	0.004	0.004	0.005	0.004	0.004
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.015	0.013	0.013	0.013	0.015	0.014	0.013
		18	0.023	0.020	0.020	0.020	0.028	0.019	0.020
		24	0.004	0.004	0.004	0.004	0.006	0.004	0.004
	M	12	0.012	0.010	0.010	0.011	0.012	0.011	0.011
		18	0.017	0.016	0.016	0.015	0.023	0.015	0.015
		24	0.004	0.004	0.004	0.004	0.005	0.004	0.004
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

Table 6.2.13
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.022	0.014	0.014	0.014	0.014	0.012	0.014
		18	0.024	0.009	0.009	0.008	0.006	0.008	0.008
		24	0.007	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.018	0.012	0.012	0.012	0.012	0.010	0.012
		18	0.017	0.007	0.007	0.006	0.006	0.005	0.006
		24	0.006	0.005	0.005	0.004	0.004	0.005	0.004
	F	12	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
LN	I	12	0.018	0.013	0.013	0.013	0.015	0.015	0.013
		18	0.023	0.012	0.012	0.012	0.011	0.010	0.012
		24	0.006	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.014	0.011	0.011	0.011	0.012	0.014	0.011
		18	0.016	0.010	0.010	0.009	0.009	0.007	0.009
		24	0.006	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
CX	I	12	0.005	0.003	0.003	0.003	0.005	0.003	0.003
		18	0.008	0.007	0.007	0.007	0.028	0.007	0.007
		24	0.005	0.005	0.005	0.005	0.007	0.005	0.005
	M	12	0.004	0.002	0.002	0.002	0.004	0.002	0.002
		18	0.006	0.005	0.005	0.005	0.024	0.005	0.005
		24	0.005	0.005	0.005	0.005	0.007	0.005	0.005
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

Table 6.2.14
Mean Squared Error for Estimates of the Tumor Incidence Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.012	0.007	0.007	0.007	0.007	0.005	0.007
		18	0.014	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.009	0.005	0.005	0.005	0.005	0.004	0.005
		18	0.009	0.004	0.004	0.003	0.003	0.003	0.003
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.009	0.005	0.005	0.005	0.005	0.005	0.005
		18	0.012	0.005	0.005	0.005	0.004	0.004	0.005
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.006	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.008	0.004	0.004	0.004	0.004	0.003	0.004
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.004	0.003	0.003	0.003	0.004	0.004	0.003
		18	0.009	0.005	0.005	0.005	0.006	0.004	0.005
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.004	0.002	0.002	0.002	0.003	0.003	0.002
		18	0.005	0.004	0.004	0.004	0.005	0.003	0.004
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- ¹ - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
² - I=incidental, M=intermediate, F=fatal
³ - sacrifice time in months

6.3 Evaluation of Estimators for More than Three Intervals

The second Monte Carlo simulation study was conducted as an extension of the three-interval discrete model evaluation discussed in the previous section to a study design with five time intervals. The aim of this simulation study was the evaluation of pooling methods for study designs in which analytic solutions for the constrained MLE's are not available, with emphasis again on the estimation of tumor incidence rates. For the three-interval study design, estimates of tumor incidence derived from the intuitive pooling algorithm were seen to compare very favorably with those of the constrained maximum likelihood estimators with respect to both the levels of bias and MSE.

The pooling algorithms involve the combination of data outcomes from one or more adjacent intervals when the observed data yields negative unconstrained estimates of tumor incidence. In this sense, there is some loss of information associated with the pooling algorithms. This is especially true in cases for which the data from a three interval study must be collapsed to two intervals, or even a single interval. In study designs consisting of a larger number of intervals, however, the data outcomes pooled from two adjacent intervals represent a smaller proportion of the total observed data than for a design with fewer intervals. Thus, it is anticipated that the intuitive pooling algorithm would compare at least as favorably to the constrained MLE's for a five-interval design as observed for a three-interval design.

The factors considered in the five-interval discrete model simulation study were identical to those described for the three-interval study, and the same method was utilized for generating the intervals during which death with and without tumor and sacrifice with and without tumor occur. Each five-interval study was assumed to include a total of 90 animals with interim sacrifices conducted at 12, 15, 18, and 21 months and a terminal sacrifice at 24 months. The percentages of live animals sacrificed at each of the interim

sacrifices were 11%, 14%, 17%, and 25% respectively; these percentages were selected in an attempt to obtain a total of 10 sacrificed animals for each interim sacrifice, as described for the three-interval study. In addition to the unconstrained MLE's, numerically constrained MLE's and intuitive pooling estimates, estimates of the tumor incidence rates and death rates were obtained by the Mean Squared Pooled Difference method described in Section 4.3 of Chapter IV. The mean estimated parameter values and MSE associated with all four of these methods of estimation were found to be unaffected by the level of background death rates just as they were for the three-interval study design discussed in Section 6.2.2; thus the results are reported only for the low background death rates.

The mean values for the tumor incidence rate as estimated by the unconstrained and numerically constrained MLE's and the intuitive and MSPD pooling methods are provided in Tables 6.3.1, 6.3.2 and 6.3.3 for cumulative tumor survival rates of 20%, 50%, and 80% respectively, along with the true values for each cross-classification of tumor survival curve shape and tumor lethality. Also included in these tables are the Williams method estimates, which coincide with the intuitive pooling estimates in the case of the tumor incidence rates. Recall that the datasets simulated under the conditions of fatal tumors for the three interval study were discovered to have non-negative tumor incidence rates under all other simulation conditions, so that estimates derived from the constrained MLE's or from pooling algorithms were always equivalent to the unconstrained estimates. This same pattern also holds for the five-interval simulated datasets, as can be verified from Tables 6.3.1, 6.3.2, and 6.3.3. For incidental and intermediate levels of tumor lethality, the unconstrained estimates seem to have the smallest mean biases among the methods of estimation. However, it will be emphasized once again that the unconstrained MLE's are not considered valid estimators of tumor incidence since they do not prevent negative estimates.

The mean values of the constrained MLE's of tumor incidence and the estimates derived from the two pooling methods are very similar, but some general patterns can be described. The mean estimates obtained from the MSPD pooling method consistently come closest to the true values for the first time interval, while the numerically constrained MLE's have the smallest mean bias for the second time interval. There appears to be no consistent pattern for the last three intervals which holds over all cross-classifications of simulation condition levels, although the intuitive pooling estimates are most often closest to the true values in the last interval. In general, all four methods of estimating tumor incidence rates produce mean values which are overestimates of the true tumor incidence rates during the last three intervals for datasets simulated under the incidental or intermediate levels of lethality.

The mean values for the estimates of the death rates for the five-interval simulation study are provided in Appendix E. With the exception of the datasets simulated with a cumulative tumor survival equal to 20%, the mean values for the unconstrained estimates of the death rate without tumor show very little bias. Examination of Table E.1, which displays the mean values for the estimates of the death rate without tumor based on a cumulative tumor survival rate of 20%, reveals that the mean estimates for all four methods are consistently larger than the true values under the incidental or intermediate levels of lethality. The mean values for the numerically constrained estimates of tumor incidence are consistently closest to the true values for datasets simulated with a cumulative tumor survival equal to 20%. The intuitive pooling estimates of the death rate without tumor for the last interval are not quite as close to the numerically constrained MLE's as they were for the three interval case, and in some cases they exhibit greater mean biases than the unconstrained estimates. Thus, the Williams method choice of using the intuitive pooling estimate of the death rate without tumor for the last interval, which appeared to be justified

for a three-interval study design, cannot be justified by the results of the five-interval simulation study.

The mean values for the estimates of the overall death rates, which are also given in Appendix E, suggest that the unconstrained MLE again estimates the overall death rate with essentially zero bias. The constrained MLE's yield mean estimates of the overall death rate almost identical to those of the unconstrained method, while the pooling methods generally produce overestimates of the overall death rate. These findings provide additional support for the use of the simple Kaplan-Meier estimator for the overall death rate even in situations in which constraints on the tumor incidence rate are necessary.

The values of the mean square error (MSE) for the estimates of the tumor incidence rates in the five-interval simulation study are displayed in Tables 6.3.4, 6.3.5, and 6.3.6 for cumulative rates of tumor survival equal to 20%, 50% and 80% respectively. The patterns observed for the five-interval study are very similar to that of the three-interval simulation study results in that the constrained MLE's and estimates derived from pooled data exhibit smaller MSE values than do the unconstrained estimates of the tumor incidence rate. In addition, the MSE values for the datasets simulated under the fatal tumor conditions are consistently smaller than those simulated under the incidental or intermediate levels of tumor lethality. The MSE levels also decrease in size as the cumulative tumor survival rate increases from 20% to 50% to 80%, as was observed for the three-interval study. The MSE values for the intuitive pooling estimates of tumor incidence are almost identical to those of the numerically constrained MLE's; the maximum difference in MSE values between these two methods is 0.002. In addition, the MSE values associated with the estimates obtained by the MSPD pooling algorithm are essentially equivalent to those of the intuitive pooling estimates and constrained MLE's.

The mean squared errors were also calculated for the death rate without tumor and the overall death rate for the five-interval simulation study, and can be found in Appendix F. For both the death rate without tumor and the overall death rate, the MSE values tend to increase in size from the first interval to the last, regardless of the shape of the tumor survival curve or the tumor lethality. In general, the MSE values for the estimates of the death rate without tumor are largest for incidental tumors and smallest for fatal tumors. The opposite pattern is observed for the numerically constrained maximum likelihood estimates of the overall death rates, for which the datasets simulated under the fatal tumor lethality condition exhibit the largest MSE values and the datasets simulated under the incidental tumor conditions exhibit the smallest MSE values. With the exception of the datasets simulated under the fatal tumor condition (for which all MSE values are equivalent to those of the unconstrained estimates), the MSE values for the intuitive pooling estimates of the death rate without tumor are only slightly larger than those of the constrained MLE's. The difference in the MSE values between intuitive pooling estimates and constrained MLE's of the overall death rate seems to be greater, and is most pronounced in datasets simulated under conditions of intermediate tumor lethality for both concave and linear tumor survival curves.

To summarize the results of the second simulation study, the MSPD and intuitive pooling estimators appear to provide reliable estimates of the tumor incidence rate in a five-interval study design under the simulation conditions considered. Although they demonstrate slightly greater bias than the numerically constrained maximum likelihood estimators, the mean squared errors for the both types of pooling estimates are essentially equivalent to those of the numerically constrained MLE's, and are consistently smaller than the MSE values for the unconstrained estimates of tumor incidence.

Table 6.3.1
Mean Values for Estimates of Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					
			true value	uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.600	0.600	0.572	0.575	0.587	0.575
		15	0.250	0.250	0.278	0.278	0.286	0.278
		18	0.200	0.213	0.220	0.235	0.200	0.235
		21	0.100	0.153	0.170	0.200	0.178	0.200
		24	0.074	0.120	0.185	0.186	0.196	0.186
	M	12	0.600	0.600	0.574	0.588	0.595	0.588
		15	0.250	0.255	0.270	0.291	0.291	0.291
		18	0.200	0.201	0.206	0.209	0.188	0.209
		21	0.100	0.126	0.158	0.139	0.124	0.139
		24	0.074	0.091	0.178	0.116	0.127	0.116
	F	12	0.600	0.600	0.600	0.600	0.600	0.600
		15	0.250	0.250	0.250	0.250	0.250	0.250
		18	0.200	0.200	0.200	0.200	0.200	0.200
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.074	0.074	0.074	0.074	0.074	0.074
LN	I	12	0.400	0.403	0.374	0.376	0.388	0.376
		15	0.167	0.161	0.195	0.196	0.212	0.196
		18	0.200	0.196	0.206	0.209	0.176	0.209
		21	0.250	0.236	0.253	0.262	0.242	0.262
		24	0.333	0.338	0.355	0.348	0.355	0.348
	M	12	0.400	0.401	0.376	0.390	0.395	0.390
		15	0.167	0.165	0.188	0.207	0.217	0.207
		18	0.200	0.196	0.205	0.200	0.184	0.200
		21	0.250	0.236	0.253	0.230	0.209	0.230
		24	0.333	0.329	0.352	0.315	0.330	0.315
	F	12	0.400	0.400	0.400	0.400	0.400	0.400
		15	0.167	0.167	0.167	0.167	0.167	0.167
		18	0.200	0.202	0.202	0.202	0.202	0.202
		21	0.250	0.248	0.248	0.248	0.248	0.248
		24	0.333	0.333	0.333	0.333	0.333	0.333
CX	I	12	0.200	0.200	0.171	0.172	0.188	0.172
		15	0.062	0.065	0.104	0.105	0.121	0.105
		18	0.168	0.163	0.157	0.158	0.119	0.158
		21	0.199	0.193	0.222	0.220	0.223	0.220
		24	0.600	0.594	0.583	0.583	0.579	0.583
	M	12	0.200	0.199	0.173	0.185	0.194	0.185
		15	0.062	0.066	0.097	0.106	0.113	0.106
		18	0.168	0.164	0.159	0.154	0.130	0.154
		21	0.199	0.198	0.218	0.204	0.206	0.204
		24	0.600	0.597	0.593	0.582	0.583	0.582
	F	12	0.200	0.200	0.200	0.200	0.200	0.200
		15	0.062	0.064	0.064	0.064	0.064	0.064
		18	0.168	0.168	0.168	0.168	0.168	0.168
		21	0.199	0.199	0.199	0.199	0.199	0.199
		24	0.600	0.599	0.599	0.599	0.599	0.599

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months

Table 6.3.2
Mean Values for Estimates of Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.400	0.400	0.343	0.346	0.356	0.346
		15	0.048	0.047	0.097	0.098	0.107	0.098
		18	0.046	0.040	0.064	0.064	0.043	0.064
		21	0.042	0.033	0.065	0.062	0.047	0.062
		24	0.042	0.030	0.089	0.083	0.082	0.083
	M	12	0.400	0.403	0.350	0.369	0.373	0.369
		15	0.048	0.042	0.089	0.101	0.104	0.101
		18	0.046	0.042	0.064	0.053	0.044	0.053
		21	0.042	0.042	0.067	0.037	0.032	0.037
		24	0.042	0.036	0.081	0.041	0.044	0.041
	F	12	0.400	0.400	0.400	0.400	0.400	0.400
		15	0.048	0.048	0.048	0.048	0.048	0.048
		18	0.046	0.044	0.044	0.044	0.044	0.044
		21	0.042	0.044	0.044	0.044	0.044	0.044
		24	0.042	0.042	0.042	0.042	0.042	0.042
LN	I	12	0.250	0.248	0.215	0.217	0.228	0.217
		15	0.083	0.084	0.108	0.110	0.124	0.110
		18	0.093	0.091	0.100	0.100	0.077	0.100
		21	0.098	0.097	0.110	0.107	0.087	0.107
		24	0.112	0.096	0.135	0.130	0.135	0.130
	M	12	0.250	0.250	0.220	0.235	0.240	0.235
		15	0.083	0.081	0.103	0.115	0.121	0.115
		18	0.093	0.094	0.100	0.093	0.081	0.093
		21	0.098	0.096	0.110	0.086	0.077	0.086
		24	0.112	0.106	0.134	0.103	0.111	0.103
	F	12	0.250	0.250	0.250	0.250	0.250	0.250
		15	0.083	0.081	0.081	0.081	0.081	0.081
		18	0.093	0.093	0.093	0.093	0.093	0.093
		21	0.098	0.099	0.099	0.099	0.099	0.099
		24	0.112	0.112	0.112	0.112	0.112	0.112
CX	I	12	0.050	0.050	0.039	0.039	0.045	0.039
		15	0.075	0.074	0.077	0.079	0.089	0.079
		18	0.100	0.099	0.109	0.110	0.101	0.110
		21	0.200	0.198	0.191	0.189	0.173	0.189
		24	0.210	0.204	0.225	0.222	0.229	0.222
	M	12	0.050	0.049	0.041	0.045	0.048	0.045
		15	0.075	0.077	0.077	0.084	0.087	0.084
		18	0.100	0.100	0.107	0.107	0.105	0.107
		21	0.200	0.199	0.195	0.186	0.176	0.186
		24	0.210	0.202	0.219	0.209	0.215	0.209
	F	12	0.050	0.050	0.050	0.050	0.050	0.050
		15	0.075	0.075	0.075	0.075	0.075	0.075
		18	0.100	0.099	0.099	0.099	0.099	0.099
		21	0.200	0.201	0.201	0.201	0.201	0.201
		24	0.210	0.212	0.212	0.212	0.212	0.212

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Table 6.3.3
Mean Values for Estimates of Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.150	0.151	0.106	0.108	0.114	0.108
		15	0.024	0.021	0.045	0.046	0.050	0.046
		18	0.016	0.016	0.030	0.029	0.021	0.029
		21	0.012	0.012	0.030	0.028	0.021	0.028
		24	0.010	0.005	0.040	0.036	0.037	0.036
	M	12	0.150	0.150	0.112	0.122	0.125	0.122
		15	0.024	0.025	0.043	0.048	0.048	0.048
		18	0.016	0.013	0.029	0.024	0.020	0.024
		21	0.012	0.011	0.025	0.015	0.012	0.015
		24	0.010	0.009	0.032	0.016	0.019	0.016
	F	12	0.150	0.150	0.150	0.150	0.150	0.150
		15	0.024	0.024	0.024	0.024	0.024	0.024
		18	0.016	0.016	0.016	0.016	0.016	0.016
		21	0.012	0.012	0.012	0.012	0.012	0.012
		24	0.010	0.010	0.010	0.010	0.010	0.010
LN	I	12	0.100	0.100	0.068	0.069	0.076	0.069
		15	0.028	0.026	0.044	0.045	0.051	0.045
		18	0.030	0.029	0.036	0.036	0.026	0.036
		21	0.028	0.029	0.040	0.039	0.029	0.039
		24	0.030	0.029	0.055	0.050	0.052	0.050
	M	12	0.100	0.099	0.073	0.081	0.085	0.081
		15	0.028	0.029	0.042	0.048	0.048	0.048
		18	0.030	0.027	0.035	0.032	0.029	0.032
		21	0.028	0.028	0.039	0.027	0.022	0.027
		24	0.030	0.029	0.047	0.033	0.035	0.033
	F	12	0.100	0.100	0.100	0.100	0.100	0.100
		15	0.028	0.029	0.029	0.029	0.029	0.029
		18	0.030	0.029	0.029	0.029	0.029	0.029
		21	0.028	0.029	0.029	0.029	0.029	0.029
		24	0.030	0.029	0.029	0.029	0.029	0.029
CX	I	12	0.050	0.050	0.030	0.030	0.035	0.030
		15	0.021	0.021	0.032	0.033	0.038	0.033
		18	0.032	0.031	0.036	0.037	0.030	0.037
		21	0.037	0.036	0.046	0.045	0.039	0.045
		24	0.077	0.076	0.083	0.080	0.080	0.080
	M	12	0.050	0.051	0.034	0.039	0.041	0.039
		15	0.021	0.021	0.031	0.034	0.038	0.034
		18	0.032	0.031	0.033	0.033	0.029	0.033
		21	0.037	0.034	0.045	0.038	0.036	0.038
		24	0.077	0.077	0.082	0.072	0.072	0.072
	F	12	0.050	0.050	0.050	0.050	0.050	0.050
		15	0.021	0.021	0.021	0.021	0.021	0.021
		18	0.032	0.032	0.032	0.032	0.032	0.032
		21	0.037	0.037	0.037	0.037	0.037	0.037
		24	0.077	0.076	0.076	0.076	0.076	0.076

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Table 6.3.4
Mean Squared Error for Estimates of the Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			unconstrained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.023	0.018	0.018	0.018	0.018
		15	0.019	0.011	0.011	0.010	0.011
		18	0.018	0.009	0.009	0.009	0.009
		21	0.018	0.008	0.010	0.010	0.010
		24	0.013	0.008	0.010	0.010	0.010
	M	12	0.020	0.015	0.017	0.017	0.017
		15	0.015	0.009	0.008	0.008	0.008
		18	0.013	0.007	0.007	0.007	0.007
		21	0.013	0.006	0.007	0.007	0.007
		24	0.010	0.006	0.007	0.007	0.007
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.003	0.003	0.003	0.003	0.003
		18	0.002	0.002	0.002	0.002	0.002
		21	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.023	0.017	0.017	0.018	0.017
		15	0.022	0.012	0.012	0.013	0.012
		18	0.021	0.010	0.010	0.010	0.010
		21	0.019	0.009	0.010	0.010	0.010
		24	0.009	0.005	0.006	0.006	0.006
	M	12	0.019	0.014	0.016	0.017	0.016
		15	0.016	0.010	0.010	0.011	0.010
		18	0.015	0.009	0.008	0.007	0.008
		21	0.012	0.007	0.007	0.007	0.007
		24	0.007	0.005	0.005	0.005	0.005
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.003	0.003	0.003	0.003	0.003
		18	0.003	0.003	0.003	0.003	0.003
		21	0.003	0.003	0.003	0.003	0.003
		24	0.004	0.004	0.004	0.004	0.004
CX	I	12	0.015	0.010	0.010	0.012	0.010
		15	0.016	0.009	0.009	0.015	0.009
		18	0.019	0.011	0.011	0.010	0.011
		21	0.020	0.014	0.014	0.014	0.014
		24	0.006	0.005	0.005	0.006	0.005
	M	12	0.013	0.008	0.009	0.011	0.009
		15	0.011	0.007	0.008	0.011	0.008
		18	0.013	0.009	0.008	0.008	0.008
		21	0.012	0.010	0.010	0.010	0.010
		24	0.005	0.005	0.005	0.005	0.005
	F	12	0.002	0.002	0.002	0.002	0.002
		15	0.002	0.002	0.002	0.002	0.002
		18	0.003	0.003	0.003	0.003	0.003
		21	0.004	0.004	0.004	0.004	0.004
		24	0.004	0.004	0.004	0.004	0.004

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

Table 6.3.5
Mean Squared Error for Estimates of the Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.024	0.015	0.014	0.014	0.014
		15	0.021	0.007	0.007	0.006	0.007
		18	0.022	0.006	0.005	0.005	0.005
		21	0.021	0.005	0.005	0.004	0.005
		24	0.011	0.006	0.006	0.006	0.006
	M	12	0.020	0.012	0.012	0.011	0.012
		15	0.015	0.006	0.005	0.005	0.005
		18	0.012	0.005	0.004	0.004	0.004
		21	0.010	0.004	0.004	0.004	0.004
		24	0.007	0.005	0.004	0.004	0.004
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.003	0.003	0.003	0.003	0.003
		18	0.003	0.003	0.003	0.003	0.003
		21	0.003	0.003	0.003	0.003	0.003
		24	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.018	0.012	0.012	0.012	0.012
		15	0.018	0.008	0.008	0.009	0.008
		18	0.020	0.007	0.007	0.006	0.007
		21	0.020	0.007	0.007	0.006	0.007
		24	0.010	0.006	0.006	0.006	0.006
	M	12	0.015	0.009	0.010	0.011	0.010
		15	0.013	0.007	0.007	0.007	0.007
		18	0.012	0.006	0.005	0.005	0.005
		21	0.011	0.006	0.005	0.005	0.005
		24	0.007	0.005	0.005	0.005	0.005
	F	12	0.002	0.002	0.002	0.002	0.002
		15	0.003	0.003	0.003	0.003	0.003
		18	0.003	0.003	0.003	0.003	0.003
		21	0.004	0.004	0.004	0.004	0.004
		24	0.005	0.005	0.005	0.005	0.005
CX	I	12	0.005	0.003	0.003	0.004	0.003
		15	0.009	0.006	0.006	0.008	0.006
		18	0.014	0.009	0.009	0.008	0.009
		21	0.018	0.011	0.011	0.010	0.011
		24	0.010	0.008	0.007	0.008	0.007
	M	12	0.003	0.002	0.003	0.003	0.003
		15	0.006	0.004	0.005	0.006	0.005
		18	0.009	0.007	0.007	0.007	0.007
		21	0.012	0.009	0.008	0.008	0.008
		24	0.008	0.007	0.007	0.007	0.007
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.004	0.004	0.004	0.004	0.004
		24	0.006	0.006	0.006	0.006	0.006

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

Table 6.3.6
Mean Squared Error for Estimates of the Tumor Incidence Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			unconstrained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.013	0.007	0.007	0.006	0.007
		15	0.012	0.004	0.004	0.003	0.004
		18	0.013	0.003	0.003	0.003	0.003
		21	0.012	0.003	0.003	0.003	0.003
		24	0.006	0.004	0.004	0.004	0.004
	M	12	0.010	0.005	0.005	0.005	0.005
		15	0.008	0.003	0.003	0.003	0.003
		18	0.006	0.002	0.002	0.002	0.002
		21	0.005	0.002	0.002	0.002	0.002
		24	0.004	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.002	0.002	0.002	0.002	0.002
		18	0.002	0.002	0.002	0.002	0.002
		21	0.002	0.002	0.002	0.002	0.002
		24	0.002	0.002	0.002	0.002	0.002
LN	I	12	0.009	0.004	0.004	0.004	0.004
		15	0.009	0.003	0.003	0.003	0.003
		18	0.011	0.003	0.003	0.003	0.003
		21	0.011	0.003	0.003	0.003	0.003
		24	0.006	0.004	0.004	0.004	0.004
	M	12	0.007	0.003	0.004	0.004	0.004
		15	0.006	0.003	0.003	0.003	0.003
		18	0.006	0.002	0.002	0.002	0.002
		21	0.005	0.003	0.002	0.002	0.002
		24	0.004	0.003	0.003	0.003	0.003
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.005	0.002	0.002	0.002	0.002
		15	0.006	0.002	0.002	0.003	0.002
		18	0.007	0.003	0.003	0.003	0.003
		21	0.009	0.004	0.003	0.003	0.003
		24	0.006	0.004	0.004	0.004	0.004
	M	12	0.004	0.001	0.002	0.002	0.002
		15	0.004	0.002	0.002	0.002	0.002
		18	0.004	0.002	0.002	0.002	0.002
		21	0.005	0.003	0.003	0.003	0.003
		24	0.004	0.004	0.004	0.004	0.004
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001
		21	0.002	0.002	0.002	0.002	0.002
		24	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

6.4 Effect of Collapsing Continuous Data into Discrete Intervals

The final simulation study was conducted to determine the effect of analyzing the data from a continuous death and tumor onset process by subdividing the length of the experiment into discrete intervals and applying the unconstrained MLE's, constrained MLE's and intuitive pooling method estimators to the resulting data counts within each time interval. The method of subdividing the length of a long-term animal study and computing estimates for each interval is used frequently in the analysis of animal carcinogenicity data; for example, the lifetable analysis (Tarone, 1975) and prevalence analysis (Hoel and Walburg, 1972) described in Chapter I both rely on this approach. However, the latter two analyses are not dependent on the availability of sacrifice data, while the methodology developed in this research requires sacrifice data within each time interval in order to estimate the tumor incidence rate. Since the number of animals which can be sacrificed is limited by the practical limitations of cost and trained personnel, it is important to determine whether the estimates obtained by collapsing the length of a long-term animal study into three intervals have desirable operating characteristics. In addition to investigating the bias and mean squared error for the estimates of the tumor incidence rate, death rate without tumor, and overall death rate, the final simulation study will also address the effect of various placements of the sacrifice times.

Datasets representing the outcomes of long-term animal studies were simulated under the same conditions described for the three and five-interval discrete model simulation studies with the exception of the two levels for the background death rate without tumor; since this factor did not appear to have any affect on the estimates of any of the parameters under any of the other simulation conditions, it was removed from consideration to simplify the presentation of results. Although the remaining three factors (tumor lethality, shape of cumulative tumor survival curve, and cumulative tumor survival rate) were maintained in the design of the simulation study, the true values of the parameter

estimates were determined in a slightly different manner due to the continuous nature of the underlying death and tumor onset process now taken into account.

The data were generated by assuming that the processes of tumor onset, death for tumor-free animals, and death for animals dying with tumors can be described by Weibull hazards. By supplying realistic values for the shape and scale parameters of these Weibull hazards, the desired data can be generated according to a method described by Portier and Bailer (1987) for simulating failure times when the event of interest cannot be observed. Basically, the idea is to generate a uniform (0,1) random number, U_1 , and use this value to determine the time of tumor onset using the well-known result from the probability integral transformation that $(1 - F_T(t)) = S_T(t) = \exp\{-\int \lambda(s) ds\}$ is distributed as a uniform (0,1) random variable. It is assumed that the continuous Weibull hazard for tumor onset, $\lambda(t)$, has the form shown below:

$$\lambda(t) = \exp\{-\alpha \eta t^{(\eta-1)}\}$$

which allows the cumulative hazard to be computed as $\Lambda(t) = \alpha t^\eta$. Setting U_1 equal to $\exp\{-\Lambda(t)\}$, the tumor onset time can be calculated as:

$$t = \left[\frac{-\log(U_1)}{\alpha} \right]^{1/\eta}$$

The time of death without tumor was calculated in a similar manner based on the generation of a second uniform (0,1) random number, and the two times are compared to determine the time to the first event E_1 . If the tumor onset time is less than the time of death without tumor, then the first event is tumor onset and it is necessary to determine the time of death with tumor for this animal. In this simulation study, the hazard for death with tumor is

assumed to be equal to the hazard for death without tumor plus an additional hazard $\gamma(t_i - t_u)$ which reflects the risk of death directly attributable to the tumor of interest given tumor onset at time t_u . Although the times of tumor onset and death without tumor can be calculated by algebraically inverting the corresponding survival function, an iterative algorithm such as the Newton-Raphson method must be employed to determine the time of death with tumor.

Sacrifices are incorporated into the study design in a manner analogous to that described for the three-interval discrete model simulation study. If the first event is death without tumor and the associated time of death exceeds the time of study termination, then the animal is noted as being sacrificed without the terminal sacrifice. Similarly, if the time of death with tumor is calculated as being after the end of the study, then the animal is noted as being sacrificed with the tumor at the terminal sacrifice. The inclusion of interim sacrifices proceeds exactly as described for the three-interval discrete model simulation study.

The data in this simulation study were generated under the assumption that the true tumor incidence rate increases over time, while the data generated in the discrete-model simulation study were based on the assumption of constant tumor incidence rates within each interval. As such, there is no "true" value in the continuous-model simulation study which is applicable to the whole interval. Instead, target values for the estimated discrete rates can be computed by transforming the survival rates associated with the end of each time interval. The target values for the estimated discrete tumor incidence rate are calculated by computing the cumulative tumor survival rate at each of the sacrifice times t_i as $\Lambda(t_i) = \exp\{-\alpha t_i^\eta\}$, then setting $\Lambda(t_i) = \prod (1 - \lambda_i)$, so that the target value for λ_i can be calculated as:

$$\begin{aligned}
\lambda_i &= 1 - \frac{\Lambda(t_i)}{\Lambda(t_{i-1})} \\
&= 1 - \frac{\exp\{-\alpha t_i^\eta\}}{\exp\{-\alpha t_{i-1}^\eta\}} \\
&= 1 - \exp\left\{-\int_{t_{i-1}}^{t_i} \alpha s^\eta ds\right\}
\end{aligned}$$

Similarly, the target value for the estimated discrete death rate for tumor can be calculated by recognizing that the survival function for the first event E_1 can be written as $S(t_i) = \exp\{-\int (\lambda(s) + \beta(s)) ds\}$ in which $\beta(s)$ is the continuous hazard for the death rate without tumor. By setting $S(t_i) = \prod (1 - \lambda_s - \beta_s)$ where the product is through the i -th interval, the term $(1 - \lambda_i - \beta_i)$ can be written as the ratio $S(t_i) / S(t_{i-1})$. Now the target value for β_i can be expressed as:

$$\begin{aligned}
\beta_i &= 1 - \lambda_i - \frac{S(t_i)}{S(t_{i-1})} \\
&= \frac{\Lambda(t_i)}{\Lambda(t_{i-1})} - \frac{S(t_i)}{S(t_{i-1})}
\end{aligned}$$

Examination of the form for $S(t_i)$ reveals that it can be written as $S(t_i) = \Lambda(t_i) \exp\{-\beta(t_i)\}$, so that the target value for β_i can also be calculated by:

$$\beta_i = \frac{\Lambda(t_i)}{\Lambda(t_{i-1})} \left[1 - \frac{\exp\{-\beta(t_i)\}}{\exp\{-\beta(t_{i-1})\}} \right]$$

The target value for ϕ_i , the overall death rate, can be calculated from expression (2.2.7) of Chapter II. This expression requires a value for γ_i to be supplied in addition to ϕ_i and β_i . The hazard for death given tumor onset is assumed to have the form $\gamma_i = \beta(t_u) + \zeta(t_{(i-u)})$ in which $\beta(t_u)$ is the continuous hazard function for death without tumor, and $\zeta(t_{(i-u)}) = \exp\{-\mu\omega t^{\omega-1}\}$ is the continuous hazard representing the additional risk directly attributable to the tumor of interest. In this simulation study, it is assumed that the shape parameter ω for the Weibull hazard is equivalent to that of the hazard for death without tumor. Under the incidental tumor lethality condition, the scale parameter μ is assumed to be 0, while levels of 5.0 and 10.0 are chosen for the intermediate and high tumor lethality conditions, respectively.

The values for the shape and scale parameters for the tumor incidence hazard were selected so as to obtain the same cumulative tumor survival rates at 12 months (52 weeks) and at the study termination (110 weeks) as for the three-interval discrete model simulation study. Similarly, the shape and scale parameters for the hazard of death for tumor-free animals were chosen in an attempt to obtain target values for the death rates without tumor at the three sacrifice times which were similar to those of the discrete model study. Since the methods of estimating tumor incidence rates based on early and late pooling were not regarded as being competitive with the remaining methods of estimation with respect to minimum variance and bias in the previous two simulation studies, these two methods were not included in the presentation of results for the simulation study under discussion.

As in the previous two simulation studies, a total of 10,000 datasets were simulated for each combination of the levels of tumor lethality, shape of the cumulative tumor survival curve, and cumulative tumor survival rate. The observed times of tumor onset, death without tumor, and death with tumor (if applicable) were generated for 70 animals in each of the 10,000 datasets. However, in this simulation study, each set of observed event

times are analyzed based on three different subdivisions of the length of the long-term animal study. In the first two simulation studies, it was found that the estimates of the tumor incidence rate generally had the highest associated MSE levels during the early time intervals. This finding was not entirely unexpected, since the number of animals which develop tumors in the early stage of a long-term animal study is typically very small. For this reason, the placement of sacrifice times can have a strong effect on the quality of the estimates of tumor incidence.

The impact of the times of sacrifice on the estimates of the tumor incidence rates and death rates was evaluated by specifying three sacrifice schemes, and evaluating the estimates for the same generated times of tumor onset and death for each sacrifice scheme. The first subdivision strategy, designated as the "early sacrifice" method, collapses the data into discrete intervals based on interim sacrifice times of 35 and 70 weeks (approximately 8 and 16 months, respectively). The second sacrifice scheme creates the counts of natural deaths and sacrifices with and without tumor assuming interim sacrifice times of 52 weeks (12 months) and 78 weeks (18 months), and is designated as the "intermediate sacrifice" method. The final method employs sacrifice times of 78 weeks (18 months) and 91 weeks (21 months), and is termed the "late sacrifice" method. All three methods assume a terminal sacrifice at 110 weeks, or just over two years. It is anticipated that the tumor incidence rates for a tumor type with a late onset (convex tumor survival curve) would be most accurately estimated by the late sacrifice scheme, while the early sacrifice method would be better suited for a tumor type with an early onset (concave tumor survival curve).

The results of the final simulation study for early sacrifice times are displayed in Table 6.4.1, 6.4.2, and 6.4.3 for cumulative tumor survival rates of 20%, 50%, and 80%, respectively. The estimates of the tumor incidence rates are extremely close among the unconstrained MLE's, analytically constrained MLE's, and intuitive pooling methods of

estimation for the 20% tumor survival rate, although all three methods tend to produce mean estimates which slightly underestimate the target values. The underestimation of the true tumor incidence rates is most pronounced in the last interval (70 - 110 weeks). The greatest bias among analytically constrained estimates of the tumor incidence rates for concave-shaped tumor survival curves is generally observed with a tumor survival rate of 50%, while a survival rate of 20% produces the highest bias for linear and convex shaped tumor survival curves. There appears to be no difference in the levels of bias associated with incidental, intermediate or fatal tumors for the early sacrifice method.

The mean values for the estimates of the tumor incidence rate based on intermediate sacrifice times are shown in Table 6.4.4, 6.4.5, and 6.4.6 for cumulative tumor survival rates of 20%, 50%, and 80%, respectively. From these three tables it is evident that, for all three methods of estimation considered in this simulation study, the mean estimates of the tumor incidence rate are consistently lower than the target values for the first time interval. The mean estimates corresponding to the last interval are also typically underestimates of the target values for the linear and convex shaped tumor survival curves, but the mean values for the analytically constrained MLE's and intuitive pooling estimates are often larger than the true values for the last interval when the data is simulated with a concave shaped tumor survival curve. Despite these patterns of differences between the target and estimated values of tumor incidence, the levels of bias are still generally small and the mean estimates for the analytic constraint and intuitive pooling methods are often closer to the true values than the unconstrained estimators. The greatest bias for the linear and convex shaped survival curves is observed for a 20% survival rate, while the 50% survival rate yields the highest bias levels for the concave shape. The level of tumor lethality also affects the level of bias, with the smallest bias consistently displayed for fatal tumors.

Last of all, the simulated datasets were analyzed by applying the late sacrifice method to the observed times of tumor onset and death. The mean values for the estimates obtained from the unconstrained MLE's, the constrained MLE's, and the intuitive pooling method based on late sacrifice times are displayed in Tables 6.4.7, 6.4.8, and 6.4.9 for rates of cumulative tumor survival equal to 20%, 50%, and 80%, respectively. The unconstrained MLE's generally have mean estimates which are closest to the target values, but it will be emphasized one final time that these estimators are not considered reasonable due to their allowance of negative tumor incidence rates. Although the constrained MLE's and intuitive pooling method estimates exhibit somewhat greater levels of bias overall, there are many instances in which one of these methods produces a mean estimate which is closer to the target value than the unconstrained mean estimate. For datasets simulated with a concave survival curve, both the analytically constrained MLE's and the intuitive pooling method appear to yield underestimates of the target tumor incidence rate during the first interval, and overestimates for the second and third interval. In fact, the mean estimates typically show decreases from the second interval to the third for these two methods, while the target values actually increase. In datasets simulated with linear and convex shaped tumor survival curves, underestimates are typically observed for the latter two methods during the first and last interval, and overestimates are observed during the middle interval. A survival rate of 20% is associated with the highest bias levels for all three shapes of the tumor survival curves. Among datasets simulated with each rate of tumor survival, the smallest levels of bias are exhibited by fatal tumors, while datasets simulated with incidental tumor lethality have the largest levels of bias.

The levels of bias exhibited by the mean estimates of the tumor incidence rate can be compared for the early, intermediate, and late sacrifice methods within levels of cumulative survival rate, shape of survival curve, and tumor lethality. Such a comparison reveals that the late sacrifice method is generally associated with the highest levels of bias while the

early sacrifice method is typically associated with the lowest levels of bias. This pattern holds for the first two time intervals but is reversed for the last time interval, in which the late sacrifice method exhibits much smaller levels of bias than either the early or intermediate method. Based on the mean estimates presented in the tables for early, intermediate and late sacrifice times, no clear preference is indicated for either the analytically constrained maximum likelihood method or the intuitive pooling method of obtaining estimates of the tumor incidence rate.

The mean values for the estimates of the death rate without tumor and overall death rate are supplied in Appendix G for the early, intermediate, and late sacrifice methods. Some of the same patterns can be observed for the three interval continuous model simulation study that were observed for the three-interval discrete model simulation study. First of all, the methods for estimating of the death rate without tumor consistently yield overestimates of the target values, especially for the last time interval. The intuitive pooling method typically produces the mean estimate which is closest to the target value for the last interval. There appears to be little difference between the unconstrained and constrained MLE's in the estimation of the overall death rates, and the mean biases exhibited by these two methods appear to be very small. In contrast, the method of pooling does not seem to provide reliable estimates of the overall death rate in comparison to the maximum likelihood methods. When the rate of cumulative tumor survival is 20%, the intermediate sacrifice method appears to yield mean estimates of the death rate without tumor displaying the smallest levels of bias; however, there is no discernible difference in the three sacrifice schemes with respect to the levels of bias for the 50% and 80% tumor survival rates, nor is any difference apparent in the estimates of the overall death rate which can be attributed to the pattern of sacrifice times.

The values for the mean squared error associated with the previously discussed estimates of tumor incidence are given in Tables 6.4.10, 6.4.11, and 6.4.12 for cumulative tumor survival rates of 20%, 50%, and 80%, respectively. Although it was anticipated that datasets simulated with a concave tumor survival curve would be most appropriately analyzed by the early sacrifice method while an analysis based on the late sacrifice times would be better suited for datasets simulated with a convex tumor survival curve, this conjecture was not entirely supported by the results of the three interval continuous model simulation study. In fact, an examination of Table 6.4.10 indicates that the MSE values for the first interval are consistently highest for the early sacrifice scheme when the shape of the tumor survival curve is concave, highest for the intermediate (or middle) sacrifice times when the shape is linear, and highest for the late sacrifice times when the shape of the survival curve is convex. However, when the shape of the survival curve is convex, the use of late sacrifice times almost always yields the smallest MSE values in both the second and third interval for all three methods of estimation.

Other patterns can be described for the MSE values which are similar to those observed in the three-interval discrete model simulation study. The MSE values for the analytically constrained parameter estimates and estimates obtained from intuitively pooled data are almost identical, and both of these methods exhibit smaller MSE values than do the unconstrained MLE's. As in the discrete model simulation, the MSE values decrease as the cumulative tumor survival rate increases from 20% to 50% to 80%, and are typically smallest in the last interval and largest in the first interval.

To summarize the results of the three-interval continuous model simulation study, the approach of collapsing data arising from a continuous tumor onset and death process into three discrete intervals is supported by the small levels of bias observed in the estimates of the tumor incidence rate computed by constrained MLE's and the intuitive

pooling method. Although the levels of bias are, in general, slightly larger than those observed in the three-interval discrete model simulation study, this difference may be explained by the fact that the discrete data was simulated under the assumption that the tumor incidence rate remained constant within each time interval, while the continuous data assumed an increase in the true tumor incidence rate over time based on a Weibull hazard. Thus, there is no legitimate "true" value for the tumor incidence rate which applies to the entire interval, and computation of the target tumor incidence rate at the end of the time interval when the sacrifices are conducted may not be appropriate in all cases. The difficulties inherent in selecting a proper value of tumor incidence as a standard of comparison for the estimates based on maximum likelihood and intuitive pooling methods may have also contributed to the unexpected pattern of MSE values observed for the early, intermediate, and late sacrifice schemes. Regardless of the levels of the conditions which were considered in this simulation study or the sacrifice scheme employed, the analytic solutions for the constrained MLE's have been demonstrated to estimate the tumor incidence rate with low levels of bias and a smaller degree of variability than the unconstrained MLE's, even in the small samples assumed by this simulation study.

Table 6.4.1
Mean Values for Estimates of Tumor Incidence Rate
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.493	0.487	0.471	0.476
		70	0.372	0.368	0.389	0.376
		110	0.371	0.346	0.362	0.370
	M	35	0.493	0.494	0.476	0.494
		70	0.372	0.362	0.380	0.377
		110	0.371	0.337	0.360	0.327
	F	35	0.493	0.492	0.478	0.495
		70	0.372	0.366	0.383	0.380
		110	0.371	0.335	0.351	0.313
LN	I	35	0.243	0.243	0.238	0.240
		70	0.409	0.402	0.406	0.401
		110	0.552	0.490	0.494	0.499
	M	35	0.243	0.243	0.239	0.243
		70	0.409	0.404	0.405	0.404
		110	0.552	0.486	0.492	0.490
	F	35	0.243	0.242	0.239	0.244
		70	0.409	0.401	0.403	0.403
		110	0.552	0.491	0.496	0.488
CX	I	35	0.076	0.075	0.074	0.074
		70	0.336	0.333	0.334	0.334
		110	0.674	0.574	0.574	0.574
	M	35	0.076	0.076	0.075	0.076
		70	0.336	0.333	0.334	0.333
		110	0.674	0.571	0.573	0.573
	F	35	0.076	0.076	0.075	0.076
		70	0.336	0.329	0.330	0.330
		110	0.674	0.577	0.577	0.577

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.2
 Mean Values for Estimates of Tumor Incidence Rate
 for a 3-Interval Study with Early Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.353	0.354	0.319	0.323
		70	0.132	0.125	0.159	0.151
		110	0.110	0.103	0.136	0.139
	M	35	0.353	0.350	0.316	0.336
		70	0.132	0.134	0.164	0.158
		110	0.110	0.099	0.131	0.098
	F	35	0.353	0.353	0.323	0.342
		70	0.132	0.130	0.167	0.163
		110	0.110	0.103	0.124	0.083
LN	I	35	0.165	0.165	0.154	0.156
		70	0.203	0.199	0.207	0.203
		110	0.248	0.230	0.238	0.242
	M	35	0.165	0.167	0.155	0.165
		70	0.203	0.196	0.207	0.204
		110	0.248	0.231	0.237	0.226
	F	35	0.165	0.165	0.156	0.166
		70	0.203	0.200	0.209	0.207
		110	0.248	0.228	0.234	0.218
CX	I	35	0.013	0.013	0.011	0.011
		70	0.123	0.122	0.124	0.124
		110	0.422	0.376	0.375	0.375
	M	35	0.013	0.013	0.011	0.012
		70	0.123	0.122	0.124	0.124
		110	0.422	0.376	0.375	0.375
	F	35	0.013	0.013	0.011	0.013
		70	0.123	0.121	0.123	0.123
		110	0.422	0.376	0.376	0.374

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.3
Mean Values for Estimates of Tumor Incidence Rate
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.128	0.128	0.098	0.100
		70	0.046	0.046	0.064	0.061
		110	0.038	0.034	0.059	0.060
	M	35	0.128	0.130	0.100	0.111
		70	0.046	0.043	0.067	0.063
		110	0.038	0.035	0.056	0.041
	F	35	0.128	0.128	0.100	0.111
		70	0.046	0.045	0.070	0.066
		110	0.038	0.035	0.053	0.035
LN	I	35	0.068	0.068	0.054	0.056
		70	0.069	0.071	0.075	0.072
		110	0.078	0.068	0.086	0.086
	M	35	0.068	0.067	0.052	0.060
		70	0.069	0.069	0.079	0.077
		110	0.078	0.072	0.084	0.074
	F	35	0.068	0.068	0.055	0.064
		70	0.069	0.068	0.078	0.076
		110	0.078	0.072	0.080	0.068
CX	I	35	0.023	0.024	0.018	0.019
		70	0.066	0.065	0.066	0.065
		110	0.123	0.112	0.119	0.119
	M	35	0.023	0.023	0.016	0.020
		70	0.066	0.066	0.070	0.068
		110	0.123	0.114	0.118	0.114
	F	35	0.023	0.023	0.017	0.021
		70	0.066	0.066	0.070	0.069
		110	0.123	0.113	0.116	0.111

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.4
 Mean Values for Estimates of Tumor Incidence Rate
 for a 3-Interval Study with Intermediate Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.600	0.592	0.567	0.572
		78	0.279	0.279	0.337	0.320
		110	0.307	0.296	0.310	0.320
	M	52	0.600	0.600	0.574	0.595
		78	0.279	0.265	0.315	0.311
		110	0.307	0.286	0.308	0.251
	F	52	0.600	0.596	0.577	0.595
		78	0.279	0.270	0.310	0.306
		110	0.307	0.281	0.298	0.238
LN	I	52	0.400	0.396	0.384	0.386
		78	0.356	0.344	0.362	0.357
		110	0.483	0.429	0.438	0.441
	M	52	0.400	0.398	0.387	0.399
		78	0.356	0.347	0.359	0.359
		110	0.483	0.422	0.435	0.413
	F	52	0.400	0.398	0.390	0.401
		78	0.356	0.344	0.356	0.359
		110	0.483	0.428	0.433	0.406
CX	I	52	0.200	0.198	0.193	0.194
		78	0.347	0.338	0.343	0.342
		110	0.617	0.522	0.525	0.525
	M	52	0.200	0.202	0.198	0.202
		78	0.347	0.337	0.340	0.342
		110	0.617	0.520	0.522	0.516
	F	52	0.200	0.198	0.196	0.200
		78	0.347	0.337	0.340	0.340
		110	0.617	0.526	0.527	0.521

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.5
 Mean Values for Estimates of Tumor Incidence Rate
 for a 3-Interval Study with Intermediate Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.400	0.399	0.355	0.358
		78	0.088	0.085	0.135	0.129
		110	0.087	0.078	0.118	0.122
	M	52	0.400	0.397	0.360	0.378
		78	0.088	0.088	0.133	0.129
		110	0.087	0.078	0.110	0.074
	F	52	0.400	0.400	0.369	0.384
		78	0.088	0.087	0.133	0.128
		110	0.087	0.080	0.101	0.065
LN	I	52	0.250	0.248	0.228	0.230
		78	0.161	0.156	0.176	0.173
		110	0.206	0.192	0.203	0.206
	M	52	0.250	0.249	0.231	0.246
		78	0.161	0.157	0.177	0.175
		110	0.206	0.190	0.199	0.174
	F	52	0.250	0.249	0.235	0.249
		78	0.161	0.156	0.174	0.173
		110	0.206	0.191	0.196	0.169
CX	I	52	0.050	0.049	0.044	0.044
		78	0.147	0.145	0.153	0.153
		110	0.383	0.342	0.341	0.340
	M	52	0.050	0.049	0.045	0.048
		78	0.147	0.144	0.150	0.150
		110	0.383	0.343	0.342	0.339
	F	52	0.050	0.050	0.047	0.050
		78	0.147	0.143	0.148	0.148
		110	0.383	0.343	0.342	0.337

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in weeks

Table 6.4.6
Mean Values for Estimates of Tumor Incidence Rate
for a 3-Interval Study with Intermediate Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.150	0.149	0.112	0.114
		78	0.030	0.031	0.057	0.055
		110	0.030	0.025	0.055	0.055
	M	52	0.150	0.151	0.118	0.128
		78	0.030	0.027	0.057	0.055
		110	0.030	0.028	0.049	0.032
	F	52	0.150	0.150	0.123	0.131
		78	0.030	0.028	0.058	0.054
		110	0.030	0.028	0.040	0.027
LN	I	52	0.100	0.100	0.078	0.079
		78	0.051	0.054	0.066	0.065
		110	0.063	0.053	0.073	0.073
	M	52	0.100	0.100	0.079	0.088
		78	0.051	0.050	0.067	0.067
		110	0.063	0.058	0.072	0.056
	F	52	0.100	0.099	0.083	0.091
		78	0.051	0.050	0.067	0.065
		110	0.063	0.060	0.067	0.053
CX	I	52	0.050	0.051	0.039	0.040
		78	0.060	0.058	0.067	0.066
		110	0.104	0.094	0.101	0.101
	M	52	0.050	0.049	0.038	0.044
		78	0.060	0.060	0.070	0.069
		110	0.104	0.096	0.101	0.092
	F	52	0.050	0.050	0.041	0.047
		78	0.060	0.058	0.068	0.066
		110	0.104	0.096	0.100	0.091

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.7
 Mean Values for Estimates of Tumor Incidence Rate
 for a 3-Interval Study with Late Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.711	0.699	0.667	0.671
		91	0.142	0.163	0.309	0.277
		110	0.193	0.238	0.235	0.252
	M	78	0.711	0.703	0.674	0.690
		91	0.142	0.135	0.239	0.223
		110	0.193	0.195	0.226	0.170
	F	78	0.711	0.704	0.683	0.696
		91	0.142	0.132	0.202	0.181
		110	0.193	0.179	0.202	0.165
LN	I	78	0.613	0.602	0.573	0.579
		91	0.224	0.198	0.300	0.283
		110	0.333	0.326	0.324	0.331
	M	78	0.613	0.601	0.579	0.595
		91	0.224	0.208	0.271	0.267
		110	0.333	0.304	0.319	0.269
	F	78	0.613	0.605	0.588	0.601
		91	0.224	0.203	0.248	0.243
		110	0.333	0.298	0.313	0.272
CX	I	78	0.478	0.464	0.444	0.447
		91	0.278	0.256	0.308	0.304
		110	0.469	0.404	0.408	0.408
	M	78	0.478	0.467	0.452	0.465
		91	0.278	0.257	0.288	0.292
		110	0.469	0.399	0.408	0.375
	F	78	0.478	0.463	0.454	0.463
		91	0.278	0.264	0.286	0.287
		110	0.469	0.405	0.408	0.381

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in weeks

Table 6.4.8
 Mean Values for Estimates of Tumor Incidence Rate
 for a 3-Interval Study with Late Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.453	0.451	0.394	0.397
		91	0.038	0.027	0.114	0.106
		110	0.050	0.041	0.097	0.100
	M	78	0.453	0.449	0.415	0.427
		91	0.038	0.033	0.089	0.080
		110	0.050	0.049	0.077	0.053
	F	78	0.453	0.452	0.433	0.440
		91	0.038	0.035	0.067	0.062
		110	0.050	0.049	0.060	0.048
LN	I	78	0.371	0.365	0.326	0.329
		91	0.088	0.077	0.138	0.131
		110	0.129	0.123	0.143	0.146
	M	78	0.371	0.366	0.340	0.354
		91	0.088	0.082	0.120	0.118
		110	0.129	0.122	0.138	0.107
	F	78	0.371	0.365	0.350	0.360
		91	0.088	0.085	0.108	0.105
		110	0.129	0.120	0.129	0.108
CX	I	78	0.189	0.185	0.168	0.169
		91	0.138	0.130	0.159	0.158
		110	0.284	0.260	0.256	0.256
	M	78	0.189	0.184	0.172	0.181
		91	0.138	0.135	0.152	0.153
		110	0.284	0.258	0.259	0.244
	F	78	0.189	0.185	0.178	0.184
		91	0.138	0.131	0.142	0.143
		110	0.284	0.260	0.261	0.249

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in weeks

Table 6.4.9
Mean Values for Estimates of Tumor Incidence Rate
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.175	0.174	0.129	0.131
		91	0.013	0.012	0.052	0.049
		110	0.017	0.013	0.047	0.047
	M	78	0.175	0.175	0.149	0.155
		91	0.013	0.011	0.038	0.034
		110	0.017	0.016	0.033	0.025
	F	78	0.175	0.174	0.161	0.164
		91	0.013	0.011	0.026	0.025
		110	0.017	0.017	0.022	0.018
LN	I	78	0.146	0.145	0.110	0.112
		91	0.026	0.028	0.056	0.054
		110	0.038	0.030	0.058	0.057
	M	78	0.146	0.144	0.123	0.129
		91	0.026	0.025	0.046	0.045
		110	0.038	0.037	0.050	0.038
	F	78	0.146	0.145	0.132	0.136
		91	0.026	0.025	0.038	0.037
		110	0.038	0.035	0.043	0.036
CX	I	78	0.107	0.107	0.081	0.083
		91	0.039	0.036	0.060	0.058
		110	0.067	0.060	0.075	0.075
	M	78	0.107	0.104	0.089	0.095
		91	0.039	0.040	0.054	0.053
		110	0.067	0.062	0.072	0.062
	F	78	0.107	0.105	0.096	0.100
		91	0.039	0.039	0.048	0.047
		110	0.067	0.063	0.069	0.062

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Table 6.4.10
Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.023	0.023	0.018	0.020	0.019	0.017	0.020	0.018	0.016
		2	0.021	0.021	0.024	0.014	0.012	0.013	0.014	0.012	0.013
		3	0.005	0.006	0.010	0.004	0.005	0.009	0.004	0.005	0.008
	M	1	0.023	0.020	0.011	0.019	0.016	0.010	0.022	0.018	0.009
		2	0.015	0.013	0.011	0.011	0.009	0.007	0.009	0.007	0.006
		3	0.005	0.005	0.006	0.004	0.004	0.005	0.004	0.004	0.005
	F	1	0.022	0.016	0.007	0.018	0.013	0.006	0.022	0.015	0.006
		2	0.010	0.008	0.006	0.008	0.006	0.004	0.007	0.005	0.004
		3	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
LN	I	1	0.017	0.022	0.021	0.016	0.019	0.018	0.016	0.019	0.017
		2	0.023	0.023	0.025	0.019	0.016	0.013	0.019	0.016	0.013
		3	0.005	0.006	0.009	0.005	0.005	0.006	0.005	0.005	0.006
	M	1	0.017	0.021	0.015	0.016	0.018	0.012	0.017	0.021	0.013
		2	0.019	0.017	0.014	0.017	0.013	0.009	0.016	0.012	0.007
		3	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005
	F	1	0.016	0.019	0.010	0.015	0.017	0.008	0.017	0.020	0.009
		2	0.015	0.011	0.008	0.013	0.009	0.006	0.013	0.009	0.005
		3	0.005	0.005	0.005	0.005	0.004	0.004	0.005	0.005	0.004

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Table 6.4.10 (cont'd)
 Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.006	0.015	0.023	0.006	0.013	0.019	0.006	0.013	0.018
		2	0.022	0.023	0.027	0.021	0.020	0.016	0.021	0.020	0.015
		3	0.009	0.008	0.009	0.008	0.007	0.007	0.008	0.007	0.007
M		1	0.006	0.015	0.017	0.006	0.013	0.013	0.006	0.014	0.015
		2	0.019	0.018	0.016	0.018	0.016	0.011	0.018	0.015	0.010
		3	0.008	0.007	0.007	0.008	0.007	0.006	0.008	0.007	0.006
F		1	0.006	0.014	0.013	0.006	0.013	0.011	0.006	0.014	0.012
		2	0.016	0.013	0.010	0.015	0.013	0.008	0.015	0.012	0.007
		3	0.008	0.007	0.006	0.008	0.007	0.005	0.008	0.007	0.006

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Table 6.4.11
Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. 3	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.022	0.023	0.022	0.015	0.015	0.015	0.015	0.015	0.014
		2	0.023	0.024	0.027	0.009	0.009	0.007	0.009	0.009	0.007
		3	0.006	0.007	0.008	0.005	0.005	0.005	0.005	0.005	0.005
	M	1	0.021	0.018	0.011	0.014	0.012	0.007	0.015	0.013	0.007
		2	0.015	0.012	0.008	0.008	0.006	0.005	0.006	0.005	0.004
		3	0.005	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.004
	F	1	0.020	0.016	0.006	0.013	0.010	0.004	0.015	0.011	0.005
		2	0.009	0.007	0.005	0.005	0.004	0.004	0.005	0.004	0.004
		3	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004
LN	I	1	0.012	0.017	0.021	0.010	0.013	0.015	0.010	0.013	0.014
		2	0.021	0.022	0.026	0.013	0.012	0.009	0.013	0.012	0.009
		3	0.006	0.006	0.007	0.005	0.005	0.005	0.005	0.005	0.005
	M	1	0.013	0.015	0.012	0.010	0.011	0.008	0.012	0.013	0.009
		2	0.015	0.013	0.011	0.011	0.009	0.006	0.010	0.008	0.005
		3	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	1	0.012	0.014	0.008	0.010	0.010	0.006	0.013	0.013	0.006
		2	0.011	0.009	0.006	0.008	0.006	0.005	0.008	0.006	0.005
		3	0.005	0.005	0.005	0.005	0.005	0.004	0.005	0.005	0.005

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Table 6.4.11(cont'd)
 Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape1	tumor lethality2	sac. int.3	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.001	0.004	0.014	0.001	0.004	0.011	0.001	0.004	0.011
		2	0.011	0.015	0.021	0.010	0.013	0.013	0.010	0.012	0.013
		3	0.007	0.007	0.007	0.007	0.007	0.006	0.007	0.007	0.006
M		1	0.001	0.004	0.009	0.001	0.003	0.007	0.001	0.004	0.008
		2	0.009	0.010	0.011	0.009	0.009	0.009	0.009	0.009	0.008
		3	0.007	0.007	0.006	0.007	0.006	0.006	0.007	0.007	0.006
F		1	0.001	0.004	0.007	0.001	0.003	0.005	0.001	0.004	0.007
		2	0.007	0.008	0.008	0.007	0.007	0.007	0.007	0.007	0.006
		3	0.007	0.007	0.006	0.007	0.006	0.006	0.007	0.007	0.006

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Table 6.4.12
 Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.011	0.012	0.013	0.006	0.007	0.007	0.006	0.007	0.007
		2	0.013	0.014	0.016	0.004	0.004	0.004	0.004	0.004	0.004
		3	0.003	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	1	0.010	0.010	0.006	0.006	0.005	0.004	0.006	0.005	0.004
		2	0.008	0.006	0.004	0.003	0.003	0.003	0.003	0.003	0.003
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	1	0.010	0.008	0.004	0.005	0.005	0.002	0.006	0.005	0.002
		2	0.005	0.004	0.003	0.003	0.002	0.002	0.003	0.002	0.002
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	1	0.006	0.008	0.011	0.004	0.005	0.006	0.004	0.005	0.006
		2	0.011	0.012	0.014	0.005	0.005	0.004	0.005	0.005	0.004
		3	0.004	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	1	0.006	0.007	0.006	0.004	0.004	0.003	0.004	0.005	0.004
		2	0.007	0.006	0.005	0.004	0.003	0.003	0.004	0.003	0.003
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	1	0.005	0.006	0.004	0.003	0.004	0.002	0.004	0.004	0.003
		2	0.005	0.004	0.003	0.003	0.003	0.002	0.003	0.003	0.002
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Table 6.4.12 (cont'd)
 Mean Squared Error for Estimates of the Tumor Incidence Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.002	0.005	0.009	0.001	0.003	0.005	0.001	0.003	0.005
		2	0.008	0.009	0.013	0.005	0.005	0.005	0.005	0.005	0.004
		3	0.003	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003
M		1	0.002	0.004	0.005	0.001	0.002	0.003	0.002	0.003	0.003
		2	0.005	0.005	0.005	0.004	0.003	0.003	0.004	0.003	0.003
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
F		1	0.002	0.003	0.003	0.001	0.002	0.002	0.002	0.003	0.002
		2	0.004	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.002
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

CHAPTER VII: DISCUSSION AND DIRECTIONS FOR FUTURE RESEARCH

In this research, general analytic solutions are derived for the maximum likelihood estimators of the tumor incidence rate, death rate without tumor, and overall death rate in a nonparametric model of tumor incidence and death. These estimators are applicable to a long-term animal study which has been subdivided into any number of time intervals, and reflect a dramatic savings in time over the iterative computer algorithms typically employed in the calculation of such estimators. More importantly, they allow the estimation and comparison of tumor incidence rates without relying on questionable assumptions regarding the relationship between tumor incidence and death, such as those required by fatal tumor analyses, incidental tumor analyses, and quantal response methods.

Although analytic expressions have also been suggested for related parameters associated with the analysis of data from long-term animal studies by McKnight and Crowley (1984) and Malani and Van Ryzin (1986), the set of estimators proposed in this research are the first to directly estimate the tumor incidence rate. The maximum likelihood estimators proposed by the latter authors address alternate functions which are only of peripheral interest in the carcinogenic evaluation of a chemical compound, and complicated transformations are often required to compute estimates of the tumor incidence rate. The availability of an analytic solution specifically for the tumor incidence rate as provided by this research allows a clear determination of what set of data outcomes would (1) prevent the calculation of the tumor incidence rate and (2) result in an improper (negative) estimate of the tumor incidence rate. The transformations necessary for the estimators proposed by McKnight and Crowley and by Malani and Van Ryzin make it difficult to recognize

possible situations in which boundary constraints would be violated. In addition, it is also difficult to formulate boundary constraints in terms of their original parameters.

The simple specification of boundary constraints for the estimators proposed in this research allowed the successful application of constrained maximization methods to obtain analytic solutions for the set of restricted maximum likelihood estimators of the tumor incidence rate, death rate without tumor, and overall death rate. These estimators are applicable to long-term animal study designs with up to three time intervals, and prevent estimation of tumor incidence rates outside the parameter space. The fact that analytic solutions for the constrained MLE's were not derived for study designs with more than three intervals is considered primarily a theoretical limitation, since almost all long-term animals studies which incorporate interim sacrifices include no more than two such planned sacrifice times.

Although long-term animal studies rarely have more than two interim sacrifices and can thus be analyzed as a three interval design, the few study designs which include more than three time intervals can be analyzed using the unconstrained MLE's of the tumor incidence rate and death rates. If the study is conducted with enough animals, then the asymptotic consistency of maximum likelihood estimators will ensure that the unconstrained maximum likelihood estimates of the tumor incidence rates are non-negative, provided they are truly non-zero. In smaller samples, the unconstrained MLE's can be applied to data pooled in such a way that the parameter estimates are within the required boundaries.

The availability of maximum likelihood estimators is desirable due to their asymptotic properties of consistency and efficiency. In addition, Monte Carlo simulations studies conducted under a variety of conditions intended to represent a wide range of

possible outcomes from long-term animal studies were clearly supportive of the constrained MLE's and the intuitive pooling estimators even for the small samples sizes typically used for animal carcinogenicity research. Whether the data arose from discrete or continuous tumor onset and death processes, the analytic solutions for the constrained MLE's provided a set of estimators which exhibited only a small degree of bias and reflected gains in precision over the unconstrained MLE's with respect to their mean squared error. The intuitive pooling estimates of tumor incidence displayed levels of bias which were only slightly greater than those of the constrained MLE's, and their degree of precision was equivalent to the constrained MLE's under the simulation conditions considered. Although the variability of estimates derived from the early pooling or late pooling methods was comparable to the other methods of estimation, the early and late pooling methods were not considered acceptable on the basis of their increased bias and mean squared error in relation to the constrained MLE's and the intuitive pooling method. For study designs with more than three intervals, the intuitive pooling estimates of the tumor incidence rate appear to offer a reliable alternative to the constrained MLE's and avoid the need for time-intensive iterative computational algorithms.

The emphasis of long-term animal studies is the estimation and comparison of tumor incidence rates, and in this respect the death rate without tumor and the overall death rate are nuisance parameters. However, the unbiased estimation of these parameters is important in the adjustment for different levels of intercurrent mortality among treatment groups. The unconstrained MLE's of the overall death rate are the familiar Kaplan-Meier estimators, yet the analytic forms for the constrained maximum likelihood estimators of the overall death rate are quite complex in some constraint cases and lack the intuitive appeal associated with the Kaplan-Meier estimators. However, on the basis of the simulation studies conducted to evaluate these estimators, the mean estimates for the unconstrained MLE's and constrained MLE's were found to be almost identical, and both methods of

estimation were demonstrated to show essentially zero bias. In addition, the variability associated with the estimates from these two methods appeared to be equivalent. These findings support the use of the Kaplan-Meier estimator for the overall death rate even in situations in which constraints on the tumor incidence rates are necessary.

The death rate without tumor was also found to be reliably estimated by the unconstrained MLE's, which displayed essentially the same mean values as did the constrained MLE's. However, both maximum likelihood methods were observed to yield small overestimates of the true death rate without tumor, especially for the last interval. For this reason, the intuitive pooling estimate of the death rate without tumor was suggested as a substitute for the last interval. This proposed estimator was supported by the results of both the discrete and continuous model three-interval simulation studies, but was not confirmed by the results of the five-interval simulation study.

It should be noted that discrete estimators of tumor incidence rates and death rates are only approximations to the underlying continuous rates. As the number of time intervals increases, the discrete tumor incidence rates and death rates theoretically approach their continuous counterparts. However, an increase in the number of time intervals brings with it an accompanying decrease in the number of animals observed as dead or sacrificed within each interval, resulting in estimates of the tumor incidence rates and death rates with a higher degree of variability. This tradeoff should be recognized in the design and analysis of long-term animal studies whose purpose is the evaluation of carcinogenic risk associated with a particular chemical compound.

Involvement in the current research has suggested a myriad of possible extensions which could improve this method of analyzing long-term animal studies, help in constructing alternative methods of analysis, or aid in the development of study designs.

One such issue which deserves attention is carcinogenicity testing through the comparison of tumor incidence rates among treatment groups. A normal approximation test statistic for comparing the tumor incidence rates among two treatment groups was proposed in Chapter V, and applied to the Kodell and Nelson data in Chapter VI. However, the operating characteristics of this test statistic need to be evaluated under various conditions of tumor lethality and treatment lethality and in relation to other commonly used tests of carcinogenicity. A new test statistic which takes into account the trend in responses over multiple treatment groups would be preferable to the pairwise comparison of treatment groups necessitated by the test statistic proposed in Chapter V, and development of such a test statistic is another possible direction which could be pursued.

Although computation of the normal approximation test statistic proposed in Chapter V could be based on the unconstrained estimates of tumor incidence in the majority of cases in the analysis of the Kodell and Nelson data, the calculation of a negative variance estimate prevented the use of the unconstrained estimate of tumor incidence in one case. It seemed to be no coincidence that this particular case also involved an improper estimate of the tumor incidence rate, so that the negative variance estimate simply reflected a lack of data or an inappropriate parameter specification. On the basis of preliminary investigations of the operating characteristics of the normal approximation test statistic, it has also been found that the observed information matrix is often near-singular or non-definite when the unconstrained maximum likelihood estimates lie on the boundaries of the parameter space. Difficulties are thus encountered in the inversion of the observed information matrix to obtain an estimate of the variance. One approach which could be pursued to overcome this difficulty is to obtain a mathematical approximation to the asymptotic variance using the delta method, such as that proposed by Malani and Van Ryzin as discussed in Section 3.3, rather than a numeric approximation based on the observed information matrix. Other possibilities are the use of a reduced information matrix based on the deletion of elements

corresponding to estimates on the boundary of the parameter space, or the calculation of a new information matrix based on a reduced set of parameters.

A semi-parametric analysis of survival and sacrifice data as described by Portier and Dinse (1987) has some benefits over the nonparametric analysis employed in this research. The assumption of a continuous parametric model for the tumor incidence rate can be justified by analyses which have demonstrated the appropriateness and flexibility of particular parametric forms for historical control animals in the NTP carcinogenicity experiments. Use of a continuous parametric model rather than a discrete model for the tumor incidence rate provides the benefits of being able to calculate the tumor incidence rate at doses between those employed in a long-term animal study and extrapolate the tumor incidence estimates to the very low doses which are of interest in determining safe levels of exposure. One additional advantage of such a semi-parametric analysis is that it requires fewer times of sacrifice in order to estimate the tumor incidence rate.

Despite all these advantages, the methodology described by Portier and Dinse requires time-consuming iterative algorithms in order to estimate the tumor incidence rate and death rates. The finding of this research that the overall death rates can be estimated without bias by the Kaplan-Meier estimates allows a reduction of the computer time necessary to calculate estimates for the semi-parametric model by almost one-half. This considerable time savings may allow some estimation problems to be pursued in terms of the semi-parametric model which were previously considered impractical.

The three-interval continuous model simulation study was somewhat inconclusive in determining the most appropriate choice of sacrifice times based on the shape of the tumor survival curve, tumor lethality, and cumulative tumor survival rate. Additional studies investigating the operating characteristics of the proposed estimators for various

numbers and placements of sacrifice times are needed in order to help develop study designs which make the best use of the animals. The discrete nonparametric model assumes that the tumor incidence rates and death rates are constant over each time interval, although the true rates do not typically remain constant. Thus, it is of interest to evaluate the effect of placing sacrifice times at various points during the time interval rather than always treating sacrifices as though they occur at the end of the interval. Preliminary investigations in this area suggest that placing sacrifices in the middle of each time interval yields estimates with greater levels of bias than those observed for sacrifices at the end of the interval. This finding may be due to the tendency for the tumors to occur as a function of a higher order power of time, so that placement of sacrifices between the middle and the end of each time interval may produce estimators with better operating characteristics.

Continued improvements in the analysis and design of long-term animal studies are important in the evaluation of the carcinogenic risk and determination of safe exposure levels for the wide array of chemicals now present in our workplace and environment. Although the extrapolation of results from animals to man and from high doses to low doses remain areas of considerable controversy, long-term animal carcinogenicity studies still remain the primary tool for carcinogenic risk assessment. Researchers from a variety of scientific disciplines must work together to obtain the best understanding of the underlying carcinogenic process and the implications of the data arising from carcinogenicity experiments.

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APPENDIX A

General Forms for the Elements of the Hessian Matrix

[Note: Because the matrix of second partial derivatives of the likelihood function is symmetric, components are provided for $m > i$ only.]

Definitions:

$$(1) A_m = \prod_{k=1}^m (1 - \phi_k) - \prod_{k=1}^m (1 - \lambda_k - \beta_k)$$

$$(2) B_m = \phi_m \prod_{k=1}^{m-1} (1 - \phi_k) - \beta_m \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)$$

Elements of Hessian Matrix:

$$\frac{\delta^2 L}{\delta \phi_i \delta \phi_m} = \frac{-c_m^+ \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^m (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \phi_i)} + \frac{d_m^+ \prod_{k=1}^{m-1} (1 - \phi_k) \beta_m \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \phi_i)} +$$

$$\sum_{j=m+1}^S \left\{ \frac{-c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \phi_i) (1 - \phi_m)} + \frac{-d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \phi_i) (1 - \phi_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \phi_i \delta \beta_m} = \frac{c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \phi_i)} + \frac{-d_m^+ \phi_m \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \phi_i)} +$$

$$\sum_{j=m+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \phi_i) (1 - \lambda_m - \beta_m)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \phi_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \phi_i \delta \lambda_m} = \frac{c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \phi_i)} + \sum_{j=m+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \phi_i) (1 - \lambda_m - \beta_m)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \phi_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \beta_i \delta \beta_m} = \frac{-c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} + \frac{d_m^+ \phi_m \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \lambda_i - \beta_i)} + \sum_{j=m+1}^S \left\{ \frac{-c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} + \frac{-d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \beta_i \delta \phi_m} = \frac{c_m^+ \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^m (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} + \frac{-d_m^+ \beta_m \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \lambda_i - \beta_i)} + \sum_{j=m+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \phi_m)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \phi_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \beta_i \delta \lambda_m} = \frac{-c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} + \sum_{j=m+1}^S \left\{ \frac{-c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} + \frac{-d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \lambda_i \delta \lambda_m} = \frac{-c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} +$$

$$\sum_{j=m+1}^S \left\{ \frac{-c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} + \frac{-d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \lambda_i \delta \phi_m} = \frac{c_m^+ \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^m (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} + \frac{-d_m^+ \beta_m \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \lambda_i - \beta_i)} +$$

$$\sum_{j=m+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \phi_m)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \phi_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \lambda_i \delta \beta_m} = \frac{-c_m^+ \prod_{k=1}^m (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{A_m^2 (1 - \lambda_i - \beta_i)} + \frac{d_m^+ \phi_m \prod_{k=1}^{m-1} (1 - \phi_k) \prod_{k=1}^{m-1} (1 - \lambda_k - \beta_k)}{B_m^2 (1 - \lambda_i - \beta_i)} +$$

$$\sum_{j=m+1}^S \left\{ \frac{-c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} + \frac{-d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \lambda_i - \beta_i) (1 - \lambda_m - \beta_m)} \right\}$$

$$\frac{\delta^2 L}{\delta \phi_i \delta \beta_i} = \frac{c_i^+ \prod_{k=1}^{i-1} (1 - \phi_k) \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{A_i^2} + \frac{d_i^+ \prod_{k=1}^{i-1} (1 - \phi_k) \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{B_i^2} +$$

$$\sum_{j=i+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \phi_i) (1 - \lambda_i - \beta_i)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \phi_i) (1 - \lambda_i - \beta_i)} \right\}$$

$$\frac{\delta^2 L}{\delta \phi_i \delta \lambda_i} = \frac{c_i^+ \prod_{k=1}^{i-1} (1 - \phi_k) \prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k)}{A_i^2} + \sum_{j=i+1}^S \left\{ \frac{c_j^+ \prod_{k=1}^j (1 - \phi_k) \prod_{k=1}^j (1 - \lambda_k - \beta_k)}{A_j^2 (1 - \phi_i) (1 - \lambda_i - \beta_i)} + \frac{d_j^+ \phi_j \prod_{k=1}^{j-1} (1 - \phi_k) \beta_j \prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k)}{B_j^2 (1 - \phi_i) (1 - \lambda_i - \beta_i)} \right\}$$

$$\frac{\delta^2 L}{\delta \beta_i \delta \lambda_i} = \frac{-c_i^-}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_i^+ \left[\prod_{k=1}^{i-1} (1 - \lambda_k - \beta_k) \right]^2}{A_i^2} + \sum_{j=i+1}^S \left\{ \frac{-(c_j^- + d_j^-)}{(1 - \lambda_i - \beta_i)^2} + \frac{-c_j^+ \left[\prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k) \right]^2}{A_j^2 (1 - \lambda_i - \beta_i)^2} + \frac{-d_j^+ \beta_j^2 \left[\prod_{k=1}^{j-1} (1 - \lambda_k - \beta_k) \right]^2}{B_j^2 (1 - \lambda_i - \beta_i)^2} \right\}$$

Appendix B
Table B.1
Number of Simulations Within Each Constraint Case
For Which No Analytic or Numeric Solution is Available
Based on Low Background Death Rates

tumor survival ¹	shape ²	tumor lethality ³	Constraint Case					
			Case II ($\lambda_2 > 0, \lambda_3 = 0$)		Case III ($\lambda_2 = 0, \lambda_3 > 0$)		Case IV ($\lambda_2 = 0, \lambda_3 = 0$)	
			Analytic	Numeric	Analytic	Numeric	Analytic	Numeric
0.8	CV	I	0	8	583	4	0	2
		M	23	0	661	0	16	0
		F	0	0	0	0	0	0
	LN	I	0	9	484	3	1	0
		M	7	1	538	2	1	2
		F	0	0	0	0	0	0
	CX	I	0	7	327	3	0	1
		M	1	1	345	0	1	0
		F	0	0	0	0	0	0
0.5	CV	I	0	4	1109	4	0	7
		M	0	0	1169	0	0	0
		F	0	0	0	0	0	0
	LN	I	0	4	541	2	0	1
		M	0	0	572	0	0	0
		F	0	0	0	0	0	0
	CX	I	0	0	328	9	0	0
		M	0	0	351	1	0	0
		F	0	0	0	0	0	0
0.2	CV	I	0	2	1403	1	0	1
		M	0	0	1452	0	0	0
		F	0	0	0	0	0	0
	LN	I	0	3	516	2	0	0
		M	0	0	523	0	0	0
		F	0	0	0	0	0	0
	CX	I	0	0	273	3	0	0
		M	0	0	256	0	0	0
		F	0	0	0	0	0	0

- 1 - probability of remaining tumor free through end of study
 2 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 3 - I=incidental, M=intermediate, F=fatal

Appendix B
Table B.2
Number of Simulations Within Each Constraint Case
For Which No Analytic or Numeric Solution is Available
Based on High Background Death Rates

tumor survival ¹	shape ²	tumor lethality ³	Constraint Case					
			Case II ($\lambda_2 > 0, \lambda_3 = 0$)		Case III ($\lambda_2 = 0, \lambda_3 > 0$)		Case IV ($\lambda_2 = 0, \lambda_3 = 0$)	
			Analytic	Numeric	Analytic	Numeric	Analytic	Numeric
0.8	CV	I	0	27	106	16	0	12
		M	23	4	153	1	18	0
		F	0	0	0	0	0	0
	LN	I	0	17	73	14	0	12
		M	10	3	88	3	6	2
		F	0	0	0	0	0	0
	CX	I	1	14	47	19	0	2
		M	2	2	79	3	1	3
		F	0	0	0	0	0	0
0.5	CV	I	0	4	373	3	0	4
		M	0	0	401	0	0	0
		F	0	0	0	0	0	0
	LN	I	0	4	139	7	0	1
		M	0	0	136	0	0	0
		F	0	0	0	0	0	0
	CX	I	0	0	72	10	0	0
		M	0	0	65	7	0	0
		F	0	0	0	0	0	0
0.2	CV	I	0	0	765	2	0	0
		M	0	0	677	0	0	0
		F	0	0	0	0	0	0
	LN	I	0	1	186	2	0	0
		M	0	0	161	0	0	0
		F	0	0	0	0	0	0
	CX	I	0	1	80	7	0	0
		M	0	6	57	4	0	0
		F	0	0	0	0	0	0

- 1 - probability of remaining tumor free through end of study
2 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
3 - I=incidental, M=intermediate, F=fatal

Appendix C
Table C.1
Mean Values for Estimates of Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.010	0.010	0.010	0.010	0.015	0.016	0.019	0.010
		18	0.030	0.040	0.040	0.035	0.052	0.078	0.031	0.040
		24	0.150	0.212	0.194	0.185	0.158	0.154	0.161	0.158
	M	12	0.010	0.010	0.010	0.010	0.015	0.015	0.019	0.010
		18	0.030	0.038	0.039	0.033	0.050	0.078	0.031	0.038
		24	0.150	0.203	0.186	0.179	0.156	0.152	0.158	0.156
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.151	0.151	0.151	0.151	0.151	0.151	0.151
LN	I	12	0.010	0.010	0.010	0.010	0.013	0.011	0.015	0.010
		18	0.030	0.033	0.033	0.031	0.040	0.059	0.029	0.033
		24	0.150	0.190	0.179	0.176	0.157	0.161	0.163	0.157
	M	12	0.010	0.010	0.010	0.010	0.012	0.011	0.014	0.010
		18	0.030	0.032	0.032	0.031	0.037	0.056	0.029	0.032
		24	0.150	0.179	0.172	0.170	0.158	0.159	0.163	0.158
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.151	0.151	0.151	0.151	0.151	0.151	0.151
CX	I	12	0.010	0.010	0.010	0.010	0.013	0.010	0.013	0.010
		18	0.030	0.031	0.031	0.030	0.031	0.048	0.030	0.031
		24	0.150	0.164	0.164	0.163	0.160	0.164	0.161	0.160
	M	12	0.010	0.010	0.010	0.010	0.013	0.010	0.013	0.010
		18	0.030	0.031	0.031	0.030	0.031	0.045	0.030	0.031
		24	0.150	0.160	0.161	0.160	0.160	0.161	0.160	0.160
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2. I=incidental, M=intermediate, F=fatal
3. sacrifice time in months
4. equivalent to unconstrained estimators for the first two intervals and intuitive pooling estimates for the last interval

Appendix C
Table C.2
Mean Values for Estimates of Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.010	0.010	0.010	0.010	0.028	0.031	0.038	0.010
		18	0.030	0.033	0.032	0.029	0.063	0.089	0.042	0.033
		24	0.150	0.165	0.147	0.146	0.124	0.120	0.122	0.124
	M	12	0.010	0.010	0.010	0.010	0.029	0.030	0.041	0.010
		18	0.030	0.032	0.032	0.029	0.065	0.089	0.046	0.032
		24	0.150	0.163	0.149	0.148	0.127	0.123	0.122	0.127
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.149	0.149	0.149	0.149	0.149	0.149	0.149
LN	I	12	0.010	0.010	0.010	0.010	0.017	0.016	0.024	0.010
		18	0.030	0.031	0.032	0.030	0.054	0.078	0.033	0.031
		24	0.150	0.163	0.153	0.152	0.138	0.139	0.141	0.138
	M	12	0.010	0.010	0.010	0.010	0.016	0.015	0.023	0.010
		18	0.030	0.031	0.032	0.030	0.050	0.074	0.034	0.031
		24	0.150	0.158	0.152	0.151	0.142	0.142	0.144	0.142
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.151	0.151	0.151	0.151	0.151	0.151	0.151
CX	I	12	0.010	0.010	0.010	0.010	0.016	0.010	0.016	0.010
		18	0.030	0.030	0.031	0.030	0.031	0.059	0.031	0.030
		24	0.150	0.151	0.153	0.152	0.152	0.155	0.152	0.152
	M	12	0.010	0.010	0.010	0.010	0.015	0.010	0.015	0.010
		18	0.030	0.031	0.031	0.030	0.031	0.056	0.031	0.031
		24	0.150	0.151	0.152	0.152	0.153	0.154	0.153	0.153
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.031	0.031	0.031	0.031	0.031	0.031	0.031
		24	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

4. equivalent to unconstrained estimators for the first two intervals
and intuitive pooling estimates for the last interval

Appendix C
Table C.3
Mean Values for Estimates of Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.010	0.010	0.010	0.010	0.038	0.044	0.056	0.010
		18	0.030	0.031	0.031	0.029	0.079	0.103	0.060	0.031
		24	0.150	0.154	0.146	0.146	0.134	0.132	0.132	0.134
	M	12	0.010	0.010	0.010	0.010	0.040	0.042	0.060	0.010
		18	0.030	0.031	0.031	0.029	0.080	0.101	0.065	0.031
		24	0.150	0.153	0.147	0.147	0.137	0.135	0.133	0.137
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.149	0.149	0.149	0.149	0.149	0.149	0.149
LN	I	12	0.010	0.010	0.010	0.010	0.028	0.031	0.043	0.010
		18	0.030	0.030	0.031	0.029	0.070	0.095	0.050	0.030
		24	0.150	0.154	0.148	0.148	0.140	0.139	0.139	0.140
	M	12	0.010	0.010	0.010	0.010	0.025	0.027	0.041	0.010
		18	0.030	0.030	0.031	0.030	0.065	0.089	0.050	0.030
		24	0.150	0.151	0.147	0.147	0.141	0.140	0.140	0.141
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.029	0.029	0.029	0.029	0.029	0.029	0.029
		24	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
CX	I	12	0.010	0.010	0.010	0.010	0.018	0.019	0.026	0.010
		18	0.030	0.030	0.030	0.029	0.051	0.075	0.037	0.030
		24	0.150	0.152	0.150	0.150	0.146	0.146	0.147	0.146
	M	12	0.010	0.010	0.010	0.010	0.017	0.016	0.023	0.010
		18	0.030	0.030	0.031	0.030	0.046	0.068	0.036	0.030
		24	0.150	0.151	0.150	0.150	0.148	0.148	0.148	0.148
	F	12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
		18	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030
		24	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to unconstrained estimators for the first two intervals and intuitive pooling estimates for the last interval

Appendix C
Table C.4
Mean Values for Estimates of Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.020	0.020	0.020	0.020	0.030	0.031	0.037	0.020
		18	0.060	0.076	0.070	0.065	0.098	0.149	0.056	0.076
		24	0.300	0.398	0.353	0.345	0.287	0.278	0.295	0.287
	M	12	0.020	0.020	0.020	0.020	0.031	0.030	0.039	0.020
		18	0.060	0.075	0.070	0.065	0.097	0.148	0.060	0.075
		24	0.300	0.381	0.341	0.335	0.287	0.277	0.291	0.287
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.059	0.059	0.059	0.059	0.059	0.059	0.059
		24	0.300	0.303	0.303	0.303	0.303	0.303	0.303	0.303
LN	I	12	0.020	0.020	0.020	0.020	0.026	0.022	0.029	0.020
		18	0.060	0.066	0.064	0.062	0.080	0.119	0.057	0.066
		24	0.300	0.378	0.348	0.346	0.306	0.310	0.318	0.306
	M	12	0.020	0.020	0.020	0.020	0.025	0.021	0.028	0.020
		18	0.060	0.064	0.062	0.061	0.074	0.110	0.057	0.064
		24	0.300	0.357	0.336	0.335	0.308	0.308	0.319	0.308
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.061	0.061	0.061	0.061	0.061	0.061	0.061
		24	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
CX	I	12	0.020	0.020	0.020	0.020	0.026	0.020	0.026	0.020
		18	0.060	0.061	0.060	0.060	0.061	0.095	0.058	0.061
		24	0.300	0.329	0.328	0.328	0.321	0.327	0.323	0.321
	M	12	0.020	0.020	0.020	0.020	0.025	0.020	0.025	0.020
		18	0.060	0.061	0.061	0.061	0.061	0.087	0.060	0.061
		24	0.300	0.322	0.321	0.320	0.320	0.322	0.321	0.320
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		24	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

4 - equivalent to unconstrained estimators for the first two intervals and intuitive pooling estimates for the last interval

Appendix C
Table C.5
Mean Values for Estimates of Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						Williams method ⁴
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.020	0.020	0.020	0.020	0.054	0.062	0.074	0.020
		18	0.060	0.065	0.059	0.057	0.125	0.172	0.082	0.065
		24	0.300	0.335	0.291	0.291	0.244	0.233	0.239	0.244
	M	12	0.020	0.020	0.020	0.020	0.057	0.059	0.080	0.020
		18	0.060	0.065	0.060	0.057	0.127	0.171	0.090	0.065
		24	0.300	0.325	0.293	0.292	0.248	0.238	0.239	0.248
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		24	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
LN	I	12	0.020	0.020	0.020	0.020	0.035	0.034	0.048	0.020
		18	0.060	0.062	0.060	0.059	0.107	0.154	0.067	0.062
		24	0.300	0.328	0.304	0.304	0.274	0.273	0.281	0.274
	M	12	0.020	0.020	0.020	0.020	0.033	0.029	0.046	0.020
		18	0.060	0.062	0.060	0.059	0.098	0.144	0.067	0.062
		24	0.300	0.318	0.303	0.303	0.283	0.280	0.288	0.283
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		24	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
CX	I	12	0.020	0.020	0.020	0.020	0.031	0.020	0.031	0.020
		18	0.060	0.060	0.059	0.059	0.061	0.116	0.060	0.060
		24	0.300	0.303	0.307	0.307	0.307	0.310	0.307	0.307
	M	12	0.020	0.020	0.020	0.020	0.030	0.020	0.030	0.020
		18	0.060	0.061	0.060	0.060	0.061	0.110	0.061	0.061
		24	0.300	0.302	0.304	0.304	0.306	0.307	0.306	0.306
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.061	0.061	0.061	0.061	0.061	0.061	0.061
		24	0.300	0.299	0.299	0.299	0.299	0.299	0.299	0.299

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

4 - equivalent to unconstrained estimators for the first two intervals and intuitive pooling estimates for the last interval

Appendix C
Table C.6
Mean Values for Estimates of Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.020	0.020	0.020	0.020	0.075	0.087	0.109	0.020
		18	0.060	0.062	0.058	0.058	0.154	0.197	0.119	0.062
		24	0.300	0.309	0.292	0.291	0.265	0.259	0.262	0.265
	M	12	0.020	0.020	0.020	0.020	0.078	0.083	0.118	0.020
		18	0.060	0.062	0.059	0.058	0.157	0.196	0.129	0.062
		24	0.300	0.306	0.294	0.294	0.269	0.264	0.262	0.269
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		24	0.300	0.298	0.298	0.298	0.298	0.298	0.298	0.298
LN	I	12	0.020	0.020	0.020	0.020	0.055	0.061	0.084	0.020
		18	0.060	0.061	0.059	0.059	0.138	0.185	0.098	0.061
		24	0.300	0.307	0.295	0.295	0.276	0.272	0.276	0.276
	M	12	0.020	0.020	0.020	0.020	0.052	0.054	0.083	0.020
		18	0.060	0.061	0.059	0.059	0.129	0.174	0.099	0.061
		24	0.300	0.304	0.295	0.295	0.281	0.277	0.279	0.281
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.059	0.059	0.059	0.059	0.059	0.059	0.059
		24	0.300	0.301	0.301	0.301	0.301	0.301	0.301	0.301
CX	I	12	0.020	0.020	0.020	0.020	0.036	0.038	0.051	0.020
		18	0.060	0.060	0.059	0.059	0.102	0.148	0.074	0.060
		24	0.300	0.304	0.301	0.301	0.290	0.288	0.293	0.290
	M	12	0.020	0.020	0.020	0.020	0.035	0.033	0.046	0.020
		18	0.060	0.060	0.060	0.059	0.091	0.133	0.072	0.060
		24	0.300	0.302	0.301	0.301	0.295	0.293	0.296	0.295
	F	12	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
		18	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		24	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2. I=incidental, M=intermediate, F=fatal
3. sacrifice time in months
4. equivalent to unconstrained estimators for the first two intervals and intuitive pooling estimates for the last interval

Appendix C
Table C.7
Mean Values for Estimates of Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						Williams method ⁴
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.025	0.025	0.025	0.025	0.043	0.049	0.055	0.025
		18	0.040	0.040	0.041	0.040	0.090	0.134	0.062	0.040
		24	0.225	0.225	0.225	0.225	0.223	0.220	0.223	0.225
	M	12	0.205	0.205	0.205	0.205	0.263	0.242	0.303	0.205
		18	0.230	0.229	0.234	0.231	0.334	0.377	0.318	0.229
		24	0.433	0.434	0.434	0.435	0.456	0.475	0.448	0.434
	F	12	0.610	0.610	0.610	0.610	0.610	0.610	0.610	0.610
		18	0.280	0.280	0.280	0.280	0.280	0.280	0.280	0.280
		24	0.483	0.484	0.484	0.484	0.484	0.484	0.484	0.484
LN	I	12	0.017	0.017	0.017	0.017	0.024	0.020	0.028	0.017
		18	0.045	0.045	0.046	0.045	0.073	0.111	0.048	0.045
		24	0.300	0.301	0.301	0.301	0.299	0.296	0.301	0.301
	M	12	0.137	0.137	0.137	0.137	0.160	0.141	0.173	0.137
		18	0.208	0.208	0.210	0.209	0.249	0.288	0.230	0.208
		24	0.518	0.516	0.517	0.517	0.520	0.523	0.517	0.516
	F	12	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.410
		18	0.363	0.362	0.362	0.362	0.362	0.362	0.362	0.362
		24	0.650	0.649	0.649	0.649	0.649	0.649	0.649	0.649
CX	I	12	0.013	0.012	0.012	0.012	0.017	0.012	0.018	0.012
		18	0.040	0.040	0.041	0.040	0.045	0.095	0.041	0.040
		24	0.450	0.449	0.449	0.449	0.448	0.441	0.449	0.449
	M	12	0.073	0.072	0.072	0.072	0.085	0.072	0.085	0.072
		18	0.148	0.149	0.150	0.149	0.156	0.209	0.154	0.149
		24	0.678	0.678	0.678	0.678	0.677	0.671	0.678	0.678
	F	12	0.210	0.209	0.209	0.209	0.209	0.209	0.209	0.209
		18	0.280	0.280	0.280	0.280	0.280	0.280	0.280	0.280
		24	0.817	0.818	0.818	0.818	0.818	0.818	0.818	0.818

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

4. equivalent to unconstrained estimators of the overall death rate

Appendix C
Table C.8
Mean Values for Estimates of Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.017	0.017	0.017	0.017	0.048	0.057	0.067	0.017
		18	0.033	0.033	0.035	0.033	0.091	0.125	0.071	0.033
		24	0.165	0.164	0.164	0.164	0.162	0.161	0.161	0.164
	M	12	0.137	0.137	0.137	0.137	0.208	0.202	0.255	0.137
		18	0.145	0.145	0.150	0.147	0.264	0.292	0.260	0.145
		24	0.272	0.272	0.276	0.276	0.314	0.332	0.313	0.272
	F	12	0.410	0.411	0.411	0.411	0.411	0.411	0.411	0.411
		18	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113
		24	0.241	0.240	0.240	0.240	0.240	0.240	0.240	0.240
LN	I	12	0.013	0.013	0.013	0.013	0.025	0.024	0.036	0.013
		18	0.036	0.036	0.038	0.036	0.074	0.106	0.048	0.036
		24	0.187	0.187	0.187	0.187	0.186	0.185	0.186	0.187
	M	12	0.088	0.088	0.088	0.088	0.115	0.102	0.141	0.088
		18	0.133	0.133	0.136	0.134	0.189	0.226	0.169	0.133
		24	0.307	0.307	0.308	0.308	0.318	0.327	0.312	0.307
	F	12	0.260	0.260	0.260	0.260	0.260	0.260	0.260	0.260
		18	0.197	0.196	0.196	0.196	0.196	0.196	0.196	0.196
		24	0.350	0.351	0.351	0.351	0.351	0.351	0.351	0.351
CX	I	12	0.011	0.011	0.011	0.011	0.017	0.011	0.017	0.011
		18	0.032	0.032	0.033	0.032	0.034	0.081	0.033	0.032
		24	0.271	0.269	0.269	0.270	0.269	0.265	0.269	0.269
	M	12	0.026	0.026	0.026	0.026	0.035	0.026	0.035	0.026
		18	0.057	0.057	0.059	0.058	0.060	0.122	0.060	0.057
		24	0.415	0.415	0.415	0.415	0.415	0.408	0.415	0.415
	F	12	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		18	0.080	0.081	0.081	0.081	0.081	0.081	0.081	0.081
		24	0.596	0.595	0.595	0.595	0.595	0.595	0.595	0.595

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

4 - equivalent to unconstrained estimators of the overall death rate

Appendix C
Table C.9
Mean Values for Estimates of Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.012	0.012	0.012	0.012	0.046	0.053	0.067	0.012
		18	0.031	0.031	0.033	0.031	0.091	0.118	0.072	0.031
		24	0.154	0.154	0.154	0.154	0.151	0.150	0.150	0.154
	M	12	0.057	0.057	0.057	0.057	0.108	0.108	0.144	0.057
		18	0.073	0.074	0.078	0.076	0.157	0.180	0.149	0.074
		24	0.191	0.191	0.194	0.194	0.210	0.216	0.210	0.191
	F	12	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
		18	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065
		24	0.175	0.174	0.174	0.174	0.174	0.174	0.174	0.174
LN	I	12	0.011	0.011	0.011	0.011	0.032	0.037	0.050	0.011
		18	0.032	0.032	0.033	0.032	0.079	0.107	0.058	0.032
		24	0.159	0.160	0.160	0.160	0.158	0.157	0.157	0.160
	M	12	0.041	0.041	0.041	0.041	0.069	0.067	0.097	0.041
		18	0.069	0.069	0.072	0.071	0.124	0.153	0.110	0.069
		24	0.202	0.201	0.203	0.203	0.210	0.215	0.207	0.201
	F	12	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110
		18	0.086	0.085	0.085	0.085	0.085	0.085	0.085	0.085
		24	0.209	0.209	0.209	0.209	0.209	0.209	0.209	0.209
CX	I	12	0.011	0.011	0.011	0.011	0.020	0.021	0.028	0.011
		18	0.032	0.031	0.032	0.031	0.057	0.084	0.041	0.031
		24	0.169	0.170	0.170	0.170	0.169	0.168	0.169	0.170
	M	12	0.026	0.026	0.025	0.026	0.038	0.035	0.047	0.026
		18	0.057	0.056	0.058	0.057	0.081	0.110	0.068	0.056
		24	0.219	0.219	0.220	0.220	0.221	0.222	0.220	0.219
	F	12	0.060	0.059	0.059	0.059	0.059	0.059	0.059	0.059
		18	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.080
		24	0.264	0.264	0.264	0.264	0.264	0.264	0.264	0.264

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2. I=incidental, M=intermediate, F=fatal
3. sacrifice time in months
4. equivalent to unconstrained estimators of the overall death rate

Appendix C
Table C.10
Mean Values for Estimates of Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.050	0.050	0.050	0.050	0.086	0.096	0.109	0.050
		18	0.080	0.080	0.082	0.080	0.178	0.263	0.123	0.080
		24	0.450	0.450	0.450	0.450	0.441	0.431	0.445	0.450
	M	12	0.230	0.231	0.230	0.231	0.301	0.280	0.349	0.231
		18	0.269	0.269	0.273	0.271	0.405	0.473	0.371	0.269
		24	0.657	0.657	0.658	0.658	0.661	0.662	0.660	0.657
	F	12	0.620	0.620	0.620	0.620	0.620	0.620	0.620	0.620
		18	0.310	0.309	0.309	0.309	0.309	0.309	0.309	0.309
		24	0.633	0.636	0.636	0.636	0.636	0.636	0.636	0.636
LN	I	12	0.033	0.033	0.033	0.033	0.047	0.041	0.057	0.033
		18	0.090	0.090	0.091	0.090	0.145	0.219	0.098	0.090
		24	0.600	0.601	0.601	0.601	0.594	0.583	0.600	0.601
	M	12	0.153	0.153	0.153	0.153	0.180	0.159	0.198	0.153
		18	0.253	0.252	0.254	0.253	0.310	0.372	0.276	0.252
		24	0.817	0.817	0.818	0.818	0.813	0.803	0.816	0.817
	F	12	0.420	0.420	0.420	0.420	0.420	0.420	0.420	0.420
		18	0.393	0.392	0.392	0.392	0.392	0.392	0.392	0.392
		24	0.800	0.799	0.799	0.799	0.799	0.799	0.799	0.799
CX	I	12	0.025	0.025	0.025	0.025	0.035	0.025	0.035	0.025
		18	0.080	0.080	0.080	0.080	0.089	0.188	0.082	0.080
		24	0.901	0.900	0.900	0.900	0.899	0.879	0.900	0.900
	M	12	0.085	0.085	0.085	0.085	0.100	0.085	0.101	0.085
		18	0.188	0.188	0.189	0.189	0.197	0.267	0.194	0.188
		24	0.976	0.976	0.976	0.976	0.975	0.960	0.976	0.976
	F	12	0.220	0.219	0.219	0.219	0.219	0.219	0.219	0.219
		18	0.310	0.310	0.310	0.310	0.310	0.310	0.310	0.310
		24	0.967	0.968	0.968	0.968	0.968	0.968	0.968	0.968

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months
- 4 - equivalent to unconstrained estimators of the overall death rate

Appendix C
Table C.11
Mean Values for Estimates of Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.033	0.033	0.033	0.033	0.095	0.110	0.131	0.033
		18	0.065	0.065	0.066	0.065	0.179	0.242	0.140	0.065
		24	0.330	0.328	0.328	0.328	0.320	0.315	0.319	0.328
	M	12	0.153	0.153	0.153	0.153	0.251	0.245	0.313	0.153
		18	0.177	0.177	0.182	0.180	0.341	0.390	0.322	0.177
		24	0.436	0.435	0.438	0.438	0.459	0.469	0.458	0.435
	F	12	0.420	0.421	0.421	0.421	0.421	0.421	0.421	0.421
		18	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
		24	0.391	0.391	0.391	0.391	0.391	0.391	0.391	0.391
LN	I	12	0.027	0.027	0.027	0.027	0.051	0.050	0.073	0.027
		18	0.072	0.072	0.073	0.072	0.148	0.210	0.096	0.072
		24	0.375	0.375	0.375	0.375	0.370	0.365	0.371	0.375
	M	12	0.102	0.102	0.102	0.102	0.138	0.123	0.173	0.102
		18	0.169	0.168	0.171	0.170	0.250	0.310	0.214	0.168
		24	0.494	0.493	0.494	0.494	0.497	0.499	0.494	0.493
	F	12	0.270	0.270	0.270	0.270	0.270	0.270	0.270	0.270
		18	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227
		24	0.500	0.499	0.499	0.499	0.499	0.499	0.499	0.499
CX	I	12	0.021	0.021	0.021	0.021	0.033	0.021	0.033	0.021
		18	0.063	0.063	0.063	0.063	0.066	0.159	0.065	0.063
		24	0.542	0.541	0.541	0.541	0.541	0.528	0.541	0.541
	M	12	0.036	0.036	0.036	0.036	0.050	0.036	0.051	0.036
		18	0.088	0.089	0.090	0.089	0.093	0.193	0.093	0.089
		24	0.685	0.686	0.686	0.686	0.686	0.670	0.686	0.686
	F	12	0.070	0.070	0.070	0.070	0.070	0.070	0.070	0.070
		18	0.110	0.111	0.111	0.111	0.111	0.111	0.111	0.111
		24	0.746	0.745	0.745	0.745	0.745	0.745	0.745	0.745

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

4. equivalent to unconstrained estimators of the overall death rate

Appendix C
Table C.12
Mean Values for Estimates of Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	true value	Method of Estimation						
				uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method ⁴
CV	I	12	0.024	0.024	0.024	0.024	0.089	0.104	0.131	0.024
		18	0.062	0.063	0.063	0.063	0.177	0.227	0.142	0.063
		24	0.308	0.307	0.308	0.308	0.299	0.294	0.296	0.307
	M	12	0.069	0.069	0.069	0.069	0.152	0.154	0.209	0.069
		18	0.104	0.105	0.108	0.107	0.240	0.282	0.220	0.105
		24	0.344	0.346	0.348	0.348	0.353	0.355	0.351	0.346
	F	12	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170
		18	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
		24	0.325	0.323	0.323	0.323	0.323	0.323	0.323	0.323
LN	I	12	0.022	0.022	0.022	0.022	0.063	0.071	0.098	0.022
		18	0.064	0.064	0.064	0.064	0.157	0.209	0.114	0.064
		24	0.319	0.319	0.319	0.319	0.312	0.308	0.311	0.319
	M	12	0.052	0.052	0.052	0.052	0.098	0.097	0.143	0.052
		18	0.101	0.101	0.103	0.102	0.194	0.246	0.164	0.101
		24	0.361	0.361	0.362	0.362	0.363	0.364	0.360	0.361
	F	12	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120
		18	0.116	0.115	0.115	0.115	0.115	0.115	0.115	0.115
		24	0.359	0.359	0.359	0.359	0.359	0.359	0.359	0.359
CX	I	12	0.021	0.021	0.021	0.021	0.039	0.042	0.056	0.021
		18	0.063	0.063	0.063	0.063	0.114	0.166	0.081	0.063
		24	0.339	0.339	0.339	0.340	0.336	0.331	0.336	0.339
	M	12	0.036	0.036	0.036	0.036	0.056	0.053	0.072	0.036
		18	0.088	0.088	0.089	0.089	0.130	0.181	0.107	0.088
		24	0.388	0.389	0.390	0.390	0.388	0.386	0.388	0.389
	F	12	0.070	0.069	0.069	0.069	0.069	0.069	0.069	0.069
		18	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110
		24	0.414	0.414	0.414	0.414	0.414	0.414	0.414	0.414

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

4. equivalent to unconstrained estimators of the overall death rate

Appendix D
Table D.1
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						
			uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method
CV	I	12	0.000	0.000	0.000	0.000	0.001	0.001	0.000
		18	0.007	0.007	0.006	0.009	0.013	0.005	0.007
		24	0.056	0.040	0.036	0.032	0.032	0.033	0.032
	M	12	0.000	0.000	0.000	0.000	0.000	0.001	0.000
		18	0.006	0.006	0.005	0.007	0.012	0.004	0.006
		24	0.048	0.035	0.031	0.028	0.027	0.028	0.028
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.010	0.010	0.010	0.010	0.010	0.010	0.010
LN	I	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.002	0.006	0.001	0.001
		24	0.033	0.020	0.020	0.016	0.015	0.016	0.016
	M	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.002	0.005	0.001	0.001
		24	0.025	0.017	0.017	0.013	0.013	0.014	0.013
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.007	0.007	0.007	0.007	0.007	0.007	0.007
CX	I	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.004	0.001	0.001
		24	0.011	0.009	0.009	0.008	0.008	0.008	0.008
	M	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.003	0.001	0.001
		24	0.009	0.008	0.008	0.007	0.007	0.007	0.007
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix D
Table D.2
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.001	0.002	0.002	0.000
		18	0.001	0.001	0.001	0.005	0.008	0.002	0.001
		24	0.013	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.000	0.002	0.002	0.003	0.000
		18	0.001	0.001	0.001	0.005	0.008	0.002	0.001
		24	0.011	0.005	0.005	0.005	0.004	0.005	0.005
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
LN	I	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.001	0.001	0.001	0.004	0.007	0.001	0.001
		24	0.010	0.005	0.005	0.004	0.004	0.005	0.004
	M	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.001	0.001	0.001	0.003	0.007	0.001	0.001
		24	0.007	0.005	0.005	0.004	0.004	0.005	0.004
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
CX	I	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.005	0.001	0.001
		24	0.003	0.004	0.004	0.003	0.003	0.003	0.003
	M	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.004	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months

Appendix D
Table D.3
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.003	0.004	0.005	0.000
		18	0.001	0.001	0.001	0.006	0.009	0.004	0.001
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.000	0.000	0.000	0.003	0.004	0.006	0.000
		18	0.001	0.001	0.001	0.006	0.009	0.004	0.001
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
LN	I	12	0.000	0.000	0.000	0.002	0.003	0.003	0.000
		18	0.001	0.001	0.001	0.005	0.009	0.003	0.001
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.000	0.000	0.000	0.002	0.002	0.003	0.000
		18	0.001	0.001	0.001	0.005	0.008	0.003	0.001
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CX	I	12	0.000	0.000	0.000	0.001	0.001	0.002	0.000
		18	0.001	0.001	0.001	0.003	0.006	0.001	0.001
		24	0.004	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.001	0.001	0.001	0.003	0.006	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix D
Table D.4
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.011	0.008	0.007	0.017	0.028	0.006	0.011
		24	0.108	0.050	0.048	0.040	0.041	0.042	0.040
	M	12	0.000	0.000	0.000	0.001	0.001	0.002	0.000
		18	0.010	0.008	0.007	0.016	0.028	0.006	0.010
		24	0.092	0.044	0.042	0.036	0.036	0.038	0.036
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		24	0.017	0.017	0.017	0.017	0.017	0.017	0.017
LN	I	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.003	0.002	0.002	0.007	0.017	0.002	0.003
		24	0.086	0.039	0.038	0.026	0.026	0.027	0.026
	M	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.002	0.002	0.002	0.006	0.015	0.002	0.002
		24	0.064	0.033	0.033	0.024	0.023	0.025	0.024
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.012	0.012	0.012	0.012	0.012	0.012	0.012
CX	I	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.002	0.011	0.001	0.001
		24	0.033	0.025	0.024	0.020	0.020	0.021	0.020
	M	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.002	0.009	0.001	0.001
		24	0.023	0.019	0.019	0.017	0.017	0.017	0.017
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.008	0.008	0.008	0.008	0.008	0.008	0.008

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2. I=incidental, M=intermediate, F=fatal
3. sacrifice time in months

Appendix D
Table D.5
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method
CV	I	12	0.000	0.000	0.000	0.005	0.007	0.007	0.000
		18	0.003	0.002	0.002	0.015	0.024	0.004	0.003
		24	0.042	0.010	0.010	0.012	0.013	0.013	0.012
	M	12	0.000	0.000	0.000	0.005	0.006	0.008	0.000
		18	0.003	0.002	0.002	0.015	0.024	0.005	0.003
		24	0.029	0.010	0.010	0.011	0.012	0.014	0.011
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.009	0.009	0.009	0.009	0.009	0.009	0.009
LN	I	12	0.000	0.000	0.000	0.002	0.003	0.004	0.000
		18	0.002	0.001	0.001	0.012	0.023	0.002	0.002
		24	0.028	0.011	0.011	0.009	0.009	0.009	0.009
	M	12	0.000	0.000	0.000	0.002	0.002	0.003	0.000
		18	0.002	0.001	0.001	0.010	0.021	0.003	0.002
		24	0.019	0.010	0.010	0.009	0.008	0.009	0.009
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.008	0.008	0.008	0.008	0.008	0.008	0.008
CX	I	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.001	0.016	0.001	0.001
		24	0.007	0.007	0.007	0.007	0.006	0.007	0.007
	M	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.001	0.015	0.001	0.001
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Appendix D
Table D.6
Mean Squared Error for Estimates of the Death Rate Without Tumor
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.011	0.015	0.017	0.000
		18	0.001	0.001	0.001	0.021	0.029	0.011	0.001
		24	0.010	0.006	0.006	0.006	0.006	0.006	0.006
	M	12	0.000	0.000	0.000	0.012	0.014	0.020	0.000
		18	0.001	0.001	0.001	0.021	0.030	0.013	0.001
		24	0.008	0.006	0.006	0.006	0.006	0.007	0.006
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006
LN	I	12	0.000	0.000	0.000	0.006	0.009	0.012	0.000
		18	0.001	0.001	0.001	0.018	0.029	0.008	0.001
		24	0.009	0.006	0.006	0.005	0.005	0.006	0.005
	M	12	0.000	0.000	0.000	0.006	0.008	0.012	0.000
		18	0.001	0.001	0.001	0.016	0.027	0.008	0.001
		24	0.007	0.006	0.006	0.005	0.005	0.006	0.005
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006
CX	I	12	0.000	0.000	0.000	0.003	0.005	0.005	0.000
		18	0.001	0.001	0.001	0.011	0.022	0.004	0.001
		24	0.007	0.006	0.006	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.000	0.002	0.004	0.004	0.000
		18	0.001	0.001	0.001	0.008	0.019	0.003	0.001
		24	0.006	0.006	0.006	0.005	0.005	0.005	0.005
	F	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

Appendix D
Table D.7
Mean Squared Error for Estimates of the Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.002	0.005	0.004	0.000
		18	0.001	0.001	0.001	0.009	0.018	0.003	0.001
		24	0.004	0.004	0.004	0.004	0.003	0.004	0.004
	M	12	0.002	0.002	0.002	0.015	0.015	0.024	0.002
		18	0.004	0.004	0.004	0.027	0.045	0.021	0.004
		24	0.008	0.008	0.008	0.009	0.009	0.009	0.008
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.009	0.009	0.009	0.009	0.009	0.009	0.009
		24	0.019	0.019	0.019	0.019	0.019	0.019	0.019
LN	I	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.001	0.001	0.001	0.007	0.017	0.001	0.001
		24	0.004	0.005	0.004	0.004	0.004	0.004	0.004
	M	12	0.002	0.002	0.002	0.005	0.003	0.008	0.002
		18	0.003	0.003	0.003	0.013	0.029	0.005	0.003
		24	0.007	0.007	0.007	0.007	0.007	0.007	0.007
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.007	0.007	0.007	0.007	0.007	0.007	0.007
		24	0.013	0.013	0.013	0.013	0.013	0.013	0.013
CX	I	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.002	0.021	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.006	0.005	0.005
	M	12	0.001	0.001	0.001	0.002	0.001	0.003	0.001
		18	0.002	0.002	0.002	0.004	0.030	0.002	0.002
		24	0.005	0.005	0.005	0.005	0.006	0.005	0.005
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2. I=incidental, M=intermediate, F=fatal
3. sacrifice time in months

Appendix D
Table D.8
Mean Squared Error for Estimates of the Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.004	0.006	0.006	0.000
		18	0.001	0.001	0.001	0.008	0.013	0.005	0.001
		24	0.003	0.003	0.003	0.003	0.002	0.003	0.003
	M	12	0.002	0.002	0.002	0.016	0.018	0.026	0.002
		18	0.002	0.003	0.003	0.025	0.034	0.024	0.002
		24	0.005	0.006	0.005	0.008	0.009	0.009	0.005
	F	12	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		24	0.007	0.007	0.007	0.007	0.007	0.007	0.007
LN	I	12	0.000	0.000	0.000	0.001	0.002	0.003	0.000
		18	0.001	0.001	0.001	0.006	0.012	0.002	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.001	0.001	0.001	0.005	0.005	0.010	0.001
		18	0.002	0.002	0.002	0.012	0.022	0.006	0.002
		24	0.006	0.006	0.006	0.005	0.006	0.005	0.006
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.008	0.008	0.008	0.008	0.008	0.008	0.008
CX	I	12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		18	0.001	0.001	0.001	0.001	0.012	0.001	0.001
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
	M	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.001	0.022	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006

1. shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2. I=incidental, M=intermediate, F=fatal

3. sacrifice time in months

Appendix D
Table D.9
Mean Squared Error for Estimates of the Overall Death Rate
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.000	0.000	0.000	0.004	0.006	0.007	0.000
		18	0.001	0.001	0.001	0.008	0.012	0.005	0.001
		24	0.003	0.003	0.003	0.002	0.002	0.002	0.003
	M	12	0.001	0.001	0.001	0.009	0.010	0.015	0.001
		18	0.001	0.001	0.001	0.014	0.018	0.012	0.001
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
LN	I	12	0.000	0.000	0.000	0.003	0.004	0.005	0.000
		18	0.001	0.001	0.001	0.007	0.011	0.004	0.001
		24	0.003	0.003	0.003	0.003	0.002	0.003	0.003
	M	12	0.001	0.001	0.001	0.004	0.005	0.009	0.001
		18	0.001	0.001	0.001	0.009	0.015	0.006	0.001
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.004	0.004	0.004	0.004	0.004	0.004	0.004
CX	I	12	0.000	0.000	0.000	0.001	0.002	0.002	0.000
		18	0.001	0.001	0.001	0.004	0.008	0.002	0.001
		24	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	M	12	0.000	0.000	0.000	0.002	0.002	0.003	0.000
		18	0.001	0.001	0.001	0.005	0.011	0.002	0.001
		24	0.004	0.004	0.004	0.004	0.003	0.004	0.004
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Appendix D
Table D.10
Mean Squared Error for Estimates of the Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.001	0.001	0.001	0.008	0.016	0.013	0.001
		18	0.001	0.001	0.001	0.032	0.064	0.010	0.001
		24	0.006	0.006	0.006	0.005	0.005	0.006	0.006
	M	12	0.003	0.003	0.003	0.022	0.024	0.036	0.003
		18	0.004	0.004	0.004	0.048	0.084	0.029	0.004
		24	0.008	0.008	0.008	0.007	0.006	0.007	0.008
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.009	0.009	0.009	0.009	0.009	0.009	0.009
		24	0.018	0.018	0.018	0.018	0.018	0.018	0.018
LN	I	12	0.001	0.001	0.001	0.002	0.004	0.004	0.001
		18	0.001	0.001	0.001	0.025	0.060	0.003	0.001
		24	0.005	0.005	0.005	0.006	0.006	0.006	0.005
	M	12	0.002	0.002	0.002	0.007	0.005	0.012	0.002
		18	0.004	0.004	0.004	0.027	0.062	0.007	0.004
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.007	0.007	0.007	0.007	0.007	0.007	0.007
		24	0.009	0.009	0.009	0.009	0.009	0.009	0.009
CX	I	12	0.000	0.000	0.000	0.001	0.001	0.001	0.000
		18	0.001	0.001	0.001	0.006	0.076	0.001	0.001
		24	0.002	0.002	0.002	0.002	0.005	0.002	0.002
	M	12	0.001	0.001	0.001	0.003	0.001	0.004	0.001
		18	0.003	0.003	0.003	0.005	0.053	0.003	0.003
		24	0.001	0.001	0.001	0.001	0.003	0.001	0.001
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.005	0.005	0.005	0.005	0.005	0.005	0.005
		24	0.001	0.001	0.001	0.001	0.001	0.001	0.001

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in months

Appendix D
Table D.11
Mean Squared Error for Estimates of the Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						Williams method
			unconstrained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	
CV	I	12	0.001	0.001	0.001	0.014	0.021	0.022	0.001
		18	0.001	0.001	0.001	0.028	0.046	0.017	0.001
		24	0.005	0.005	0.005	0.004	0.004	0.004	0.005
	M	12	0.002	0.002	0.002	0.029	0.033	0.047	0.002
		18	0.003	0.003	0.003	0.047	0.068	0.040	0.003
		24	0.007	0.007	0.007	0.007	0.006	0.007	0.007
	F	12	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.010	0.010	0.010	0.010	0.010	0.010	0.010
LN	I	12	0.000	0.000	0.000	0.004	0.008	0.009	0.000
		18	0.001	0.001	0.001	0.022	0.041	0.006	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	M	12	0.001	0.001	0.001	0.009	0.009	0.018	0.001
		18	0.003	0.003	0.003	0.026	0.050	0.011	0.003
		24	0.007	0.007	0.007	0.006	0.006	0.006	0.007
	F	12	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		18	0.004	0.004	0.004	0.004	0.004	0.004	0.004
		24	0.009	0.009	0.009	0.009	0.009	0.009	0.009
CX	I	12	0.000	0.000	0.000	0.001	0.000	0.001	0.000
		18	0.001	0.001	0.001	0.001	0.042	0.001	0.001
		24	0.005	0.005	0.005	0.005	0.006	0.005	0.005
	M	12	0.001	0.001	0.001	0.002	0.001	0.002	0.001
		18	0.001	0.001	0.001	0.002	0.056	0.002	0.001
		24	0.005	0.005	0.005	0.005	0.006	0.005	0.005
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix D
Table D.12
Mean Squared Error for Estimates of the Overall Death Rate
Based on High Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation						
			uncon- strained	analytic constraint	numeric constraint	intuitive pooling	early pooling	late pooling	Williams method
CV	I	12	0.000	0.000	0.000	0.015	0.021	0.024	0.000
		18	0.001	0.001	0.001	0.028	0.040	0.017	0.001
		24	0.005	0.005	0.005	0.004	0.004	0.004	0.005
	M	12	0.001	0.001	0.001	0.022	0.025	0.037	0.001
		18	0.002	0.002	0.002	0.035	0.048	0.028	0.002
		24	0.006	0.006	0.006	0.005	0.004	0.004	0.006
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006
LN	I	12	0.000	0.000	0.000	0.009	0.013	0.016	0.000
		18	0.001	0.001	0.001	0.024	0.037	0.011	0.001
		24	0.005	0.005	0.005	0.004	0.004	0.005	0.005
	M	12	0.001	0.001	0.001	0.011	0.014	0.022	0.001
		18	0.002	0.002	0.002	0.025	0.041	0.015	0.002
		24	0.005	0.005	0.005	0.005	0.004	0.005	0.005
	F	12	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006
CX	I	12	0.000	0.000	0.000	0.003	0.006	0.006	0.000
		18	0.001	0.001	0.001	0.014	0.028	0.004	0.001
		24	0.005	0.005	0.005	0.005	0.004	0.005	0.005
	M	12	0.001	0.001	0.001	0.004	0.006	0.007	0.001
		18	0.001	0.001	0.001	0.012	0.029	0.005	0.001
		24	0.005	0.005	0.005	0.005	0.005	0.005	0.005
	F	12	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		24	0.006	0.006	0.006	0.006	0.006	0.006	0.006

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months

Appendix E: Table E.1
Mean Values for Estimates of Death Rate Without Tumor for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.010	0.010	0.010	0.019	0.017	0.010
		15	0.040	0.055	0.047	0.067	0.070	0.055
		18	0.080	0.124	0.104	0.128	0.131	0.124
		21	0.100	0.181	0.142	0.192	0.194	0.181
		24	0.150	0.251	0.166	0.211	0.214	0.211
	M	12	0.010	0.010	0.010	0.019	0.016	0.010
		15	0.040	0.052	0.045	0.071	0.074	0.052
		18	0.080	0.113	0.095	0.130	0.137	0.113
		21	0.100	0.158	0.122	0.177	0.184	0.158
		24	0.150	0.212	0.153	0.187	0.191	0.187
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.039	0.039	0.039	0.039	0.039
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.150	0.150	0.150	0.150	0.150
LN	I	12	0.010	0.010	0.010	0.021	0.016	0.010
		15	0.040	0.044	0.039	0.057	0.059	0.044
		18	0.080	0.091	0.082	0.102	0.106	0.091
		21	0.100	0.125	0.107	0.135	0.137	0.125
		24	0.150	0.206	0.162	0.171	0.173	0.171
	M	12	0.010	0.010	0.010	0.020	0.014	0.010
		15	0.040	0.043	0.040	0.057	0.060	0.043
		18	0.080	0.088	0.081	0.104	0.110	0.088
		21	0.100	0.118	0.105	0.133	0.137	0.118
		24	0.150	0.184	0.155	0.164	0.165	0.164
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.150	0.150	0.150	0.150	0.150
CX	I	12	0.010	0.010	0.010	0.027	0.018	0.010
		15	0.040	0.041	0.039	0.057	0.058	0.041
		18	0.080	0.083	0.082	0.102	0.101	0.083
		21	0.100	0.108	0.102	0.115	0.115	0.108
		24	0.150	0.171	0.168	0.164	0.165	0.164
	M	12	0.010	0.010	0.010	0.024	0.015	0.010
		15	0.040	0.041	0.039	0.054	0.057	0.041
		18	0.080	0.081	0.080	0.098	0.098	0.081
		21	0.100	0.104	0.101	0.111	0.111	0.104
		24	0.150	0.159	0.159	0.161	0.160	0.161
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.151	0.151	0.151	0.151	0.151

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix E: Table E.2
Mean Values for Estimates of Death Rate Without Tumor for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.010	0.010	0.010	0.042	0.039	0.010
		15	0.040	0.044	0.037	0.086	0.090	0.044
		18	0.080	0.087	0.076	0.126	0.128	0.087
		21	0.100	0.113	0.097	0.153	0.152	0.113
		24	0.150	0.167	0.143	0.160	0.161	0.160
	M	12	0.010	0.010	0.010	0.045	0.039	0.010
		15	0.040	0.043	0.037	0.094	0.097	0.043
		18	0.080	0.084	0.075	0.135	0.140	0.084
		21	0.100	0.105	0.095	0.158	0.160	0.105
		24	0.150	0.156	0.144	0.165	0.166	0.165
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.099	0.099	0.099	0.099	0.099
		24	0.150	0.149	0.149	0.149	0.149	0.149
LN	I	12	0.010	0.010	0.010	0.031	0.025	0.010
		15	0.040	0.042	0.039	0.071	0.073	0.042
		18	0.080	0.083	0.078	0.119	0.121	0.083
		21	0.100	0.107	0.098	0.149	0.148	0.107
		24	0.150	0.165	0.148	0.161	0.161	0.161
	M	12	0.010	0.010	0.010	0.028	0.022	0.010
		15	0.040	0.041	0.039	0.071	0.073	0.041
		18	0.080	0.081	0.078	0.120	0.123	0.081
		21	0.100	0.105	0.099	0.148	0.147	0.105
		24	0.150	0.156	0.148	0.164	0.162	0.164
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.149	0.149	0.149	0.149	0.149
CX	I	12	0.010	0.010	0.010	0.022	0.016	0.010
		15	0.040	0.040	0.039	0.059	0.057	0.040
		18	0.080	0.081	0.079	0.100	0.102	0.081
		21	0.100	0.104	0.102	0.129	0.127	0.104
		24	0.150	0.160	0.150	0.152	0.151	0.152
	M	12	0.010	0.010	0.010	0.017	0.013	0.010
		15	0.040	0.040	0.039	0.056	0.054	0.040
		18	0.080	0.081	0.079	0.095	0.096	0.081
		21	0.100	0.101	0.100	0.122	0.121	0.101
		24	0.150	0.158	0.152	0.154	0.153	0.154
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.101	0.101	0.101	0.101	0.101
		24	0.150	0.150	0.150	0.150	0.150	0.150

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Appendix E: Table E.3
Mean Values for Estimates of Death Rate Without Tumor for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.010	0.010	0.010	0.059	0.054	0.010
		15	0.040	0.041	0.039	0.109	0.111	0.041
		18	0.080	0.082	0.077	0.150	0.151	0.082
		21	0.100	0.102	0.096	0.172	0.170	0.102
		24	0.150	0.154	0.145	0.176	0.175	0.176
	M	12	0.010	0.010	0.010	0.060	0.055	0.010
		15	0.040	0.041	0.038	0.117	0.116	0.041
		18	0.080	0.081	0.077	0.158	0.158	0.081
		21	0.100	0.101	0.097	0.176	0.175	0.101
		24	0.150	0.151	0.147	0.182	0.181	0.182
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.150	0.150	0.150	0.150	0.150
LN	I	12	0.010	0.010	0.010	0.048	0.042	0.010
		15	0.040	0.041	0.039	0.096	0.097	0.041
		18	0.080	0.081	0.078	0.141	0.142	0.081
		21	0.100	0.101	0.097	0.165	0.163	0.101
		24	0.150	0.154	0.148	0.172	0.171	0.172
	M	12	0.010	0.010	0.010	0.044	0.037	0.010
		15	0.040	0.040	0.039	0.095	0.094	0.040
		18	0.080	0.080	0.078	0.141	0.142	0.080
		21	0.100	0.101	0.098	0.165	0.164	0.101
		24	0.150	0.151	0.147	0.176	0.174	0.176
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.081	0.081	0.081	0.081	0.081
		21	0.100	0.101	0.101	0.101	0.101	0.101
		24	0.150	0.150	0.150	0.150	0.150	0.150
CX	I	12	0.010	0.010	0.010	0.039	0.033	0.010
		15	0.040	0.040	0.039	0.084	0.081	0.040
		18	0.080	0.081	0.079	0.128	0.129	0.081
		21	0.100	0.101	0.098	0.152	0.150	0.101
		24	0.150	0.152	0.150	0.165	0.164	0.165
	M	12	0.010	0.010	0.010	0.033	0.029	0.010
		15	0.040	0.040	0.039	0.078	0.075	0.040
		18	0.080	0.080	0.079	0.122	0.122	0.080
		21	0.100	0.101	0.099	0.145	0.144	0.101
		24	0.150	0.151	0.149	0.163	0.162	0.163
	F	12	0.010	0.010	0.010	0.010	0.010	0.010
		15	0.040	0.040	0.040	0.040	0.040	0.040
		18	0.080	0.080	0.080	0.080	0.080	0.080
		21	0.100	0.100	0.100	0.100	0.100	0.100
		24	0.150	0.149	0.149	0.149	0.149	0.149

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix E: Table E.4
Mean Values for Estimates of Overall Death Rate for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.025	0.025	0.025	0.055	0.047	0.025
		15	0.053	0.054	0.054	0.115	0.110	0.054
		18	0.100	0.100	0.100	0.169	0.163	0.100
		21	0.111	0.111	0.111	0.187	0.180	0.111
		24	0.162	0.162	0.161	0.195	0.190	0.162
	M	12	0.205	0.205	0.205	0.282	0.256	0.205
		15	0.243	0.242	0.243	0.397	0.373	0.242
		18	0.290	0.290	0.293	0.463	0.450	0.290
		21	0.285	0.284	0.290	0.464	0.452	0.284
		24	0.317	0.315	0.322	0.439	0.424	0.315
	F	12	0.610	0.610	0.610	0.610	0.610	0.610
		15	0.290	0.290	0.290	0.290	0.290	0.290
		18	0.280	0.280	0.280	0.280	0.280	0.280
		21	0.200	0.201	0.201	0.201	0.201	0.201
		24	0.224	0.223	0.223	0.223	0.223	0.223
LN	I	12	0.017	0.017	0.017	0.039	0.029	0.017
		15	0.048	0.047	0.048	0.088	0.085	0.047
		18	0.100	0.100	0.100	0.149	0.146	0.100
		21	0.133	0.133	0.133	0.192	0.184	0.133
		24	0.225	0.224	0.224	0.240	0.237	0.224
	M	12	0.137	0.137	0.137	0.189	0.159	0.137
		15	0.177	0.177	0.178	0.271	0.257	0.177
		18	0.242	0.241	0.243	0.342	0.336	0.241
		21	0.292	0.293	0.295	0.383	0.376	0.293
		24	0.410	0.410	0.412	0.440	0.434	0.410
	F	12	0.410	0.410	0.410	0.410	0.410	0.410
		15	0.207	0.207	0.207	0.207	0.207	0.207
		18	0.280	0.281	0.281	0.281	0.281	0.281
		21	0.350	0.348	0.348	0.348	0.348	0.348
		24	0.483	0.483	0.483	0.483	0.483	0.483
CX	I	12	0.013	0.012	0.013	0.035	0.024	0.012
		15	0.043	0.043	0.043	0.072	0.073	0.043
		18	0.096	0.096	0.096	0.130	0.125	0.096
		21	0.125	0.125	0.125	0.153	0.151	0.125
		24	0.375	0.375	0.374	0.375	0.374	0.375
	M	12	0.073	0.073	0.073	0.111	0.086	0.073
		15	0.103	0.104	0.105	0.159	0.158	0.104
		18	0.183	0.183	0.184	0.235	0.230	0.183
		21	0.236	0.237	0.239	0.268	0.264	0.237
		24	0.589	0.588	0.588	0.588	0.588	0.588
	F	12	0.210	0.210	0.210	0.210	0.210	0.210
		15	0.103	0.103	0.103	0.103	0.103	0.103
		18	0.247	0.248	0.248	0.248	0.248	0.248
		21	0.300	0.300	0.300	0.300	0.300	0.300
		24	0.750	0.750	0.750	0.750	0.750	0.750

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix E: Table E.5
Mean Values for Estimates of Overall Death Rate for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.017	0.017	0.017	0.073	0.066	0.017
		15	0.042	0.042	0.042	0.125	0.124	0.042
		18	0.084	0.083	0.084	0.169	0.166	0.083
		21	0.104	0.105	0.105	0.193	0.187	0.105
		24	0.157	0.156	0.156	0.195	0.191	0.156
	M	12	0.137	0.137	0.137	0.247	0.226	0.137
		15	0.147	0.147	0.150	0.322	0.306	0.147
		18	0.175	0.174	0.178	0.357	0.350	0.174
		21	0.182	0.181	0.187	0.362	0.356	0.181
		24	0.223	0.223	0.229	0.343	0.335	0.223
	F	12	0.410	0.410	0.410	0.410	0.410	0.410
		15	0.088	0.088	0.088	0.088	0.088	0.088
		18	0.125	0.125	0.125	0.125	0.125	0.125
		21	0.143	0.142	0.142	0.142	0.142	0.142
		24	0.192	0.192	0.192	0.192	0.192	0.192
LN	I	12	0.013	0.014	0.014	0.044	0.036	0.014
		15	0.044	0.044	0.044	0.094	0.093	0.044
		18	0.088	0.088	0.088	0.149	0.147	0.088
		21	0.111	0.111	0.111	0.182	0.175	0.111
		24	0.169	0.168	0.168	0.196	0.191	0.168
	M	12	0.088	0.089	0.089	0.143	0.122	0.089
		15	0.120	0.121	0.123	0.215	0.203	0.121
		18	0.168	0.167	0.170	0.275	0.267	0.167
		21	0.193	0.194	0.197	0.299	0.290	0.194
		24	0.256	0.256	0.259	0.306	0.297	0.256
	F	12	0.260	0.259	0.259	0.259	0.259	0.259
		15	0.123	0.122	0.122	0.122	0.122	0.122
		18	0.172	0.172	0.172	0.172	0.172	0.172
		21	0.199	0.199	0.199	0.199	0.199	0.199
		24	0.262	0.262	0.262	0.262	0.262	0.262
CX	I	12	0.011	0.011	0.011	0.024	0.017	0.011
		15	0.043	0.043	0.043	0.068	0.065	0.043
		18	0.089	0.089	0.089	0.117	0.118	0.089
		21	0.125	0.126	0.126	0.165	0.160	0.126
		24	0.190	0.190	0.190	0.204	0.200	0.190
	M	12	0.026	0.026	0.026	0.039	0.031	0.026
		15	0.076	0.075	0.076	0.106	0.102	0.075
		18	0.140	0.139	0.140	0.174	0.174	0.139
		21	0.214	0.212	0.213	0.253	0.248	0.212
		24	0.304	0.305	0.306	0.322	0.317	0.305
	F	12	0.060	0.060	0.060	0.060	0.060	0.060
		15	0.115	0.115	0.115	0.115	0.115	0.115
		18	0.180	0.180	0.180	0.180	0.180	0.180
		21	0.300	0.301	0.301	0.301	0.301	0.301
		24	0.360	0.361	0.361	0.361	0.361	0.361

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix E: Table E.6
Mean Values for Estimates of Overall Death Rate for a 5-interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation					Williams method
			true value	unconstrained	numeric constraint	intuitive pooling	MSPD pooling	
CV	I	12	0.012	0.012	0.012	0.070	0.064	0.012
		15	0.041	0.041	0.042	0.126	0.127	0.041
		18	0.081	0.081	0.082	0.170	0.168	0.081
		21	0.101	0.101	0.101	0.191	0.187	0.101
		24	0.152	0.152	0.152	0.193	0.190	0.152
	M	12	0.057	0.057	0.057	0.135	0.126	0.057
		15	0.080	0.080	0.082	0.205	0.197	0.080
		18	0.113	0.113	0.116	0.246	0.242	0.113
		21	0.127	0.127	0.130	0.257	0.253	0.127
		24	0.172	0.172	0.176	0.252	0.247	0.172
	F	12	0.160	0.160	0.160	0.160	0.160	0.160
		15	0.064	0.064	0.064	0.064	0.064	0.064
		18	0.095	0.095	0.095	0.095	0.095	0.095
		21	0.112	0.112	0.112	0.112	0.112	0.112
		24	0.160	0.160	0.160	0.160	0.160	0.160
LN	I	12	0.011	0.011	0.012	0.055	0.048	0.011
		15	0.041	0.041	0.041	0.108	0.107	0.041
		18	0.082	0.083	0.083	0.156	0.155	0.083
		21	0.103	0.103	0.103	0.182	0.177	0.103
		24	0.155	0.155	0.155	0.189	0.185	0.155
	M	12	0.041	0.041	0.041	0.092	0.081	0.041
		15	0.070	0.070	0.072	0.156	0.149	0.070
		18	0.111	0.111	0.113	0.208	0.205	0.111
		21	0.131	0.131	0.133	0.230	0.226	0.131
		24	0.182	0.182	0.185	0.234	0.229	0.182
	F	12	0.110	0.110	0.110	0.110	0.110	0.110
		15	0.068	0.068	0.068	0.068	0.068	0.068
		18	0.109	0.109	0.109	0.109	0.109	0.109
		21	0.129	0.130	0.130	0.130	0.130	0.130
		24	0.180	0.180	0.180	0.180	0.180	0.180
CX	I	12	0.011	0.010	0.011	0.042	0.035	0.010
		15	0.041	0.041	0.041	0.090	0.087	0.041
		18	0.083	0.083	0.083	0.139	0.138	0.083
		21	0.104	0.103	0.104	0.164	0.161	0.103
		24	0.163	0.163	0.163	0.182	0.180	0.163
	M	12	0.026	0.025	0.026	0.057	0.050	0.025
		15	0.058	0.058	0.058	0.111	0.105	0.058
		18	0.104	0.103	0.105	0.164	0.162	0.103
		21	0.129	0.129	0.131	0.191	0.189	0.129
		24	0.201	0.202	0.203	0.222	0.219	0.202
	F	12	0.060	0.060	0.060	0.060	0.060	0.060
		15	0.061	0.061	0.061	0.061	0.061	0.061
		18	0.112	0.112	0.112	0.112	0.112	0.112
		21	0.137	0.137	0.137	0.137	0.137	0.137
		24	0.227	0.226	0.226	0.226	0.226	0.226

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in months

Appendix F: Table F.1
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.001	0.000	0.000
		15	0.009	0.007	0.010	0.011	0.009
		18	0.034	0.028	0.029	0.030	0.034
		21	0.075	0.051	0.067	0.068	0.075
		24	0.107	0.043	0.073	0.074	0.073
	M	12	0.000	0.000	0.001	0.001	0.000
		15	0.008	0.007	0.010	0.011	0.008
		18	0.026	0.021	0.024	0.026	0.026
		21	0.055	0.035	0.048	0.049	0.055
		24	0.074	0.033	0.048	0.049	0.048
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.004	0.004	0.004	0.004	0.004
		21	0.008	0.008	0.008	0.008	0.008
		24	0.021	0.021	0.021	0.021	0.021
LN	I	12	0.000	0.000	0.001	0.000	0.000
		15	0.002	0.001	0.003	0.003	0.002
		18	0.006	0.003	0.005	0.006	0.006
		21	0.019	0.010	0.014	0.015	0.019
		24	0.063	0.025	0.033	0.034	0.033
	M	12	0.000	0.000	0.001	0.000	0.000
		15	0.001	0.001	0.003	0.004	0.001
		18	0.005	0.003	0.005	0.006	0.005
		21	0.014	0.008	0.011	0.012	0.014
		24	0.042	0.020	0.023	0.023	0.023
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.005	0.005	0.005	0.005	0.005
		24	0.015	0.015	0.015	0.015	0.015
CX	I	12	0.000	0.000	0.001	0.001	0.000
		15	0.001	0.001	0.002	0.002	0.001
		18	0.002	0.002	0.003	0.003	0.002
		21	0.006	0.004	0.005	0.005	0.006
		24	0.022	0.015	0.013	0.013	0.013
	M	12	0.000	0.000	0.001	0.001	0.000
		15	0.001	0.001	0.002	0.002	0.001
		18	0.002	0.002	0.003	0.003	0.002
		21	0.004	0.003	0.004	0.004	0.004
		24	0.013	0.011	0.011	0.011	0.011
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.003	0.003	0.003	0.003	0.003
		24	0.008	0.008	0.008	0.008	0.008

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix F: Table F.2
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.003	0.003	0.000
		15	0.002	0.001	0.006	0.007	0.002
		18	0.004	0.002	0.007	0.008	0.004
		21	0.008	0.004	0.009	0.009	0.008
		24	0.017	0.007	0.007	0.007	0.007
	M	12	0.000	0.000	0.004	0.003	0.000
		15	0.001	0.001	0.008	0.009	0.001
		18	0.003	0.002	0.008	0.009	0.003
		21	0.005	0.003	0.009	0.009	0.005
		24	0.011	0.008	0.007	0.007	0.007
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.003	0.003	0.003	0.003	0.003
		24	0.008	0.008	0.008	0.008	0.008
LN	I	12	0.000	0.000	0.002	0.001	0.000
		15	0.001	0.001	0.004	0.005	0.001
		18	0.002	0.002	0.006	0.006	0.002
		21	0.005	0.003	0.008	0.008	0.005
		24	0.015	0.007	0.007	0.008	0.007
	M	12	0.000	0.000	0.002	0.001	0.000
		15	0.001	0.001	0.004	0.005	0.001
		18	0.002	0.002	0.006	0.007	0.002
		21	0.004	0.003	0.008	0.008	0.004
		24	0.010	0.007	0.007	0.007	0.007
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.003	0.003	0.003	0.003	0.003
		24	0.007	0.007	0.007	0.007	0.007
CX	I	12	0.000	0.000	0.001	0.001	0.000
		15	0.001	0.001	0.002	0.002	0.001
		18	0.001	0.001	0.003	0.003	0.001
		21	0.003	0.003	0.005	0.005	0.003
		24	0.011	0.007	0.007	0.007	0.007
	M	12	0.000	0.000	0.001	0.000	0.000
		15	0.001	0.001	0.002	0.002	0.001
		18	0.001	0.001	0.003	0.003	0.001
		21	0.003	0.002	0.005	0.004	0.003
		24	0.009	0.007	0.006	0.006	0.006
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001
		21	0.002	0.002	0.002	0.002	0.002
		24	0.006	0.006	0.006	0.006	0.006

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix F: Table F.3
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.007	0.006	0.000
		15	0.001	0.001	0.010	0.011	0.001
		18	0.002	0.001	0.010	0.010	0.002
		21	0.003	0.002	0.009	0.009	0.003
		24	0.006	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.008	0.007	0.000
		15	0.001	0.001	0.012	0.012	0.001
		18	0.002	0.001	0.011	0.011	0.002
		21	0.003	0.002	0.010	0.010	0.003
		24	0.005	0.005	0.005	0.005	0.005
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001
		21	0.002	0.002	0.002	0.002	0.002
		24	0.005	0.005	0.005	0.005	0.005
LN	I	12	0.000	0.000	0.005	0.004	0.000
		15	0.001	0.001	0.008	0.008	0.001
		18	0.002	0.001	0.008	0.008	0.002
		21	0.003	0.002	0.009	0.009	0.003
		24	0.006	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.005	0.004	0.000
		15	0.001	0.001	0.008	0.008	0.001
		18	0.001	0.001	0.009	0.009	0.001
		21	0.002	0.002	0.009	0.009	0.002
		24	0.005	0.005	0.005	0.005	0.005
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001
		21	0.002	0.002	0.002	0.002	0.002
		24	0.005	0.005	0.005	0.005	0.005
CX	I	12	0.000	0.000	0.004	0.003	0.000
		15	0.001	0.001	0.006	0.006	0.001
		18	0.001	0.001	0.006	0.006	0.001
		21	0.002	0.002	0.007	0.007	0.002
		24	0.005	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.003	0.003	0.000
		15	0.001	0.001	0.005	0.005	0.001
		18	0.001	0.001	0.006	0.006	0.001
		21	0.002	0.002	0.006	0.006	0.002
		24	0.005	0.004	0.005	0.005	0.005
	F	12	0.000	0.000	0.000	0.000	0.000
		15	0.001	0.001	0.001	0.001	0.001
		18	0.001	0.001	0.001	0.001	0.001
		21	0.002	0.002	0.002	0.002	0.002
		24	0.004	0.004	0.004	0.004	0.004

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix F: Table F.4
Mean Squared Error for Estimates of the Overall Death Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.004	0.004	0.000
		15	0.001	0.001	0.010	0.009	0.001
		18	0.001	0.001	0.010	0.009	0.001
		21	0.002	0.002	0.012	0.010	0.002
		24	0.004	0.004	0.007	0.006	0.004
	M	12	0.002	0.002	0.025	0.018	0.002
		15	0.003	0.003	0.046	0.038	0.003
		18	0.005	0.005	0.047	0.042	0.005
		21	0.008	0.009	0.051	0.046	0.008
		24	0.018	0.018	0.039	0.035	0.018
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.007	0.007	0.007	0.007	0.007
		18	0.011	0.011	0.011	0.011	0.011
		21	0.015	0.015	0.015	0.015	0.015
		24	0.029	0.029	0.029	0.029	0.029
LN	I	12	0.000	0.000	0.002	0.002	0.000
		15	0.001	0.001	0.005	0.005	0.001
		18	0.001	0.001	0.007	0.007	0.001
		21	0.002	0.002	0.010	0.009	0.002
		24	0.006	0.006	0.006	0.006	0.006
	M	12	0.001	0.001	0.012	0.006	0.001
		15	0.002	0.002	0.021	0.017	0.002
		18	0.004	0.004	0.023	0.021	0.004
		21	0.007	0.007	0.023	0.021	0.007
		24	0.016	0.015	0.016	0.016	0.016
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.004	0.004	0.004	0.004	0.004
		18	0.006	0.006	0.006	0.006	0.006
		21	0.012	0.012	0.012	0.012	0.012
		24	0.028	0.028	0.028	0.028	0.028
CX	I	12	0.000	0.000	0.002	0.001	0.000
		15	0.001	0.001	0.004	0.004	0.001
		18	0.001	0.001	0.004	0.004	0.001
		21	0.002	0.002	0.006	0.006	0.002
		24	0.007	0.007	0.007	0.007	0.007
	M	12	0.001	0.001	0.006	0.003	0.001
		15	0.001	0.001	0.009	0.009	0.001
		18	0.003	0.003	0.009	0.009	0.003
		21	0.005	0.005	0.008	0.008	0.005
		24	0.011	0.011	0.011	0.011	0.011
	F	12	0.002	0.002	0.002	0.002	0.002
		15	0.001	0.001	0.001	0.001	0.001
		18	0.004	0.004	0.004	0.004	0.004
		21	0.007	0.007	0.007	0.007	0.007
		24	0.012	0.012	0.012	0.012	0.012

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
- 2 - I=incidental, M=intermediate, F=fatal
- 3 - sacrifice time in months

Appendix F: Table F.5
Mean Squared Error for Estimates of the Overall Death Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			unconstrained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.009	0.008	0.000
		15	0.001	0.001	0.013	0.013	0.001
		18	0.001	0.001	0.012	0.012	0.001
		21	0.002	0.002	0.012	0.011	0.002
		24	0.004	0.004	0.006	0.006	0.004
	M	12	0.001	0.001	0.031	0.025	0.001
		15	0.002	0.002	0.045	0.041	0.002
		18	0.003	0.003	0.044	0.042	0.003
		21	0.004	0.005	0.043	0.041	0.004
		24	0.008	0.009	0.028	0.026	0.008
	F	12	0.003	0.003	0.003	0.003	0.003
		15	0.002	0.002	0.002	0.002	0.002
		18	0.003	0.003	0.003	0.003	0.003
		21	0.005	0.005	0.005	0.005	0.005
		24	0.009	0.009	0.009	0.009	0.009
LN	I	12	0.000	0.000	0.004	0.003	0.000
		15	0.001	0.001	0.007	0.007	0.001
		18	0.001	0.001	0.009	0.008	0.001
		21	0.002	0.002	0.010	0.009	0.002
		24	0.004	0.004	0.006	0.006	0.004
	M	12	0.001	0.001	0.011	0.008	0.001
		15	0.002	0.002	0.019	0.016	0.002
		18	0.003	0.003	0.021	0.019	0.003
		21	0.004	0.004	0.021	0.019	0.004
		24	0.008	0.008	0.012	0.011	0.008
	F	12	0.002	0.002	0.002	0.002	0.002
		15	0.002	0.002	0.002	0.002	0.002
		18	0.003	0.003	0.003	0.003	0.003
		21	0.005	0.005	0.005	0.005	0.005
		24	0.011	0.011	0.011	0.011	0.011
CX	I	12	0.000	0.000	0.001	0.001	0.000
		15	0.001	0.001	0.003	0.003	0.001
		18	0.001	0.001	0.004	0.004	0.001
		21	0.002	0.002	0.007	0.006	0.002
		24	0.005	0.005	0.005	0.005	0.005
	M	12	0.000	0.000	0.002	0.001	0.000
		15	0.001	0.001	0.005	0.004	0.001
		18	0.002	0.002	0.006	0.006	0.002
		21	0.004	0.004	0.009	0.008	0.004
		24	0.008	0.008	0.009	0.009	0.008
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.003	0.003	0.003	0.003	0.003
		21	0.005	0.005	0.005	0.005	0.005
		24	0.011	0.011	0.011	0.011	0.011

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix F: Table F.6
Mean Squared Error for Estimates of the Overall Death Rate for a 5-Interval Study Design
Based on Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. time ³	Method of Estimation				
			uncon- strained	numeric constraint	intuitive pooling	MSPD pooling	Williams method
CV	I	12	0.000	0.000	0.009	0.009	0.000
		15	0.001	0.001	0.014	0.014	0.001
		18	0.001	0.001	0.013	0.012	0.001
		21	0.002	0.002	0.012	0.011	0.002
		24	0.004	0.004	0.006	0.006	0.004
	M	12	0.001	0.001	0.017	0.016	0.001
		15	0.001	0.001	0.026	0.024	0.001
		18	0.002	0.002	0.024	0.023	0.002
		21	0.003	0.003	0.023	0.022	0.003
		24	0.005	0.005	0.013	0.013	0.005
	F	12	0.002	0.002	0.002	0.002	0.002
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.002	0.002	0.002	0.002	0.002
		24	0.005	0.005	0.005	0.005	0.005
LN	I	12	0.000	0.000	0.006	0.005	0.000
		15	0.001	0.001	0.010	0.010	0.001
		18	0.001	0.001	0.010	0.010	0.001
		21	0.002	0.002	0.011	0.010	0.002
		24	0.004	0.004	0.006	0.006	0.004
	M	12	0.000	0.000	0.010	0.008	0.000
		15	0.001	0.001	0.016	0.015	0.001
		18	0.002	0.002	0.016	0.016	0.002
		21	0.003	0.003	0.016	0.015	0.003
		24	0.005	0.005	0.009	0.009	0.005
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.003	0.003	0.003	0.003	0.003
		24	0.005	0.005	0.005	0.005	0.005
CX	I	12	0.000	0.000	0.004	0.003	0.000
		15	0.001	0.001	0.007	0.007	0.001
		18	0.001	0.001	0.007	0.007	0.001
		21	0.002	0.002	0.008	0.008	0.002
		24	0.004	0.004	0.005	0.005	0.004
	M	12	0.000	0.000	0.005	0.004	0.000
		15	0.001	0.001	0.009	0.008	0.001
		18	0.001	0.002	0.009	0.009	0.001
		21	0.002	0.002	0.009	0.009	0.002
		24	0.005	0.005	0.006	0.006	0.005
	F	12	0.001	0.001	0.001	0.001	0.001
		15	0.001	0.001	0.001	0.001	0.001
		18	0.002	0.002	0.002	0.002	0.002
		21	0.003	0.003	0.003	0.003	0.003
		24	0.006	0.006	0.006	0.006	0.006

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in months

Appendix G: Table G.1
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.003	0.004	0.004	0.009
		70	0.038	0.051	0.049	0.062
		110	0.136	0.228	0.197	0.168
	M	35	0.003	0.004	0.004	0.009
		70	0.038	0.051	0.049	0.060
		110	0.136	0.210	0.186	0.165
	F	35	0.003	0.004	0.004	0.010
		70	0.038	0.051	0.049	0.058
		110	0.136	0.192	0.177	0.159
LN	I	35	0.002	0.003	0.003	0.004
		70	0.032	0.041	0.041	0.049
		110	0.131	0.227	0.210	0.189
	M	35	0.002	0.003	0.003	0.004
		70	0.032	0.040	0.040	0.048
		110	0.131	0.221	0.208	0.190
	F	35	0.002	0.003	0.003	0.004
		70	0.032	0.041	0.041	0.046
		110	0.131	0.210	0.203	0.192
CX	I	35	0.002	0.002	0.002	0.003
		70	0.035	0.042	0.042	0.045
		110	0.128	0.246	0.243	0.236
	M	35	0.002	0.002	0.002	0.002
		70	0.035	0.043	0.042	0.044
		110	0.128	0.243	0.241	0.236
	F	35	0.002	0.002	0.002	0.002
		70	0.035	0.042	0.042	0.043
		110	0.128	0.236	0.235	0.232

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.2
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.003	0.003	0.003	0.019
		70	0.037	0.042	0.040	0.068
		110	0.148	0.173	0.153	0.134
	M	35	0.003	0.003	0.003	0.023
		70	0.037	0.041	0.039	0.071
		110	0.148	0.165	0.153	0.133
	F	35	0.003	0.003	0.003	0.026
		70	0.037	0.042	0.041	0.069
		110	0.148	0.161	0.154	0.132
LN	I	35	0.002	0.002	0.002	0.007
		70	0.032	0.035	0.035	0.053
		110	0.146	0.174	0.166	0.154
	M	35	0.002	0.002	0.002	0.008
		70	0.032	0.036	0.036	0.049
		110	0.146	0.171	0.167	0.159
	F	35	0.002	0.003	0.003	0.007
		70	0.032	0.035	0.035	0.045
		110	0.146	0.169	0.167	0.162
CX	I	35	0.002	0.002	0.002	0.003
		70	0.034	0.036	0.036	0.038
		110	0.143	0.191	0.191	0.190
	M	35	0.002	0.002	0.002	0.003
		70	0.034	0.036	0.036	0.037
		110	0.143	0.191	0.191	0.190
	F	35	0.002	0.002	0.002	0.003
		70	0.034	0.036	0.036	0.036
		110	0.143	0.191	0.191	0.191

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.3
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.002	0.002	0.002	0.028
		70	0.032	0.034	0.034	0.078
		110	0.154	0.161	0.154	0.142
	M	35	0.002	0.002	0.002	0.035
		70	0.032	0.033	0.034	0.079
		110	0.154	0.158	0.153	0.141
	F	35	0.002	0.002	0.002	0.037
		70	0.032	0.033	0.033	0.078
		110	0.154	0.157	0.154	0.140
LN	I	35	0.002	0.002	0.002	0.014
		70	0.031	0.032	0.033	0.066
		110	0.153	0.163	0.158	0.150
	M	35	0.002	0.002	0.002	0.016
		70	0.031	0.032	0.033	0.061
		110	0.153	0.160	0.157	0.151
	F	35	0.002	0.002	0.002	0.019
		70	0.031	0.032	0.033	0.059
		110	0.153	0.160	0.157	0.152
CX	I	35	0.003	0.003	0.003	0.007
		70	0.034	0.036	0.036	0.055
		110	0.149	0.160	0.158	0.154
	M	35	0.003	0.003	0.003	0.008
		70	0.034	0.035	0.035	0.049
		110	0.149	0.158	0.157	0.155
	F	35	0.003	0.003	0.003	0.008
		70	0.034	0.035	0.036	0.047
		110	0.149	0.159	0.158	0.157

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix G: Table G.4
 Mean Values for Estimates of Death Rate Without Tumor
 for a 3-Interval Study with Intermediate Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.002	0.002	0.002	0.028
		70	0.032	0.034	0.034	0.078
		110	0.154	0.161	0.154	0.142
	M	35	0.002	0.002	0.002	0.035
		70	0.032	0.033	0.034	0.079
		110	0.154	0.158	0.153	0.141
	F	35	0.002	0.002	0.002	0.037
		70	0.032	0.033	0.033	0.078
		110	0.154	0.157	0.154	0.140
LN	I	35	0.002	0.002	0.002	0.014
		70	0.031	0.032	0.033	0.066
		110	0.153	0.163	0.158	0.150
	M	35	0.002	0.002	0.002	0.016
		70	0.031	0.032	0.033	0.061
		110	0.153	0.160	0.157	0.151
	F	35	0.002	0.002	0.002	0.019
		70	0.031	0.032	0.033	0.059
		110	0.153	0.160	0.157	0.152
CX	I	35	0.003	0.003	0.003	0.007
		70	0.034	0.036	0.036	0.055
		110	0.149	0.160	0.158	0.154
	M	35	0.003	0.003	0.003	0.008
		70	0.034	0.035	0.035	0.049
		110	0.149	0.158	0.157	0.155
	F	35	0.003	0.003	0.003	0.008
		70	0.034	0.035	0.036	0.047
		110	0.149	0.159	0.158	0.157

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.5
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Intermediate Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.010	0.011	0.011	0.032
		78	0.047	0.053	0.048	0.076
		110	0.136	0.159	0.138	0.123
	M	52	0.010	0.011	0.011	0.036
		78	0.047	0.051	0.048	0.078
		110	0.136	0.148	0.138	0.123
	F	52	0.010	0.011	0.011	0.036
		78	0.047	0.051	0.049	0.074
		110	0.136	0.145	0.140	0.123
LN	I	52	0.010	0.011	0.011	0.021
		78	0.043	0.048	0.047	0.068
		110	0.139	0.165	0.154	0.144
	M	52	0.010	0.011	0.011	0.022
		78	0.043	0.049	0.048	0.064
		110	0.139	0.160	0.155	0.150
	F	52	0.010	0.011	0.011	0.022
		78	0.043	0.048	0.047	0.059
		110	0.139	0.157	0.155	0.151
CX	I	52	0.010	0.010	0.010	0.015
		78	0.047	0.051	0.051	0.056
		110	0.140	0.184	0.183	0.180
	M	52	0.010	0.010	0.010	0.015
		78	0.047	0.051	0.051	0.054
		110	0.140	0.182	0.182	0.181
	F	52	0.010	0.010	0.010	0.014
		78	0.047	0.051	0.051	0.052
		110	0.140	0.182	0.182	0.182

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.6
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Intermediate Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.010	0.010	0.010	0.043
		78	0.042	0.044	0.043	0.087
		110	0.139	0.146	0.138	0.133
	M	52	0.010	0.010	0.010	0.050
		78	0.042	0.043	0.042	0.089
		110	0.139	0.142	0.138	0.133
	F	52	0.010	0.010	0.010	0.050
		78	0.042	0.043	0.043	0.080
		110	0.139	0.141	0.139	0.132
LN	I	52	0.010	0.010	0.010	0.031
		78	0.041	0.043	0.042	0.080
		110	0.140	0.149	0.143	0.140
	M	52	0.010	0.010	0.010	0.034
		78	0.041	0.043	0.042	0.076
		110	0.140	0.146	0.143	0.140
	F	52	0.010	0.010	0.010	0.035
		78	0.041	0.043	0.043	0.071
		110	0.140	0.145	0.143	0.141
CX	I	52	0.012	0.012	0.012	0.023
		78	0.044	0.046	0.045	0.070
		110	0.136	0.147	0.144	0.142
	M	52	0.012	0.012	0.012	0.024
		78	0.044	0.045	0.045	0.063
		110	0.136	0.144	0.143	0.143
	F	52	0.012	0.013	0.013	0.024
		78	0.044	0.045	0.045	0.059
		110	0.136	0.144	0.144	0.144

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix G: Table G.7
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.027	0.035	0.035	0.044
		91	0.055	0.109	0.105	0.105
		110	0.110	0.218	0.207	0.179
	M	78	0.027	0.035	0.035	0.045
		91	0.055	0.085	0.082	0.085
		110	0.110	0.159	0.148	0.131
	F	78	0.027	0.035	0.035	0.043
		91	0.055	0.069	0.069	0.070
		110	0.110	0.134	0.127	0.117
LN	I	78	0.035	0.046	0.046	0.057
		91	0.065	0.096	0.086	0.098
		110	0.134	0.234	0.211	0.187
	M	78	0.035	0.045	0.045	0.056
		91	0.065	0.087	0.079	0.091
		110	0.134	0.202	0.185	0.172
	F	78	0.035	0.046	0.046	0.055
		91	0.065	0.082	0.076	0.084
		110	0.134	0.180	0.170	0.163
CX	I	78	0.049	0.062	0.062	0.074
		91	0.079	0.107	0.097	0.115
		110	0.154	0.280	0.251	0.224
	M	78	0.049	0.062	0.062	0.072
		91	0.079	0.102	0.096	0.108
		110	0.154	0.252	0.234	0.224
	F	78	0.049	0.061	0.061	0.069
		91	0.079	0.098	0.094	0.101
		110	0.154	0.232	0.224	0.221

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix G: Table G.8
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.037	0.039	0.039	0.060
		91	0.046	0.053	0.047	0.078
		110	0.100	0.119	0.100	0.097
	M	78	0.037	0.039	0.039	0.060
		91	0.046	0.049	0.048	0.077
		110	0.100	0.105	0.100	0.101
	F	78	0.037	0.039	0.039	0.054
		91	0.046	0.049	0.048	0.063
		110	0.100	0.104	0.102	0.102
LN	I	78	0.041	0.045	0.045	0.063
		91	0.050	0.055	0.051	0.083
		110	0.111	0.132	0.118	0.114
	M	78	0.041	0.045	0.045	0.062
		91	0.050	0.053	0.052	0.078
		110	0.111	0.123	0.118	0.119
	F	78	0.041	0.045	0.045	0.058
		91	0.050	0.053	0.053	0.072
		110	0.111	0.120	0.118	0.119
CX	I	78	0.053	0.058	0.058	0.070
		91	0.058	0.064	0.062	0.084
		110	0.123	0.154	0.150	0.146
	M	78	0.053	0.058	0.058	0.068
		91	0.058	0.062	0.061	0.076
		110	0.123	0.150	0.148	0.149
	F	78	0.053	0.058	0.058	0.065
		91	0.058	0.063	0.062	0.071
		110	0.123	0.148	0.147	0.148

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.9
Mean Values for Estimates of Death Rate Without Tumor
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.045	0.047	0.047	0.078
		91	0.044	0.045	0.043	0.094
		110	0.102	0.107	0.100	0.111
	M	78	0.045	0.046	0.046	0.075
		91	0.044	0.045	0.044	0.083
		110	0.102	0.103	0.101	0.113
	F	78	0.045	0.046	0.046	0.061
		91	0.044	0.044	0.044	0.061
		110	0.102	0.103	0.102	0.107
LN	I	78	0.046	0.048	0.048	0.075
		91	0.045	0.047	0.045	0.093
		110	0.105	0.111	0.105	0.114
	M	78	0.046	0.048	0.047	0.073
		91	0.045	0.046	0.046	0.083
		110	0.105	0.107	0.105	0.115
	F	78	0.046	0.048	0.048	0.065
		91	0.045	0.046	0.046	0.068
		110	0.105	0.107	0.106	0.112
CX	I	78	0.053	0.055	0.055	0.076
		91	0.046	0.047	0.047	0.088
		110	0.102	0.109	0.106	0.113
	M	78	0.053	0.054	0.054	0.071
		91	0.046	0.047	0.047	0.078
		110	0.102	0.106	0.104	0.112
	F	78	0.053	0.055	0.055	0.067
		91	0.046	0.047	0.047	0.068
		110	0.102	0.107	0.106	0.112

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.10
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.007	0.007	0.007	0.019
		70	0.060	0.060	0.061	0.099
		110	0.216	0.216	0.216	0.218
	M	35	0.057	0.050	0.050	0.120
		70	0.453	0.424	0.427	0.488
		110	0.706	0.699	0.702	0.714
	F	35	0.102	0.089	0.089	0.163
		70	0.586	0.556	0.560	0.591
		110	0.606	0.621	0.623	0.656
LN	I	35	0.003	0.003	0.003	0.006
		70	0.055	0.055	0.055	0.076
		110	0.294	0.293	0.293	0.293
	M	35	0.012	0.010	0.010	0.022
		70	0.314	0.273	0.273	0.308
		110	0.796	0.780	0.781	0.778
	F	35	0.021	0.017	0.017	0.031
		70	0.441	0.392	0.393	0.409
		110	0.757	0.762	0.763	0.764
CX	I	35	0.002	0.002	0.002	0.003
		70	0.053	0.053	0.053	0.060
		110	0.393	0.393	0.393	0.393
	M	35	0.003	0.002	0.002	0.005
		70	0.196	0.160	0.160	0.167
		110	0.852	0.828	0.828	0.827
	F	35	0.004	0.003	0.003	0.006
		70	0.282	0.230	0.230	0.234
		110	0.840	0.836	0.836	0.836

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.11
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.004	0.004	0.004	0.031
		70	0.042	0.043	0.044	0.092
		110	0.167	0.166	0.166	0.167
	M	35	0.035	0.032	0.032	0.144
		70	0.288	0.274	0.281	0.368
		110	0.417	0.419	0.425	0.460
	F	35	0.063	0.059	0.059	0.183
		70	0.369	0.358	0.368	0.424
		110	0.327	0.340	0.344	0.421
LN	I	35	0.003	0.003	0.003	0.009
		70	0.040	0.040	0.040	0.068
		110	0.194	0.193	0.193	0.193
	M	35	0.012	0.010	0.010	0.036
		70	0.212	0.187	0.190	0.226
		110	0.500	0.494	0.495	0.499
	F	35	0.020	0.016	0.016	0.045
		70	0.286	0.256	0.259	0.280
		110	0.449	0.460	0.461	0.469
CX	I	35	0.002	0.002	0.002	0.003
		70	0.039	0.039	0.039	0.041
		110	0.247	0.246	0.246	0.246
	M	35	0.002	0.002	0.002	0.005
		70	0.097	0.077	0.078	0.080
		110	0.594	0.557	0.558	0.557
	F	35	0.002	0.002	0.002	0.006
		70	0.129	0.100	0.101	0.101
		110	0.584	0.574	0.574	0.574

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix G: Table G.12
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Early Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	35	0.003	0.003	0.003	0.032
		70	0.034	0.034	0.036	0.088
		110	0.160	0.161	0.161	0.158
	M	35	0.010	0.010	0.010	0.083
		70	0.121	0.116	0.122	0.191
		110	0.254	0.254	0.258	0.270
	F	35	0.017	0.016	0.016	0.094
		70	0.155	0.149	0.157	0.213
		110	0.220	0.224	0.228	0.254
LN	I	35	0.002	0.002	0.002	0.015
		70	0.033	0.033	0.034	0.073
		110	0.166	0.166	0.166	0.165
	M	35	0.006	0.005	0.005	0.036
		70	0.101	0.091	0.095	0.140
		110	0.278	0.277	0.280	0.282
	F	35	0.009	0.008	0.008	0.045
		70	0.130	0.118	0.123	0.158
		110	0.254	0.260	0.262	0.270
CX	I	35	0.003	0.003	0.003	0.008
		70	0.037	0.037	0.037	0.059
		110	0.169	0.170	0.170	0.170
	M	35	0.004	0.004	0.004	0.014
		70	0.084	0.072	0.073	0.096
		110	0.298	0.290	0.291	0.291
	F	35	0.006	0.005	0.005	0.017
		70	0.103	0.090	0.092	0.107
		110	0.284	0.287	0.287	0.289

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.13
 Mean Values for Estimates of Overall Death Rate
 for a 3-Interval Study with Intermediate Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.025	0.025	0.025	0.051
		78	0.071	0.071	0.072	0.117
		110	0.192	0.192	0.192	0.199
	M	52	0.220	0.197	0.197	0.309
		78	0.481	0.461	0.467	0.552
		110	0.624	0.619	0.624	0.662
	F	52	0.350	0.316	0.316	0.402
		78	0.547	0.534	0.543	0.601
		110	0.502	0.518	0.522	0.594
LN	I	52	0.017	0.017	0.017	0.028
		78	0.075	0.075	0.075	0.108
		110	0.269	0.268	0.268	0.270
	M	52	0.090	0.074	0.074	0.117
		78	0.405	0.369	0.371	0.413
		110	0.743	0.729	0.731	0.734
	F	52	0.150	0.122	0.122	0.164
		78	0.510	0.477	0.481	0.502
		110	0.680	0.690	0.691	0.700
CX	I	52	0.012	0.013	0.013	0.018
		78	0.082	0.082	0.082	0.099
		110	0.368	0.367	0.367	0.367
	M	52	0.034	0.027	0.027	0.042
		78	0.311	0.267	0.268	0.287
		110	0.820	0.798	0.798	0.797
	F	52	0.052	0.040	0.040	0.054
		78	0.410	0.357	0.358	0.365
		110	0.795	0.796	0.797	0.797

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in weeks

Appendix G: Table G.14
 Mean Values for Estimates of Overall Death Rate
 for a 3-Interval Study with Intermediate Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.017	0.017	0.017	0.054
		78	0.051	0.051	0.053	0.105
		110	0.148	0.148	0.148	0.155
	M	52	0.137	0.128	0.127	0.249
		78	0.294	0.286	0.295	0.396
		110	0.342	0.344	0.350	0.426
	F	52	0.221	0.207	0.207	0.310
		78	0.314	0.313	0.325	0.410
		110	0.256	0.267	0.272	0.383
LN	I	52	0.013	0.013	0.013	0.029
		78	0.052	0.052	0.052	0.086
		110	0.175	0.174	0.174	0.177
	M	52	0.070	0.059	0.059	0.112
		78	0.254	0.235	0.240	0.287
		110	0.437	0.434	0.437	0.451
	F	52	0.113	0.094	0.094	0.145
		78	0.300	0.283	0.289	0.321
		110	0.379	0.391	0.392	0.413
CX	I	52	0.011	0.011	0.011	0.016
		78	0.055	0.055	0.056	0.062
		110	0.227	0.226	0.226	0.227
	M	52	0.018	0.015	0.015	0.026
		78	0.156	0.129	0.130	0.136
		110	0.557	0.525	0.525	0.525
	F	52	0.024	0.019	0.019	0.029
		78	0.194	0.163	0.164	0.166
		110	0.540	0.534	0.534	0.535

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.15
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Intermediate Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	52	0.012	0.012	0.012	0.050
		78	0.043	0.044	0.045	0.099
		110	0.143	0.145	0.144	0.148
	M	52	0.048	0.045	0.045	0.127
		78	0.133	0.129	0.136	0.210
		110	0.213	0.214	0.219	0.251
	F	52	0.075	0.070	0.070	0.144
		78	0.143	0.141	0.149	0.209
		110	0.182	0.185	0.189	0.228
LN	I	52	0.011	0.011	0.011	0.035
		78	0.044	0.044	0.045	0.088
		110	0.150	0.150	0.150	0.153
	M	52	0.033	0.029	0.029	0.079
		78	0.121	0.114	0.119	0.170
		110	0.240	0.240	0.243	0.258
	F	52	0.051	0.044	0.044	0.093
		78	0.136	0.131	0.136	0.179
		110	0.216	0.221	0.223	0.242
CX	I	52	0.013	0.013	0.013	0.025
		78	0.047	0.047	0.048	0.076
		110	0.152	0.153	0.153	0.156
	M	52	0.025	0.021	0.021	0.043
		78	0.107	0.096	0.099	0.126
		110	0.264	0.258	0.259	0.264
	F	52	0.035	0.028	0.028	0.051
		78	0.120	0.112	0.114	0.135
		110	0.248	0.252	0.252	0.258

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix G: Table G.16
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.094	0.094	0.094	0.127
		91	0.065	0.064	0.066	0.132
		110	0.136	0.137	0.137	0.156
	M	78	0.606	0.567	0.566	0.629
		91	0.329	0.339	0.354	0.542
		110	0.417	0.424	0.430	0.535
	F	78	0.711	0.681	0.680	0.710
		91	0.248	0.278	0.292	0.474
		110	0.324	0.333	0.342	0.434
LN	I	78	0.090	0.090	0.090	0.122
		91	0.084	0.084	0.086	0.150
		110	0.202	0.202	0.201	0.214
	M	78	0.473	0.415	0.415	0.482
		91	0.374	0.376	0.384	0.508
		110	0.571	0.567	0.571	0.608
	F	78	0.593	0.541	0.540	0.580
		91	0.350	0.373	0.382	0.491
		110	0.496	0.505	0.508	0.552
CX	I	78	0.093	0.093	0.093	0.120
		91	0.109	0.109	0.110	0.167
		110	0.290	0.290	0.290	0.297
	M	78	0.346	0.287	0.287	0.339
		91	0.383	0.371	0.375	0.448
		110	0.696	0.679	0.680	0.689
	F	78	0.452	0.383	0.382	0.417
		91	0.402	0.408	0.413	0.465
		110	0.649	0.655	0.656	0.666

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice time in weeks

Appendix G: Table G.17
 Mean Values for Estimates of Overall Death Rate
 for a 3-Interval Study with Late Sacrifice Times
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.067	0.067	0.067	0.105
		91	0.048	0.048	0.050	0.118
		110	0.105	0.105	0.105	0.130
	M	78	0.396	0.377	0.376	0.438
		91	0.165	0.173	0.184	0.365
		110	0.203	0.208	0.216	0.342
	F	78	0.468	0.455	0.455	0.484
		91	0.112	0.124	0.129	0.258
		110	0.158	0.164	0.169	0.266
LN	I	78	0.064	0.064	0.064	0.094
		91	0.054	0.054	0.056	0.112
		110	0.128	0.128	0.128	0.142
	M	78	0.315	0.279	0.279	0.335
		91	0.189	0.196	0.204	0.320
		110	0.292	0.296	0.300	0.352
	F	78	0.385	0.351	0.350	0.385
		91	0.161	0.178	0.185	0.288
		110	0.252	0.259	0.261	0.305
CX	I	78	0.065	0.065	0.065	0.080
		91	0.067	0.067	0.067	0.099
		110	0.172	0.171	0.171	0.176
	M	78	0.180	0.142	0.141	0.170
		91	0.191	0.184	0.187	0.225
		110	0.440	0.419	0.419	0.424
	F	78	0.220	0.178	0.178	0.199
		91	0.200	0.203	0.206	0.234
		110	0.420	0.416	0.416	0.420

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
 2 - I=incidental, M=intermediate, F=fatal
 3 - sacrifice time in weeks

Appendix G: Table G.18
Mean Values for Estimates of Overall Death Rate
for a 3-Interval Study with Late Sacrifice Times
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation			
			true value	uncon- strained	analytic constraint	intuitive pooling
CV	I	78	0.055	0.055	0.055	0.093
		91	0.044	0.045	0.046	0.109
		110	0.103	0.105	0.105	0.126
	M	78	0.177	0.169	0.168	0.215
		91	0.086	0.088	0.092	0.181
		110	0.137	0.138	0.144	0.202
	F	78	0.209	0.201	0.201	0.221
		91	0.068	0.071	0.072	0.118
		110	0.122	0.123	0.126	0.157
LN	I	78	0.054	0.054	0.054	0.086
		91	0.046	0.046	0.047	0.105
		110	0.109	0.109	0.109	0.126
	M	78	0.154	0.140	0.140	0.182
		91	0.090	0.094	0.098	0.175
		110	0.160	0.161	0.165	0.204
	F	78	0.182	0.169	0.169	0.194
		91	0.079	0.085	0.087	0.137
		110	0.146	0.149	0.152	0.181
CX	I	78	0.059	0.059	0.059	0.083
		91	0.048	0.048	0.049	0.097
		110	0.110	0.111	0.110	0.124
	M	78	0.134	0.115	0.115	0.144
		91	0.091	0.093	0.096	0.152
		110	0.183	0.181	0.183	0.203
	F	78	0.154	0.137	0.137	0.158
		91	0.087	0.093	0.095	0.136
		110	0.174	0.175	0.177	0.194

- 1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex
2 - I=incidental, M=intermediate, F=fatal
3 - sacrifice time in weeks

Appendix H: Table H.1
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. 3	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.001	0.003
		2	0.003	0.011	0.043	0.002	0.008	0.043	0.005	0.009	0.038
		3	0.051	0.061	0.087	0.032	0.043	0.084	0.029	0.039	0.072
	M	1	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.001	0.003
		2	0.004	0.010	0.023	0.003	0.007	0.021	0.004	0.009	0.019
		3	0.038	0.041	0.039	0.024	0.028	0.035	0.022	0.026	0.029
	F	1	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.001	0.003
		2	0.003	0.006	0.011	0.002	0.004	0.009	0.004	0.005	0.009
		3	0.028	0.024	0.020	0.020	0.019	0.017	0.019	0.018	0.015
LN	I	1	0.000	0.000	0.003	0.000	0.000	0.003	0.000	0.000	0.002
		2	0.001	0.003	0.016	0.001	0.002	0.011	0.002	0.004	0.012
		3	0.038	0.045	0.064	0.024	0.026	0.048	0.024	0.024	0.043
	M	1	0.000	0.000	0.003	0.000	0.000	0.003	0.000	0.000	0.002
		2	0.001	0.003	0.010	0.001	0.002	0.007	0.002	0.004	0.008
		3	0.033	0.036	0.039	0.023	0.022	0.028	0.022	0.021	0.025
	F	1	0.000	0.000	0.003	0.000	0.000	0.003	0.000	0.000	0.002
		2	0.001	0.003	0.007	0.001	0.002	0.005	0.002	0.003	0.006
		3	0.026	0.024	0.023	0.021	0.019	0.018	0.021	0.018	0.016

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.1 (cont'd)
 Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.002
		2	0.001	0.002	0.009	0.001	0.002	0.005	0.001	0.003	0.007
		3	0.039	0.042	0.061	0.036	0.032	0.034	0.037	0.031	0.032
M		1	0.000	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.002
		2	0.001	0.002	0.007	0.001	0.002	0.004	0.001	0.003	0.006
		3	0.037	0.035	0.040	0.036	0.030	0.026	0.036	0.029	0.024
F		1	0.000	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.002
		2	0.001	0.002	0.005	0.001	0.002	0.004	0.001	0.002	0.005
		3	0.037	0.032	0.028	0.036	0.029	0.022	0.036	0.029	0.021

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.2
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. 3	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.002	0.001
		2	0.001	0.002	0.003	0.001	0.001	0.001	0.005	0.004	0.003
		3	0.015	0.014	0.011	0.006	0.006	0.005	0.006	0.005	0.004
	M	1	0.000	0.000	0.001	0.000	0.000	0.001	0.002	0.002	0.002
		2	0.001	0.002	0.002	0.001	0.001	0.001	0.004	0.004	0.003
		3	0.008	0.007	0.005	0.005	0.005	0.004	0.005	0.005	0.003
	F	1	0.000	0.000	0.001	0.000	0.000	0.001	0.002	0.002	0.001
		2	0.002	0.002	0.002	0.001	0.001	0.001	0.004	0.004	0.002
		3	0.007	0.006	0.004	0.005	0.005	0.004	0.006	0.005	0.003
LN	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.003	0.004	0.004
		3	0.010	0.010	0.010	0.006	0.006	0.005	0.006	0.005	0.004
	M	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.003	0.003	0.003
		3	0.007	0.007	0.005	0.006	0.006	0.004	0.006	0.005	0.004
	F	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.001
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.002	0.003	0.003
		3	0.006	0.006	0.004	0.006	0.005	0.004	0.005	0.005	0.004

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.2 (cont'd)
 Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.003
		3	0.008	0.008	0.008	0.008	0.007	0.006	0.008	0.007	0.005
	M	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.003
		3	0.008	0.007	0.006	0.008	0.007	0.005	0.008	0.007	0.005
	F	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
		3	0.008	0.007	0.005	0.007	0.007	0.005	0.007	0.006	0.005

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.3
Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.003	0.004	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.005	0.005
		3	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.002
	M	1	0.000	0.000	0.001	0.000	0.000	0.001	0.004	0.004	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.005	0.004
		3	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.002
	F	1	0.000	0.000	0.001	0.000	0.000	0.001	0.004	0.005	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.004	0.003
		3	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.002
LN	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.002	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.005	0.005
		3	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	M	1	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.003	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.004	0.004
		3	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003
	F	1	0.000	0.000	0.001	0.000	0.000	0.001	0.002	0.003	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.004	0.003
		3	0.004	0.003	0.003	0.004	0.003	0.003	0.003	0.003	0.003

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.3 (cont'd)
 Mean Squared Error for Estimates of the Death Rate Without Tumor for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004
		3	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003
M		1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.004
		3	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
F		1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.003
		3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.4
 Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.001	0.006	0.000	0.001	0.006	0.001	0.003	0.005
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.007	0.007	0.009
		3	0.004	0.004	0.003	0.004	0.004	0.003	0.004	0.004	0.004
	M	1	0.001	0.004	0.015	0.001	0.004	0.015	0.029	0.041	0.011
		2	0.006	0.006	0.010	0.006	0.006	0.009	0.021	0.019	0.063
		3	0.011	0.014	0.020	0.011	0.013	0.019	0.008	0.009	0.032
	F	1	0.001	0.005	0.012	0.001	0.005	0.013	0.036	0.029	0.009
		2	0.006	0.007	0.011	0.006	0.006	0.011	0.010	0.012	0.094
		3	0.015	0.018	0.021	0.015	0.018	0.020	0.012	0.020	0.049
LN	I	1	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.001	0.005
		2	0.001	0.001	0.002	0.001	0.001	0.001	0.006	0.008	0.010
		3	0.009	0.007	0.004	0.009	0.007	0.004	0.009	0.006	0.004
	M	1	0.000	0.002	0.016	0.000	0.002	0.016	0.003	0.013	0.017
		2	0.007	0.007	0.007	0.007	0.007	0.007	0.019	0.018	0.034
		3	0.019	0.017	0.019	0.019	0.017	0.018	0.019	0.016	0.014
	F	1	0.000	0.003	0.015	0.000	0.003	0.015	0.004	0.016	0.013
		2	0.009	0.008	0.009	0.009	0.007	0.009	0.014	0.011	0.040
		3	0.021	0.020	0.022	0.021	0.020	0.021	0.020	0.017	0.020

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.4 (cont'd)
 Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 20%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.003	0.000	0.000	0.003	0.000	0.001	0.004
		2	0.001	0.002	0.002	0.001	0.002	0.002	0.003	0.006	0.011
		3	0.031	0.018	0.007	0.031	0.019	0.007	0.031	0.018	0.007
	M	1	0.000	0.001	0.014	0.000	0.001	0.014	0.000	0.004	0.016
		2	0.005	0.008	0.007	0.005	0.008	0.007	0.009	0.015	0.019
		3	0.050	0.038	0.026	0.050	0.038	0.025	0.050	0.039	0.022
	F	1	0.000	0.001	0.016	0.000	0.001	0.016	0.001	0.004	0.016
		2	0.008	0.010	0.007	0.008	0.010	0.007	0.010	0.013	0.016
		3	0.051	0.042	0.028	0.051	0.041	0.027	0.051	0.041	0.024

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.5
Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.000	0.002	0.000	0.000	0.002	0.003	0.004	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.007	0.007	0.008
		3	0.003	0.003	0.002	0.003	0.003	0.002	0.003	0.003	0.003
	M	1	0.001	0.002	0.006	0.001	0.002	0.006	0.040	0.037	0.008
		2	0.004	0.004	0.004	0.004	0.004	0.004	0.021	0.021	0.062
		3	0.007	0.008	0.006	0.007	0.007	0.006	0.007	0.014	0.040
	F	1	0.001	0.003	0.005	0.001	0.003	0.005	0.049	0.029	0.006
		2	0.004	0.005	0.003	0.004	0.005	0.004	0.011	0.019	0.060
		3	0.008	0.007	0.006	0.007	0.007	0.006	0.017	0.030	0.042
LN	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.002	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.005	0.007
		3	0.004	0.004	0.003	0.004	0.004	0.003	0.004	0.003	0.003
	M	1	0.000	0.001	0.006	0.000	0.001	0.006	0.005	0.013	0.010
		2	0.004	0.004	0.004	0.003	0.004	0.004	0.014	0.013	0.032
		3	0.009	0.008	0.007	0.008	0.008	0.007	0.008	0.007	0.012
	F	1	0.000	0.002	0.007	0.000	0.002	0.007	0.007	0.014	0.007
		2	0.005	0.004	0.004	0.004	0.004	0.004	0.010	0.009	0.035
		3	0.008	0.008	0.008	0.008	0.008	0.007	0.007	0.008	0.015

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.5 (cont'd)
 Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
 Based on Continuous Weibull Hazards for Tumor Incidence and Death,
 Low Background Death Rates and Cumulative Tumor Survival = 50%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.002
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.004
		3	0.012	0.009	0.004	0.012	0.009	0.004	0.012	0.009	0.004
	M	1	0.000	0.000	0.004	0.000	0.000	0.004	0.000	0.001	0.006
		2	0.002	0.003	0.003	0.002	0.003	0.003	0.003	0.005	0.009
		3	0.023	0.018	0.012	0.023	0.018	0.011	0.023	0.018	0.011
	F	1	0.000	0.000	0.005	0.000	0.000	0.005	0.000	0.002	0.006
		2	0.003	0.004	0.003	0.003	0.004	0.003	0.003	0.005	0.007
		3	0.018	0.014	0.010	0.018	0.014	0.010	0.018	0.014	0.010

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.6
Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CV	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.004	0.005	0.004
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.007	0.007
		3	0.003	0.003	0.002	0.003	0.003	0.002	0.003	0.002	0.003
	M	1	0.000	0.001	0.002	0.000	0.001	0.002	0.016	0.017	0.007
		2	0.002	0.002	0.002	0.002	0.002	0.002	0.013	0.013	0.020
		3	0.004	0.004	0.003	0.004	0.004	0.003	0.004	0.005	0.012
	F	1	0.000	0.001	0.003	0.000	0.001	0.003	0.018	0.015	0.004
		2	0.002	0.002	0.001	0.002	0.002	0.001	0.010	0.010	0.013
		3	0.004	0.004	0.003	0.004	0.004	0.003	0.005	0.007	0.009
LN	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.003	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.006	0.007
		3	0.003	0.003	0.002	0.003	0.003	0.002	0.003	0.003	0.003
	M	1	0.000	0.000	0.002	0.000	0.000	0.002	0.005	0.009	0.006
		2	0.002	0.002	0.002	0.002	0.002	0.002	0.010	0.010	0.015
		3	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.007
	F	1	0.000	0.001	0.002	0.000	0.001	0.002	0.007	0.010	0.005
		2	0.002	0.002	0.002	0.002	0.002	0.002	0.008	0.008	0.012
		3	0.005	0.004	0.003	0.004	0.004	0.003	0.004	0.005	0.007

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks

Appendix H: Table H.6 (cont'd)
Mean Squared Error for Estimates of the Overall Death Rate for a 3-Interval Study
Based on Continuous Weibull Hazards for Tumor Incidence and Death,
Low Background Death Rates and Cumulative Tumor Survival = 80%

shape ¹	tumor lethality ²	sac. int. ³	Method of Estimation								
			unconstrained			analytic constraint			intuitive pooling		
			early	middle	late	early	middle	late	early	middle	late
CX	I	1	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.003
		2	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.004	0.006
		3	0.003	0.003	0.002	0.003	0.003	0.002	0.003	0.003	0.003
	M	1	0.000	0.000	0.002	0.000	0.000	0.002	0.001	0.003	0.004
		2	0.001	0.002	0.002	0.001	0.002	0.002	0.006	0.006	0.010
		3	0.005	0.005	0.004	0.005	0.005	0.004	0.005	0.004	0.004
	F	1	0.000	0.000	0.002	0.000	0.000	0.002	0.001	0.003	0.004
		2	0.002	0.002	0.002	0.002	0.002	0.002	0.005	0.005	0.009
		3	0.005	0.004	0.004	0.005	0.004	0.004	0.005	0.004	0.005

1 - shape of cumulative tumor survival curve: CV=concave, LN=linear, CX=convex

2 - I=incidental, M=intermediate, F=fatal

3 - sacrifice interval: early sacrifices performed at 35, 70, and 110 weeks, middle sacrifices performed at 52, 78, and 110 weeks, and late sacrifices performed at 78, 91, and 110 weeks