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# A Bayesian statistical analysis of mouse dermal tumor promotion assay data for evaluating cigarette smoke condensate

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ABSTRACT

The mouse dermal assay has long been used to assess the dermal tumorigenicity of cigarette smoke condensate (CSC). This mouse skin model has been developed for use in carcinogenicity testing utilizing the SENCAR mouse as the standard strain. Though the model has limitations, it remains as the most relevant method available to study the dermal tumor promoting potential of mainstream cigarette smoke. In the typical SENCAR mouse CSC bioassay, CSC is applied for 29 weeks following the application of a tumor initiator such as 7,12-dimethylbenz[*a*]anthracene (DMBA). Several endpoints are considered for analysis including: the percentage of animals with at least one mass, latency, and number of masses per animal. In this paper, a relatively straightforward analytic model and procedure is presented for analyzing the time course of the incidence of masses. The procedure considered here takes advantage of Bayesian statistical techniques, which provide powerful methods for model fitting and simulation. Two datasets are analyzed to illustrate how the model fits the data, how well the model may perform in predicting data from such trials, and how the model may be used as a decision tool when comparing the dermal tumorigenicity of cigarette smoke condensate from multiple cigarette types. The analysis presented here was developed as a statistical decision tool for differentiating between two or more prototype products based on the dermal tumorigenicity.



## 1. Introduction

In Bayesian statistical approaches, all uncertainty is measured by probability. Unknown values for model parameters are assigned a probability distribution. Before the data from a trial are utilized, prior probability distributions are assigned to the parameters. These *prior distributions* are usually uninformative, meaning that they are relatively flat over a range of plausible values, when little is known in advance of conducting a trial. Once the data from a trial are available, the distributions of the parameters are updated by conditioning on the results of the trial, forming what are commonly termed *posterior distributions*. Bayesian statistical conclusions about parameters are made in terms of probability statements based on the posterior distributions. In cases where the form of the posterior distribution is exceedingly complex, Markov Chain Monte Carlo techniques are used to generate random samples from the distribution. These random samples may then be used to characterize the posterior distribution; to determine the mean, median, quantiles, or to make various probability statements. For the interested reader, the mathematics of the Bayesian approach has been well described (Gelman et al., 2003; Berry, 2006).

The mouse dermal assay has long been used to assess the dermal tumorigenicity of cigarette smoke condensate (CSC). This mouse skin model has been developed for use in carcinogenicity testing utilizing the SENCAR mouse as the standard strain. Though the model has limitations, it remains as the most relevant method available to study the dermal tumor promoting potential of mainstream cigarette smoke (Smith et al., 2006; Walaszek et al., 2007). Background on the assay and recommendations for standardizing the procedures for conducting the studies may be found elsewhere (Meckley et al., 2004a). An example of such a study evaluating the ECLIPSE® cigarette has been published (Meckley et al., 2004b).

When comparing the responses of various groups using this assay several endpoints may be of interest with the most common being: the number or percentage of animals with at least one mass at a given time point; latency – time to appearance of one or more masses; and number of masses per animal (at risk or mass bearing). Though all of these are likely to be related in some way, there are some potential differences. The number of masses per animal appears to be the most challenging to assess. First, this measure is sensitive to the fact that some animals may die or be removed from the study early, and thus are not likely to achieve their potential maximum number of masses. Second, this measure is sensitive to latency in that animals developing masses later in the trial are less likely to develop many masses. Third, based on datasets that

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were considered for analysis here, this measure appeared to be highly variable. In particular, within a single treatment group, there seemed to be some mice that would develop a small number of masses, while others appeared to be overly sensitive in that they would develop many masses (sometimes more than 20 on a particular animal). Finally, masses may be prone to merging, splitting, or disappearing. Thus the focus here will be on the percentage of animals with at least one mass and latency, though some thoughts for working with the number of masses will be shared in the discussion.

No attempt is made here to consider the whether or not the masses are malignant. It has been argued elsewhere (Meckley et al., 2004a) that the length of the study is sufficient for papilloma development, but that a longer period would be required to assess malignant neoplasms.

In this paper, a relatively straightforward analytic model and procedure is presented for analyzing the time course of the incidence of masses. Two datasets were analyzed to illustrate how the model fits the data, how well the model may perform in predicting data from such trials, and how the model may be used as a decision tool when comparing the dermal tumorigenicity of CSC from multiple cigarette types. The focus here is on trials analyzing CSC applied to SENCAR mice, but it is expected that the illustrated approaches may be applicable to other test articles and other similarly conducted studies.

## 2. Material and methods

### 2.1. Dermal tumorigenicity studies

The in-life portion of the mouse dermal tumorigenicity studies were essentially conducted as previously described (Meckley et al., 2004a). Animals were housed and cared for according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996) and the protocols were reviewed and approved by an Institutional Animal Care and Use Committee prior to the conduct of the studies. All cigarettes were machine smoked using a standard Cambridge filter method, which consists of a 35 ml puff of 2 s duration taken once per minute (i.e., 35/60/2). Cigarette smoke condensates (CSCs) were collected by the use of a cold trap and dosing solutions were subsequently prepared in acetone/water (see Meckley et al., 2004a for a description of the methods used). These studies are performed assuming the concept of 2-stage carcinogenesis (Armitage, 1985). Female SENCAR mice were treated with a single application of 75 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator, followed a week later by promotion with either a low-, mid- or high-dose CSC application three times/week for 29 weeks.

The Female SENCAR mice were obtained from the National Cancer Institute.

Background information on the strain is found elsewhere (Meckley et al., 2004a). Support for the use of the SENCAR strain is based upon a large historical database of initiation/promotion studies using this strain (Slaga, 1986).

The first study (Study 1) considered in this evaluation compared the responses of two cigarettes, a Reference Cigarette and a Test Cigarette (a nonstandard, experimental cigarette design), in the mouse dermal tumorigenicity assay, as summarized in Table 1.

The test cigarette was tobacco burning, yielding tar and nicotine values similar to those of the Kentucky 2R4F Reference Cigarette. In this study, groups of 40 SENCAR mice were initiated with DMBA and then treated with CSC doses of either 7 (low), 14 (mid) or 21 (high) mg "tar"/application thrice weekly for 29 weeks. As an objective of this study was to evaluate the 'within-study' variability of dermal tumor responses associated with the assay, duplicate subgroups of 40 mice were treated with low-, mid- and high-dose Reference and Test CSCs.

**Table 1**
Summary of the experimental groups assigned to Study 1.

| Group | Subgroup | Initiator/promoter | mg "tar"/application |
|---|---|---|---|
| 1 | 1 | DMBA/Reference CSC | 7 (low) |
| | 2 | DMBA/Reference CSC | 7 (low) |
| 2 | 1 | DMBA/Reference CSC | 14 (mid) |
| | 2 | DMBA/Reference CSC | 14 (mid) |
| 3 | 1 | DMBA/Reference CSC | 21 (high) |
| | 2 | DMBA/Reference CSC | 21 (high) |
| 4 | 1 | DMBA/Test CSC | 7 (low) |
| | 2 | DMBA/Test CSC | 7 (low) |
| 5 | 1 | DMBA/Test CSC | 14 (mid) |
| | 2 | DMBA/Test CSC | 14 (mid) |
| 6 | 1 | DMBA/Test CSC | 21 (high) |
| | 2 | DMBA/Test CSC | 21 (high) |

CSC, cigarette smoke condensate; DMBA, 7,12-dimethylbenz[*a*]anthracene.

**Table 2**
Summary of the experimental groups assigned to Study 2.

| Group | Initiator/promoter | mg "tar"/application |
|---|---|---|
| 1 | DMBA/Reference CSC | 9 (low) |
| 2 | DMBA/Reference CSC | 18 (mid) |
| 3 | DMBA/Reference CSC | 27 (high) |
| 4 | DMBA/Test A CSC | 9 (low) |
| 5 | DMBA/Test A CSC | 18 (mid) |
| 6 | DMBA/Test A CSC | 27 (high) |
| 7 | DMBA/Test B CSC | 9 (low) |
| 8 | DMBA/Test B CSC | 18 (mid) |
| 9 | DMBA/Test B CSC | 27 (high) |

CSC, cigarette smoke condensate; DMBA, 7,12-dimethylbenz[*a*]anthracene.

The second study (Study 2) compared the responses of three cigarettes, a Reference Cigarette, Test Cigarette A and Test Cigarette B, as summarized in Table 2. The test cigarettes were tobacco burning, yielding tar and nicotine values similar to those of the Kentucky 2R4F Reference Cigarette. In this study, groups of 40 SENCAR mice were initiated with DMBA and then treated with CSC doses of either 9 (low), 18 (mid) or 27 (high) mg "tar"/application thrice weekly for 29 weeks.

Data recorded in both studies included mortality; body and organ weights; clinical observations; weekly observations of all new masses, abnormalities or changes to a previously observed mass; and gross necropsy observations.

### 2.2. Analytic approach

The approach considered here is to model the number of animals developing masses over the time course of the study, where the model focuses on the number of mice developing masses at a given time point. This can be modeled by assuming that the number of "at risk" animals developing a tumor at week $i$ follows a binomial distribution with

$$p_i = \frac{E_{\max} * Week^{\gamma}}{EC_{50}^{\gamma} + Week^{\gamma}} \quad (1)$$

At risk animals are tumor free at the beginning of week $i$, and have not been previously removed from the study. In Eq. (1), $p_i$ denotes the probability that an at risk mouse will develop a mass during week $i$. The size for the binomial distribution at week $i$ ($n_i$) is the number of at risk animals. The model for $p_i$ is the Emax model (Gabrielsson and Weiner, 2006), where the $p_i$ will increase over time up to some maximum value ($E_{\max}$). Here $E_{\max}$ must be greater than zero and less than or equal to one since it is a