Monotonic Nonparametric Dose Response Model

Faten S. Alamri* Edward L. Boone† David J. Edwards‡

Abstract

Toxicologists are often concerned with determining the dosage to which an individual can be exposed with an acceptable risk of adverse effect. These types of studies have been conducted widely in the past, and many novel approaches have been developed. Parametric techniques utilizing ANOVA and non-linear regression models are well represented in the literature. The biggest drawback of parametric approaches is the need to specify the correct model. Recently, there has been an interest in non-parametric approaches to tolerable dosage estimation. In this work, we focus on the monotonically decreasing dose response model where the response is a percent to control. This poses two constraints to the non-parametric approach. The dose-response function must be one at control (dose = 0), and the function must always be positive. Here we propose a Bayesian solution to this problem using a novel class of non-parametric models. A basis function developed in this research is the Alamri Monotonic spline (AM-spline). Our approach is illustrated using both simulated data and an experimental dataset from pesticide related research at the US Environmental Protection Agency.

Key Words: Bayesian Statistics; Nonparametric modelling; Alamri Monotonic spline; Toxicology data; Benchmark tolerable region.

1. Introduction

Evaluating the risk of exposure to multiple chemicals starts by measuring the side effects of those chemicals on an experiment specimen by regression modelling. Benchmark dose (BMD) is a method that finds the maximum tolerable dose, which produces a prespecified accepted side effect level on the experiment specimen. There are many threshold types in a dose-response model, which determine the safest dosage, the dangerous dosage or any other inquiry; ED_γ, where γ could be $\{50, 75, 90\}$ or any other number. ED_{50} the median effective dosage in the study that determines the safe and the lethal dosage, is a one type that we will use in this research. For more about benchmark dose estimation, see Shao and Shapiro [2018]. Many toxicology studies are performed on rodents and in some cases require sacrificing the rodent to get the measurement. Therefore, the sampling method is the best fit for these types of studies. Studying the chemical effect has different aspects and methods, such as Stork et al. [2007] considered the interaction of a large number of chemicals for additivity consideration using the fixed-ratio design. On the other hand, Yeatts et al. [2010] considered the additive and piecewise model along the fixed-ratio mixture ray to determine the dosage and the interaction thresholds in a toxicology data analysis. We consider the Bayesian framework using a normal likelihood and stick breaking prior to estimate the posterior predictive by the Markov Chain Monte Carlo (MCMC) sampling method. In this paper, we propose a new non-parametric model as an alternative to the parametric model for cases where the parametric models do not fit the data. In this research, a new spline model (AM-Spline) is developed, which matches

*Princess Nourah Bint Abdulrahman University,Riyadh ,Saudi Arabia, PO Box 84428 and Virginia Commonwealth University, Richmond, VA 23284, USA

†Virginia Commonwealth University, Richmond, VA 23284, USA

‡Virginia Commonwealth University, Richmond, VA 23284, USA

the pathological example well. The AM-spline was used as a dose-response model, which we develop an algorithm to define the tolerable region that contains the safest chemical dosage with an acceptable side effects.

Many authors have contributed to this area in the past. Smooth monotone functions and their properties are introduced by Ramsay and Abrahamowicz [1989] such as the I-splines. I-spline is a monotone spline constructed by a non-negative linear combination coefficient constricted by Ramsay et al. [1988] who used the integrated B-spline as a basis function to maintain monotonicity. Users of non-parametric smoothing techniques should utilize their judgement in deciding the estimated regression curve and the smoothing level. The noise level which controls the smoothness of the curve is a subjective decision as Härdle [1990] mentions the decision is subjectively using a software tool. Comparisons of different non-parametric methods are introduced in Bhattacharya and Lin.

Contrarily, binary dose-response data have been estimated using non-parametric curves with some constraints as presented in Gelfand and Kuo [1991]. In non-parametric dose response models there are two approaches to estimate monotone functions that were introduced by Shively et al. [2009]. The first approach that utilized the power of the Bayesian method was introduced by Ramsay et al. [1988], which uses unconstrained estimation. This model was used as the hierarchical Bayes framework. The second is the Bayesian regression spline model from Smith and Kohn [1996]. This enforced monotonicity by a mixture of constraints on the normal prior distribution on the regression coefficient. Furthermore, Hastie and Tibshirani [1986] introduce generalized additive models and examples for various data.

Methods for non-parametric monotone based on Bayesian analysis of the isotonic regression were developed by Neelon and Dunson [2004], who defined the flat region of the dose-response curve constructed using a piecewise linear model with a restricted prior distribution, along with the latent Markov process formulation that was used to simplify the computation and to form a smooth regression line. Another approach is the Semiparametric, which was suggested for dose-response analysis Wheeler and Bailer [2012]. A semiparametric estimation has a constrained shape, which was introduced by Wu and Sickles [2018] for elasticity. They used penalized splines when applying the shape constraint on the fitted model, and their work was inspired by Ramsay et al. [1988]. Assumption of monotonicity on the dose-response curve and continuous observation to define the confidence bands for isotonic dose-response curves are found by Korn [1982], and is modified by Lee [1996] while keeping monotonicity.

Monotonic increasing function in the Bayesian framework considers a mixture of triangular distribution with unspecified dimension during the analysis the method is introduced by Perron and Mengersen [2001]. Their approach is not restricted to Bayesian, but could have other applications in the frequentest perspective. The test of monotonic regression function based on the critical bandwidth and the smoothing level imposes the non-parametric estimate to be monotonic in Bowman et al. [1998]. Curves of a dose response model estimated by contracting a combination of smoothing spline and the non-negative properties of cubic B-spline was used as in Kong and Eubank [2006]. Different non-parametric methods introduced to estimate the monotonic dose-response curve using the bootstrap confidence interval were introduced in Dilleen et al. [2003]. Delecroix et al. [1996] used the kernel method to estimate the monotonic dose-response curve under general shape restriction. Our approach is similar to Clyde and Wolpert [2007] where they used a regression function model as a linear combination of kernels. However, they used the general Lévy processes as the prior distribution on the measure, whereas we used the stick breaking prior distribution. Their approach is also different than ours, since they are not considering the monotone regression in their

model constraint, along with the utilization of different processes. Another approach that is similar is Bornkamp and Ickstadt [2009], but they used a monotone increasing function and the Two-Sided Power distribution (TSP) as the spline bases function. They concluded that TSP is 10 to 15 times faster than using the Beta distribution function. More on TSP can be found inVan Dorp and Kotz [2002].

Several researchers have discovered other approaches in isotonic regression with smoothing consideration. For instance, Wright et al. [1980], Mammen [1991], Härdle [1990], Friedman and Tibshirani [1984], and Kim et al. [2018] used the hierarchical Bayes framework and characterization of stick-breaking process that allows unconstrained estimation of the monotone function. What motivated us is that all parametric and non-parametric models struggle in fitting sinuous monotonic decreasing data.This paper is organized as follows: Section 2 Motivating Example, Section 3 cover the Methodology. We applied our method to possible applications in section 4, and ended with discussion in section 5.

2. Motivating Example

To motivate our approach, we reanalyzed the study of the Organophosphate Pesticide (OP) data by Moser et al. [2005]. A neurotoxic study was conducted using 349 rats to investigate the effects of OP, which is a common active pesticide in agriculture. OP data represent the side effects on the nervous system of the rats that have been measured and called the endpoints. Moser et al. [2005] tested multiple neurotoxicity endpoints in which we only consider the Blood cholinesterase (BloodCHE). This endpoint was measured at the effect of two different pesticides Acephate (ACE) and Diazinon (DIA) with different levels of doses. ACE dose level from 0 to 120 (mg/kg) and DIA dose level from 0 to 250 (mg/kg). Each pesticide was consumed by the rat and absorbed into their system while the other pesticide had a dose level of zero. The continuous endpoint BloodCHE is measured and recorded from the rat's system. In our research we considered each pesticide dosage individually and BloodCHE endpoint measurement, which is available for each rat. See Moser et al. [2005] for more details about the data collection and measurement process. Figure 1 represents the OP data is monotonic decreasing data. In panel (a) the OP data is considering the ACE pesticide. We see the data is monotonically decreasing with variation at zero dosage and the remaining data are gradually decreasing, most of the data are in the range of 0 to 0.7 percent to control. Panel (b) is the DIA pesticide data, which is dramatically decreasing with some variation at zero dosage; most of the data fall in the range of 0 to 0.7 percent to control. The Figure shows that DIA data is denser than ACE data. The x-axis is the chemical dosage and the y-axis is the percent to control. which is the percent to corresponding controls required to consider the variance of these controls. More about percent to control in Feuerstein et al. [1997].

(a) (b)

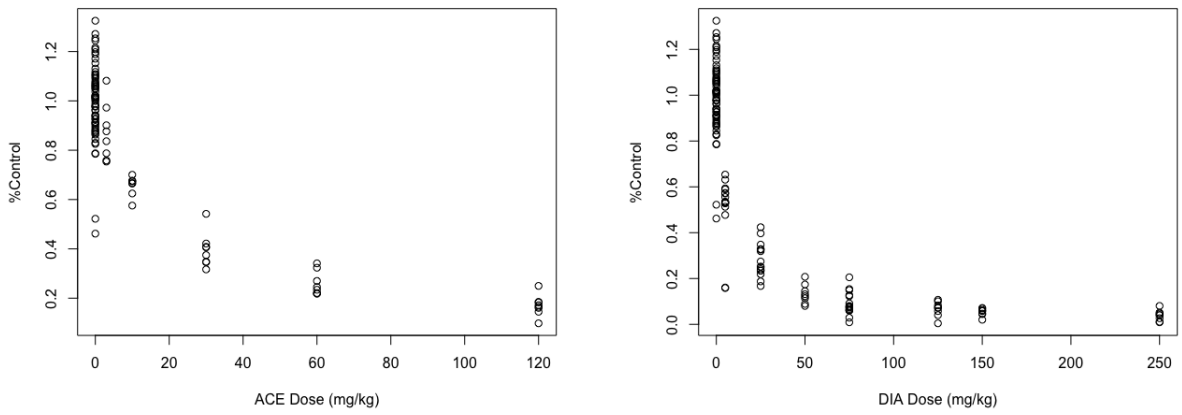

Figure 1: OP Data Blood considering the Blood Cholinesterase (BloodCHE) endpoint. In panel (a) is the ACE pesticide data and in panel (b) is DIA pesticide data, both data are monotonically decreasing. The x-axis is the chemical dosage (mg/kg) and the y-axis is the percent to control which is the percent to corresponding controls required to consider the variance of these controls.

Fitting a model allows us to identify the region of safe dose level. Invert the spline function using the adverse effect level to determine the corresponding dose, which bounds the region under the fitted spline. Understanding this region could help to address environmental public health questions, such as what is the safest dose of pesticide? The literature describes many dose-response models that fit the type of data we have but, to our best knowledge, none have used our proposed model with the specific constraints and none have used it in the dose-response perspective.

3. Methodology

3.1 Nonparametric Regression

The Nonparametric regression is a type of regression analysis known as a distribution-free with models that are infinite-dimensional, as in the following format

$$y_i = m(x_i) + \varepsilon_i, \quad i = 1, ..., n$$

Where $0 \leq x_1 \leq x_2 \leq .... \leq x_N \leq 1$ and the ε_i are independent draws from normal$(0, \sigma^2)$ with unknown $\sigma > 0$ and m(.) is the unknown smooth and flexible function. Nonparametric regression is different than the parametric regression by its ability to capture unexpected features of the data using a different shape of the functional relationship. Different types of nonparametric modelling are introduced in Ruppert et al. [2003] as regression splines, smoothing splines, kernel methods including local regression, series-based smothers, and wavelets.

3.2 AM-Spline

Spline is a nonparametric regression technique that is written as a combination of basis functions. It fits a smooth curve between points in the data called knots that are in the interval [L, U] with specific constraints. All splines follows this model

$$y_i = f(x_i) + \varepsilon_i, \quad x_i \in [0,1], \quad i = 1,2,.....,n$$

where $f(x_i)$ is a piecewise polynomial spline and ε_i is the white noise Wegman and Wright [1983]. The knots are a sequence of points that divide the spline interval to subintervals and are different in values and locations for different spline. It is known in the literature that the spline smoothness is controlled by the number of knots and their locations, thus number of knots are less than the data points. Knots have different selection methods Wold [1974] states some recommendations for knots selection. His recommendations are upon the assumption of the cubic spline are which needs modifications for a spline with degree greater than three. Cubic spline frequently used since no lower degree spline can interpolate through data endpoints that have an exact derivative at each point Wolberg and Alfy [1999]. Some knots properties: knots are located on the data points. The Minimum of 4 to 5 observation should be between knots. Knots should not have more than one extrema, and one inflection point, both should be between knots. The extrema should be centred in the interval and inflection points should be close to knot points. A general introduction of the theory in interpolating and smoothing splines is given by Wasserman [2006] and Green and Silverman [1993]. DiMatteo et al. [2001] used fully Bayesian methods for curve fitting with free-knot splines using the reversible-jump MCMC as a posterior sampling tool. While the literature was studying the constraints on the parameters of B-splines, Wood [1994] used piecewise polynomial properties of a spline with conditions on monotonicity. That is considered the cubic piecewise from Hyman [1983] and extends the work to the cross-validation and confidence interval techniques.

There are many types of splines such as M, B, P, cubic, linear and quadratic splines, for more about spline types read Ruppert et al. [2006]. Every spline has, t which are the set of basis functions that connect linearly, which differ in each spline. This article will contribute to a new monotonic spline. Several monotonic splines are in the literature but our proposed spline is different as it has specific constraints and it has a more general structure. I-spline is a monotone spline constructed by a non-negative linear combination coefficient as Ramsay et al. [1988] used the integrated B-spline as basis function to maintain monotonicity. Ramsay et al. [1988] fitted the constrained curve using non-Bayesian methods. He and Shi [1998] mentioned that I-spline faces uncertainty when fitting it to real data. So, they proposed monotonic spline using a quadratic spline as the basis function. An isotonic spline is a monotonic spline that depends on the cubic spline, and it is non-decreasing on a specific integral by certain constraints as mentioned in Wang and Li [2008]. Xue and Wang [2010] moved to higher than the quadratic spline order using He and Shi [1998] methods, which is computationally longer but they made monotonicity possible for any penalized splines (PS) order.

Spline function is known as

$$f(x) = \sum_{i=1}^{k} a_i x_i \tag{1}$$

where a_i set of non negative weights sums up to one and x_i are the basis function over the knots K_i. Smoothing spline is a popular technique as spline usage introduced by Silverman [1985] that provide a review of all possible smoothing methods. We used a smoothing parameter to control the smoothing fit of our proposed spline.

Selecting the something parameter λ or the knots k is crucial and differs in each spline. Different selection methods are proposed in the literature as Xue and Wang [2010] used AIC criteria to select the number of interior knots K_n. Fitting spline using cross-validation was covered by Wahba and Wold [1975], a valid mean square error method used to determine the correct degree of smoothing to discrete data, and used MCMC to estimate the true smooth function and its derivative. In our proposed spline we picked a fixed λ and specific the knots but the previous methods could be used in a future paper.

AM-spline is our novel approach, using the CDF as the basis functions, any continuous CDF is capable to our spline. Provide that CDF is in the constructed support. The probability density function of the normal distribution with parameters μ and σ^2 is given by

Normal $\sim (\mu, \sigma^2)$

$$f(x) = \frac{1}{\sqrt{2\pi\sigma x}} exp(-\frac{(x-\mu)^2}{2\sigma^2}), x > 0 \tag{2}$$

For illustration we used the following normal CDF as basis function of AM-spline

$$CDF_{(normal)} = \frac{1}{2}\left[1 + \text{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right] \tag{3}$$

where

$$erf(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt$$

The monotonic decreasing AM-spline is as follows

$$f_{(norm)} = \sum a_i (1 - F(x)), \qquad f(x) = (1 - F(x)) \tag{4}$$

where a_i are the weights and $F(x)$ is the Normal CDF. The basis function could be any CDF of any statistical distribution. Figure 2 represents the basis functions of AM-spline. Each curve is one base function $f(x)$ in equation 4.

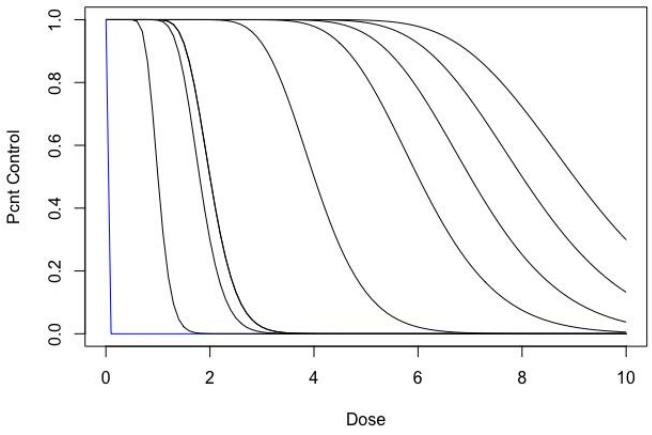

Figure 2: Spline Basis Functions

General representation of the AM-spline in Figure 3 shows the monotonic decreasing spline that is fitted in the percent to control interval of $[0,1]$. The solid curve and the dashed line represent the ED_{50} under the fitted curve as if the spline used as a dose response model.

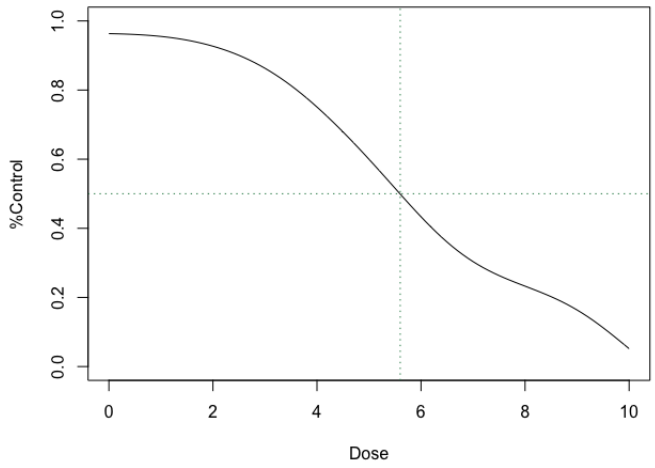

Figure 3: Fit of smooth monotonic decreasing function on the percent to control interval [0,1]. Two-dotted lines define the tolerable region under the fitted model as the following, The dotted horizontal line in the y-axis is the ED_{50} and the dotted vertical line in the x-axis is the dose correspond with the ED_{50}

3.3 BMD/BMDL estimation

Dose response model has several famous thresholds such as the Bench mark dose (BMD) and the No Observable Adverse Effect Level (NOAEL). Slob et al. [2005] provides a comparison between BMD and NOAEL in multiple simulated studies. Bayesian BMD (BBMD) is a benchmark dose technique that incorporates the prior information. This can lead to saving animals' lives in a study and improving the accuracy of the research Slob and Setzer [2014].

In this article the tolerable region is bounded by the ED_{50} threshold, the region of the safest dose using the single chemical and the adverse effect. MCMC sampler is a sampling method across the models, we suggest Metropolis-Hasting algorithm. Algorithm steps: the chain start with one set of mean μ_1 in the simplest model and the variance drawn from the prior. Change is then introduced to the model, the changes are in adding a new set or rotating change points or deleting an existing set in the model. Accepted changes have probability Q

$$Q = min\Big\{1, \frac{p(\beta'|Y)S(\beta|\beta')}{p(\beta|Y)S(\beta'|\beta)}\Big\} \tag{5}$$

Where β is the model parameters current model and β' is the new model with change parameters. $p(\beta|Y)$ is the posterior probability for the model parameters β conditioning on the data. S is the proposal distribution for the change parameters. When the changes are accepted then the chain moves to the new β' and if not the chain stays at it is steady state β. These steps repeat a large

number of times to get samples $\beta_1, \beta_2, ..., \beta_i$. Success MCMC sampling method relies on the proposal distribution *S*, where it is effective in alternate the dimension samplers. Since adding and removing parameters from the model impact the likelihood of the new model Holmes and Heard [2003].

4. Application

In this section, we apply our proposed approach to evaluate its effectiveness on two simulated datasets and to the OP data introduced in Section 2.

4.1 Simulated Example

To assess the viability of our proposed technique we applied it to two simulated datasets named $\{$sim 1$\}$ and $\{$sim 2$\}$. These are pathological examples as they do not exhibit traditional parametric model shapes. Sim 1 has 140 observations. and Sim 2 has 234 observations. Figure 4 shows the scatter plots of the two datasets; the x-axis is Dose, and the y-axis is the percent to control. In panel (a) Sim 1, the percent to control data decreases linearly as dose increases from dosage 0 to 3. After dosage of 3 the pattern levels out sharply to around 0.2. In panel (b) Sim 2 has multiple inflection points at dosages 3, 5 and 7. The percent to control is near zero from dosages 7 to 10. Notice that both of these simulated data are monotonically decreasing on the percent to control as the dose increases.

(a) (b)

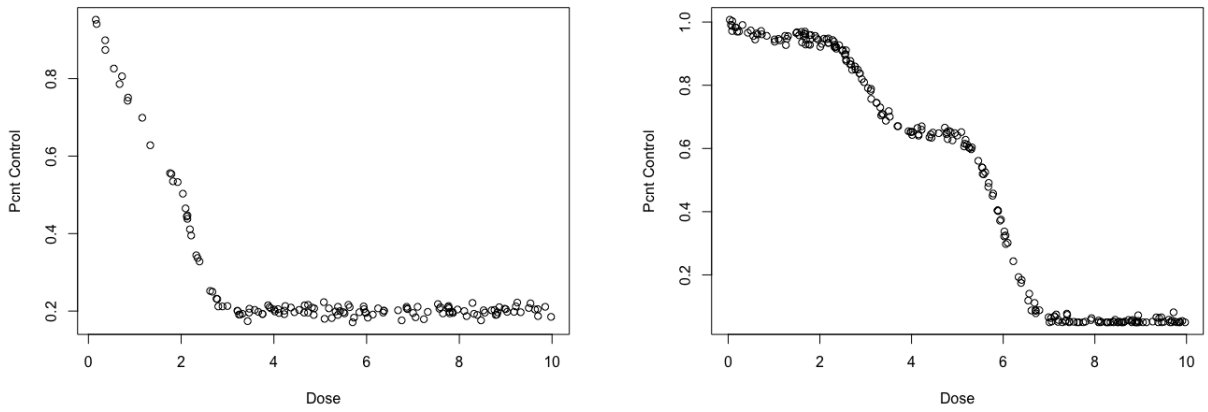

Figure 4: Two simulated datasets Sim 1 (a) and Sim 2 (b).

4.1.1 Sim 1 data analysis

To define the basis functions of the AM-spline model when applying it to the sim 1, we use the following knots k=$\{$ 0, 0.1, 0.25, 0.5, 1,1.2, 2, 3, 4, 5, 6, 7, 10$\}$ and the bandwidth $\lambda = 0.8$. We consider the normal likelihood with the stick-breaking prior distribution on the a_i parameters, normal prior distribution $N \sim (0,1)$ on the α parameters and chi-square distribution on the σ parameters with

2 degrees of freedom. These priors combined with the likelihood are used to draw samples of the parameters from the posterior distribution. The following parameters were estimated: the weights a_i where $\sum_{i=1}^{k} a_i = 1$, the standard deviation σ and the lower support α using MCMC sampling methods. Python 3.6 is used to implement the sampling scheme which takes 30 minutes to obtain the samples. The Metropolis-Hastings sampling method was employed to obtain $10,000$ MCMC samples. Before running a long chain from the sampler we ran several short chains of $1,000$ to tune the sampler, and then discarded these samples as burn-in samples. Table 1 shows the estimate summaries of the posterior parameter samples of α, σ^2 and the weights $a_{i=k}$. This shows that there are three parameters with heavyweights at the two knots ($k = \{0.5, 2\}$) and the α. Fitting the AM-spline model to the simulated data sim 1 results in the parameters estimate represented in Table 1 and the fit of the model in Figure 5, which shows the AM-spline is fit to Sim 1 data. In panel (a) the red solid line is the AM-spline mean, and the dashed lines are the 0.025 and the 0.975 quantiles of the posterior predictive distribution. We examined the convergence of the parameters of MCMC samples from the posterior distribution by visual inspection of the trace plots. These samples were then used to generate samples from posterior predictive distribution which are then used to find the samples of the ED_{50} distribution. ED_{50} is the dosage with the 50% corresponding response, which define the tolerable region that is calculated by Equation (5). Panel (b) represents the histogram of the tolerable region and the vertical dashed line on the histogram is the estimated ED_{50}. This is calculated as the 0.05 quantile of the distribution effective dose which is at Dose= 1.95. This demonstrates that the AM-spline is capable of finding an ED_{50} for Sim 1.

Table 1: Sim 1 Parameter estimates for the AM-Spline based on 10000 MCMC samples from the posterior distribution, $\lambda = 0.8$, $\sigma = 0.001$ and knots at $k = \{0, 0.1, 0.25, 0.5, 1, 1.2, 2, 3, 4, 5, 6, 7, 10\}$

Parameter	Mean	Median	StDev	95% Credible interval
α	0.1989	0.1989	0.0017	**(0.1978, 0.2022)**
σ^2	0.0002	0.0002	0.00003	(0.0002, 0.0003)
a_1 $(k = 0)$	0.0179	0.0142	0.0148	(0.0062, 0.0513)
a_2 $(k = 0.1)$	0.0307	0.0246	0.0261	(0.0102,0.0930)
a_3 $(k = 0.25)$	0.0541	0.0494	0.0372	(0.0238,0.1307)
a_4 $(k = 0.5)$	0.2287	0.2322	0.0434	**(0.1989,0.3032)**
a_5 $(k = 1)$	0.0157	0.0109	0.0148	(0.0046,0.0528)
a_6 $(k = 1.2)$	0.0097	0.0067	0.0096	(0.0028,0.0354)
a_7 $(k = 2)$	0.6166	0.6170	0.0198	**(0.6027,0.6523)**
a_8 $(k = 3)$	0.0236	0.0242	0.0118	(0.0143,0.0444)
$a_9(k = 4)$	0.0013	0.0008	0.0015	(0.0003,0.0052)
a_{10} $(k = 5)$	0.0012	0.0008	0.0012	(0.0003,0.0042)
a_{11} $(k = 6)$	0.0003	0.00008	0.0005	(0.00001,0.0017)
$a_{12}(k = 7)$	0.0002	0.00003	0.0005	(0.000008,0.0018)
a_{13} $(k = 10)$	0.00001	0.000005	0.00001	(0.0000003,0.0001)

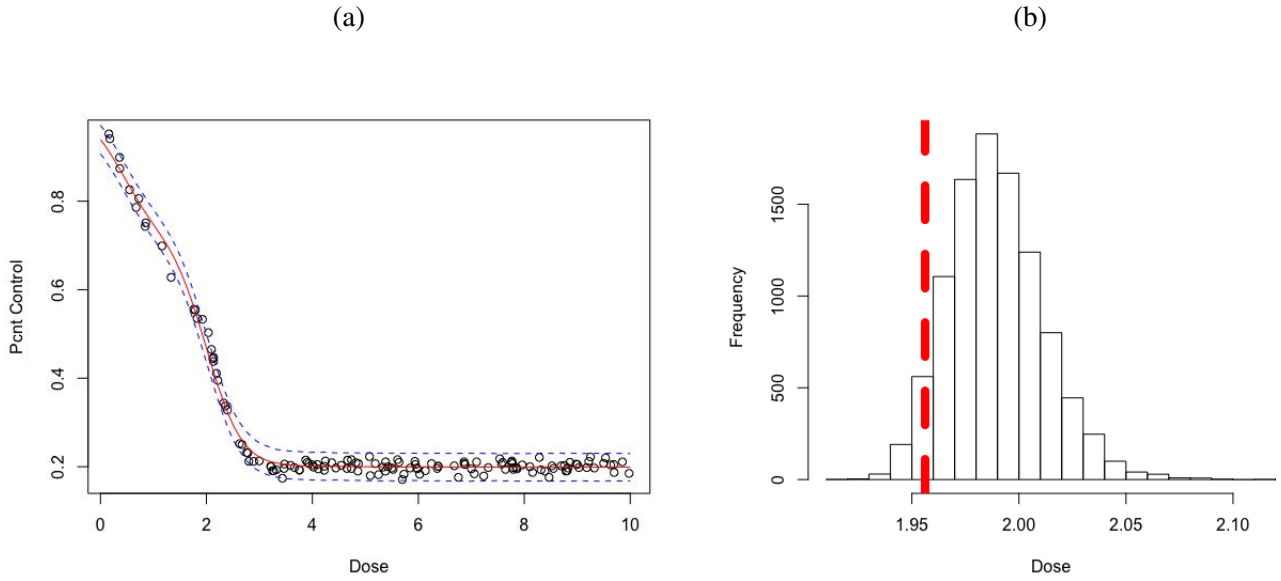

Figure 5: Panel (a) AM-spline model fitted to the simulated Sim 1, the solid line represents the fit of the AM-spline and the dotted lines are the credible intervals. Panel (b) histogram represents the samples of the tolerable region and the vertical dashed line represents the ED_{50}.

4.2 Organophosphate Data

The US EPA is interested in researching the effects of exposure to pesticides, especially Organophosphates (OP) such as Acephate (ACE) and Diazinon (DIA). Moser et al. [2005] conducted a laboratory study looking at Blood Cholinesterase as the endpoint when rats were dosed with these two chemicals. More about the data in Section 2. Here we are applying the univariate AM-spline model to the ACE data. The goal here is to evaluate efficiency of our model and determine the ED_{50}.

4.2.1 ACE data

The AM-spline model requires defining the smoothing parameter λ, the set of knots and the standard deviation for each data. We fit the following bandwidth :$\{2, 6, 10, 20\}$ to determine the ideal bandwidth for the ACE data. Figure 6 shows the different proposed bandwidth when fitted to the ACE data, the x-axis represents the chemical dosage and the y-axis represents the percent to control. This Figure also shows that when the bandwidth increases the smoothness of the model increases. and when the bandwidth exceeds the data limit the fitted model no longer fits the data adequately. Consequently, we picked $\lambda = 10$ as the smoothing parameter when fitting AM-spline model in this application. We are using the Metropolis Hastings algorithm, and we consider the coefficient step value when changing the bandwidth.

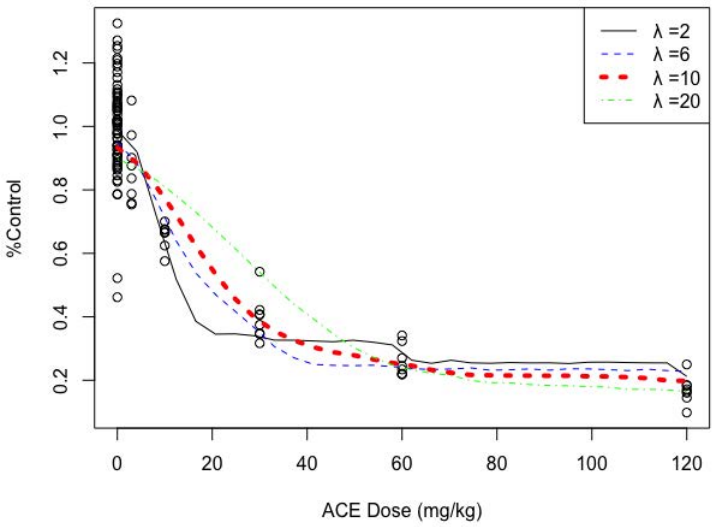

Figure 6: Comparing the fit of AM-spline model when considering the following four bandwidths $\{\lambda = (2,6,10,20)\}$. We found $\lambda = 10$ matched the data the most, so we used $\lambda = 10$ as our bandwidth in this research

Applying the AM-spline to the ACE data using the knots $k = \{0,5,9,15,30,60,120\}$ and the smoothing parameter $\lambda = 10$. Bayesian framework used to estimate the weights a_i where $\sum_{i=1}^{k} a_i = 1$, the standard deviation σ and the lower support α using MCMC sampling methods. We assumed normal likelihood with stick-breaking prior distribution on the a_i, normal prior distribution $N \sim (0,1)$ on the α parameters and chi-square distribution on the σ parameters with 2 degrees of freedom. Python 3.6 was used to implement the sampling scheme which takes 45 minutes to obtain the samples. The Metropolis-Hastings sampling method was employed to obtain $10,000$ MCMC samples. Before running a long chain from the sampler, we ran several short chains of $1,000$ to tune the sampler and then discarded these samples as burn-in samples. Table 2 shows the estimate summaries of the posterior parameter samples of α, σ^2 and the weights $a_{i=k}$. This shows that there are three parameters with heavyweights at the knot $(k = \{15,30\})$ and the α. Fitting the AM-spline model to the simulated data Sim 1 results in the parameters estimate represented in Table 2 and the fit of the model in Figure 7, which shows the AM-spline is fit to ACE 1. In panel (a) the red solid line is the AM-spline mean, and the dashed lines are the 0.025 and the 0.975 quantiles of the posterior predictive distribution. We examine these convergence of the parameters of MCMC samples from the posterior distribution by visual inspection of the trace plots. These samples were then used to generate samples from posterior predictive distribution, which are then used to find the samples of the ED_{50} distribution. ED_{50} is the dosage with the 50% corresponding response, which define the tolerable region that is calculated by Equation (5). Panel (b) represents the histogram of the tolerable region, and the vertical dashed line on the histogram is the estimated ED_{50}. This is calculated as the 0.05 quantile of the distribution effective dose which is at Dose = 18. This demonstrates that the AM-spline is capable of finding an ED_{50} for ACE data.

Table 2: ACE data Parameter estimates for the AM-Spline fit considering 10,000 MCMC samples from the posterior distribution with $\lambda = 10$ and knots at $k = \{0, 5, 9, 15, 30, 60, 120\}$

Parameter	Mean	Median	StDev	95% credible interval
α	0.1776	0.1830	0.0576	**(0.1409, 0.2742)**
σ^2	0.0212	0.0210	0.0030	(0.0190, 0.0276)
a_1 $(k = 0)$	0.0226	0.0180	0.0197	(0.0082, 0.0696)
a_2 $(k = 5)$	0.0476	0.0350	0.0439	(0.0153, 0.1547)
a_3 $(k = 9)$	0.1024	0.0819	0.0853	(0.0343, 0.3100)
a_4 $(k = 15)$	0.5341	0.5487	0.1700	**(0.4214, 0.8265)**
a_5 $(k = 30)$	0.1660	0.1374	0.1280	**(0.0618, 0.4560)**
a_6 $(k = 60)$	0.0846	0.0692	0.0693	(0.0265, 0.2394)
a_7 $(k = 120)$	0.0427	0.0216	0.0521	(0.0056, 0.1840)

(a) (b)

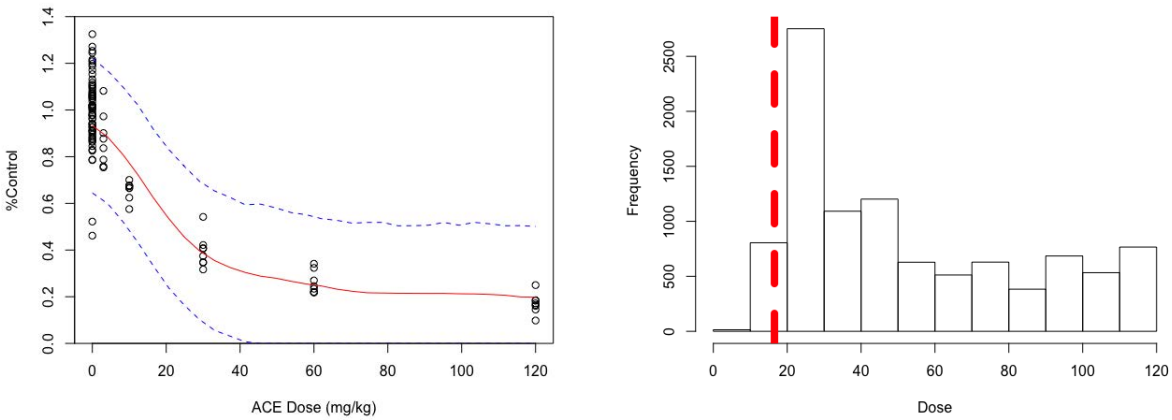

Figure 7: Panel (a) AM-spline fitted to the ACE data as the solid line and the two dashed lines are the credible intervals and in panel (b) is the ACE-histogram of the samples of the tolerable region with the dashed line that represents the corresponding dose to the ED_{50}, the stressor (ACE) and the endpoint (BloodCHE).

5. Discussion

In this article, a new monotonic spline has been presented. We found that constrained smoothing splines are useful since we can customize the methods to any specific study and can choose the proper smoothing parameter λ. Our nonparametric monotonically decreasing spline is flexible and effective in different statistical approaches and applications. Analysis and computation of nonparametric models are not as easy as mentioned in the literature. The process and the fit of the model are sensitive to multiple properties: the step value, the size of the MCMC sample, the sampling

starting value effect, the number of knots and the smoothing value. Our approach produced the new spline (AM-spline) with constraints on the distribution with values between zero and one. Fitting of the dose-response model to toxicology experiment was used to determine the tolerable region that defines the safest dose combination. This work could be expanded to experimental design, applied to different parameter selection method, spatially adaptive model and could be used as a model in the optimal follow-up design criteria.

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