A finite sample comparison of nonparametric estimates of the effective dose in quantal bioassay

Holger Dette
Ruhr-Universität Bochum
Fakultät für Mathematik
44780 Bochum, Germany
e-mail: holger.dette@rub.de
FAX: +49 234 3214 559

Regine Scheder
Ruhr-Universität Bochum
Fakultät für Mathematik
44780 Bochum, Germany
e-mail: regine.scheder@rub.de

February 29, 2008

Abstract

To estimate the effective dose level ED_α in the common binary response model, several parametric and nonparametric estimators have been proposed in the literature. In the present paper, we focus on nonparametric methods and present a detailed numerical comparison of four different approaches to estimate the ED_α nonparametrically. The methods are briefly reviewed and their finite sample properties are studied by means of a detailed simulation study. Moreover, a data example is presented to illustrate the different concepts.

Keywords and Phrases: Binary response model, effective dose level, nonparametric regression, isotonic regression, order restricted inference, local linear regression

1 Introduction

In pharmacology or toxicology, dose-response relationships are often studied to investigate effects of a chemical drug. In quantal bioassay experiments different subjects are treated at different dose levels, and it is observed if the subject reacts or not. Therefore the response of such experiments is binary, which motivates the name quantal in this context. In many situations the main objective of the experiment is to identify the effective dose level given $\alpha \in (0, 1)$ such that $100\alpha\%$ of the subjects react. This value is denoted by ED_α and shows the effectiveness of the chemical drug.
Traditionally, parametric models like the probit or the logit model are used to estimate the dose response curve

$$p(x) = P(Y = 1|X = x)$$

which describes the probability of success as a function of the dose level x. The ED_α is then defined by

$$\mathrm{ED}_\alpha = p^{-1}(\alpha).$$

Parametric models have a long history and are frequently used in this context [see Berkson (1944) or Bliss (1934) for early references]. Many different parameterizations have been proposed in the literature to model the dependence $x \to p(x)$ [see e.g. Woutersen et al. (2001), Slob (2001), Krewski, Smythe and Fung (2002) or Bretz, Pinheiro, Branson (2005) among many others].
However, in many applications, the specific parametric form of the success probability is not known by the experimenter, since the biological relation between the response and the predictor is not easy to understand [see e.g. Bretz, Pinheiro and Branson (2005)]. In such situations parametric models can lead to wrong conclusions about the effective dose level if the underlying parametric model is wrong [see Hamilton (1979) or Dette, Neumeyer and Pilz (2005)]. Therefore several authors proposed nonparametric estimates of the effective dose in the literature [see Müller and Schmitt (1988), Dette et al. (2005), Park and Park (2006), Bhattacharya and Kong (2007)].
The purpose of the present paper is to compare the finite sample properties of four different nonparametric estimates for the effective dose level, which have been recently suggested in the literature. In Section 2 we review the basic properties of the different estimates. In Section 3 we present a detailed investigation of the finite sample properties of the different methods by means of a simulation study. In particular, we study the finite sample bias, variance and mean squared error of the estimates under a repeated and non-repeated measurement design. A data example is briefly discussed in Section 4, while some conclusions are given in Section 5.

2 Nonparametric estimates of the effective dose level

Consider the binary response model in quantal bioassay applications, where the single covariate x_i gives the investigated dose level of the ith subject, and the binary response Y_i is coded by $Y_i = 0$ for "no reaction" and $Y_i = 1$ for "reaction". Each observation is taken as the outcome of a Bernoulli experiment with success probability $p(x)$ at the dose level x, i.e. $Y \sim \mathrm{Bin}(1, p(x))$, which implies

$$P(Y_i = 1|X_i = x_i) = p(x_i) = 1 - P(Y_i = 0|X_i = x_i) \tag{2.1}$$

for $i = 1, \ldots, m$ as underlying statistical model, where the observations are supposed to be independent. Throughout this paper it is assumed that the explanatory variable varies in a compact interval without loss of generality given by the interval $[0, 1]$. The function p is called the dose-response curve. In many applications it can be assumed that the function p is strictly increasing, and in this case the ED_α is simply the inverse of the function p at the point α, which will be assumed throughout this paper. However, it is also worthwhile to mention that there exist situations where monotonicity can not be guaranteed by biological and physical backgrounds [see e.g. Hunt and Bowmann (2004) or Chen and Kodell (1989)]. For a given $\alpha \in (0, 1)$, the effective dose level is defined as

$$\mathrm{ED}_\alpha = p^{-1}(\alpha) := \inf\{x \in [0, 1] \mid p(x) \geq \alpha\},$$

where $100\%\alpha$ of the subjects react to the treatment. In the following, we introduce four different nonparametric approaches to estimate the effective dose level ED_α for a given $\alpha \in (0,1)$. For the sake of brevity only equidistant designs with and without replications are considered in the study of the finite sample properties of the different estimates, which will now be introduced.

2.1 The "pool-adjacent-violators" (PAV) algorithm

Bhattacharya and Kong (2007) proposed a PAVA estimator of ED_α using minimal assumptions. They used an experimental design, where n_i subjects are tested at different dose levels x_i for $i = 1, \ldots, k$. To keep things simple, we assume that for each dose level n different subjects are analyzed. In order to make the design comparable to the equidistant design with no replications, we follow the suggestion of these authors and set $m = nk$.
For the ordered equidistant dose levels $x_j = \frac{j}{k}$ $(j = 1, \ldots, k)$, the corresponding responses are denoted by $r_1, \ldots, r_k$, respectively, and give the number of positive reactions at each dose level. Mathematically, the number of responses is modeled by a binomial distribution, i.e. $r_j \sim \mathrm{Bin}(n, p(x_j))$. The non-decreasing dose-response function p is estimated by the pool-adjacent-violators algorithm using the observed frequencies $\frac{r_i}{n}$ as response for the probability of success at level x_i. Then the estimate of the effective dose level for a given α can be obtained by an "inversion" of the function $\hat{p}_{\mathrm{PAV}}$, where $\hat{p}_{\mathrm{PAV}}$ denotes the PAVA estimate of p. In a seminal paper, Ayer et al. (1955) firstly introduced a max-min formula of the monotone maximum likelihood estimate for a monotonic non-decreasing function. Applied to the dose-response model (2.1), we obtain

$$\hat{p}_{\mathrm{PAV}}(x_i) = \max_{u \le i} \min_{v \ge i} \frac{1}{(v-u+1)n} \sum_{j=u}^{v} r_j \qquad i = 1, \ldots, k,$$

which forms a set of monotone increasing points $\hat{p}_{\mathrm{PAV}}(x_1) \le \ldots \le \hat{p}_{\mathrm{PAV}}(x_k)$. The PAVA estimate can be easily calculated by the *pool-adjacent-violators* algorithm [see Barlow et al. (1972)]. Between the design points x_i, the estimate of the dose-response curve is constructed by linear interpolation, i.e. for $x_i \le x \le x_{i+1}$

$$\hat{p}_{\mathrm{PAV}}(x) = \hat{p}_{\mathrm{PAV}}(x_i) + \frac{\hat{p}_{\mathrm{PAV}}(x_{i+1}) - \hat{p}_{\mathrm{PAV}}(x_i)}{x_{i+1} - x_i}(x - x_i).$$

Therefore the estimate of the effective dose level can be obtained as the generalized inverse of the dose-response estimate. In particular, this means

$$\begin{aligned} \hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})} &= \hat{p}_{\mathrm{PAV}}^{-1}(\alpha) = \inf\{x : \hat{p}_{\mathrm{PAV}}(x) \ge \alpha\} \\ &= \begin{cases} x_1 & \text{if } \alpha < \hat{p}_{\mathrm{PAV}}(x_1), \\ x_i + \frac{\alpha - \hat{p}_{\mathrm{PAV}}(x_i)}{\hat{p}_{\mathrm{PAV}}(x_{i+1}) - \hat{p}_{\mathrm{PAV}}(x_i)}(x_{i+1} - x_i) & \text{if } \hat{p}_{\mathrm{PAV}}(x_i) < \alpha \le \hat{p}_{\mathrm{PAV}}(x_{i+1}) \text{ for some } i, \\ x_k & \text{if } \alpha > \hat{p}_{\mathrm{PAV}}(x_k) \end{cases} \end{aligned} \tag{2.2}$$

Throughout this paper this nonparametric estimate of the effective dose is denoted by $\mathrm{ED}_\alpha^{(\mathrm{BK})}$. Bhattacharya and Kong (2007) showed that the above estimate is consistent and derived its asymptotic distribution.

2.2 A local smoothing estimator for ED_α

Another nonparametric approach to estimate the effective dose level is to incorporate kernel methods which yield a smooth estimator. Müller and Schmitt (1988) proposed a kernel estimator for the dose-response curve p. In particular, they considered a design with no replications, say $0 \leq x_1 < \ldots < x_m \leq 1$, and used the Gasser-Müller estimator [see Gasser and Müller (1984)]

$$\hat{p}_{GM}(x) = \frac{1}{h} \sum_{i=1}^{m} \int_{s_{i-1}}^{s_i} K\left(\frac{x-u}{h}\right) du Y_i,$$

where $s_0 = 0, s_m = 1$, and $s_i = \frac{1}{2}(x_i + x_{i+1})$ for $1 \leq i < m$. The function K is called kernel and denotes a continuous, symmetric function with existing second moments. The quantity h is called bandwidth and converges to 0 with increasing sample size m. Furthermore, the bandwidth h fulfills $mh \to \infty$ for $m \to \infty$. This estimator has nice asymptotic properties which were derived in Müller and Schmitt (1988). On the other hand, the estimate $\hat{p}_{GM}$ is not necessarily monotone, which means that the inverse $\hat{p}_{GM}^{-1}$ might not be uniquely defined. Secondly, the effective dose estimate might be outside of the dose range for small or large values of α. The last problem can be handled using a specific kernel K to extrapolate beyond the range $[0, 1]$. To address the monotonicity issue, Müller and Schmitt (1988) suggested to average over the smallest and the largest value of all x coordinates with $\hat{p}_{GM}(x) = \alpha$ for a given α. To be precise, we define the estimate of the effective dose level by

$$\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})} = \frac{1}{2}(\inf M_\alpha + \sup M_\alpha),$$

where $M_\alpha = \{x \in [0,1] \mid \hat{p}_{GM}(x) = \alpha, \hat{p}_{GM}^{(1)}(x) > 0)\}$. Note that for small or large values of α, it might happen that $M_\alpha = \emptyset$. In this case the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ of Müller and Schmitt (1988) is not defined.

2.3 A locally weighted quasi-likelihood estimator for ED_α

Similarly, Park and Park (2006) proposed a kernel method using the local quasi-likelihood approach. The idea is to maximize the function

$$\sum_{i=1}^{m} Q[g^{-1}(\beta_0 + \beta_1(x_i - x)), Y_i] K\left(\frac{x_i - x}{h}\right), \tag{2.3}$$

where h is a bandwidth, K the kernel function, and g a known link function, to obtain an estimate for $\eta(x) = g(p(x))$. In the context of the present paper the quasi-likelihood function $Q(p(x), y)$ satisfies

$$\frac{\partial}{\partial w} Q(w, y) = \frac{y - w}{V(w)} = \frac{y - w}{w(w-1)},$$

since $\mathrm{Var}(Y|X = x) = p(x)(1 - p(x))$. Furthermore, the logit function

$$\mathrm{logit}(p(x)) = \log\left(\frac{p(x)}{1 - p(x)}\right)$$

is used as link function, which coincides with the local Bernoulli log-likelihood method. The maximum local linear quasi-likelihood estimate is given by

$$\hat{\eta}(x) = \hat{\beta}_0,$$

where $(\hat{\beta}_0, \hat{\beta}_1)$ maximizes (2.3), and the dose-response curve estimate is computed by

$$\hat{p}_{QL}(x) = g^{-1}(\hat{\eta}(x)).$$

Again, we have to face the problem that the resulting dose-response estimate is not necessarily monotone in x. Park and Park (2006) suggest to monotonize this estimate and compute the generalized inverse of the monotonized estimate. We denote the monotonized estimate by $\tilde{p}_{QL}(x)$. In particular, Park and Park (2006) considered two methods to calculate a monotonized estimate. The first method applies the PAV algorithm discussed in Section 2.1. In this case, the pool-adjacent-violators algorithm calculates the maximum likelihood estimate under monotonicity constraints for the observations $\{(x_i, \hat{p}_{QL}(x_i))\}_{i=1}^m$. This yields a monotone estimate of the function p at the dose levels $x_1, \ldots, x_m$, and the estimate of the dose response curve at an arbitrary dose level is obtained by linear interpolation. Throughout this paper we denote the estimate of the effective dose level obtained through the inversion of the PAVA-monotonized estimate by $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$.
Park and Park (2006) discussed another method to monotonize $\hat{p}_{QL}(x)$ proposed by Kappenman (1987) [see also Silvermann (1981)], where the bandwidth h of the weighted quasi-likelihood estimator in (2.3) is increased to determine the smallest h_0 such that $\hat{p}_{QL}(x)$ is monotone for all $h \geq h_0$. Then h_0 is used as bandwidth. We call this estimate for the effective dose level $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$, which is obtained as the generalized inverse of the quasi-likelihood estimate with the bandwidth h_0.

2.4 A strictly monotone estimator for ED_α

Dette et al. (2005) proposed an estimator for the effective dose level ED_α, which is strictly monotone and is a combination of a regression and an integrated kernel density estimate. The method consists of two steps. First, the dose-response curve is estimated by local linear techniques, i.e. the weighted sum of squares

$$\sum_{i=1}^m \{Y_i - \beta_0 - \beta_1(x_i - x)\}^2 K\left(\frac{x_i - x}{h}\right) \tag{2.4}$$

is minimized with respect to the parameters β_0 and β_1. Here K is a kernel function and h denotes a bandwidth, which converges to 0 with increasing sample size. The resulting estimate is given by $\hat{p}_{LL}(x) = \hat{\beta}_0$ if $(\hat{\beta}_0, \hat{\beta}_1)$ minimizes the equation (2.4). As in the last two sections this estimate is not necessarily monotone in x. Dette et al. (2005) apply an operator to $\hat{p}_{LL}$ which deals simultaneously with this lack and the issue of inversion to obtain an estimate of the effective dose level. To be precise, we define the effective dose level estimate for $\alpha \in (0, 1)$ by

$$\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})} = \hat{p}_I^{-1}(\alpha) := \frac{1}{Nh_d} \sum_{i=1}^N \int_{-\infty}^{\alpha} K_d\left(\frac{\hat{p}_{LL}(\frac{i}{N}) - u}{h_d}\right) du, \tag{2.5}$$

where the kernel K_d is positive, symmetric, twice continuously differentiable, and supported on the interval $[-1, 1]$. The corresponding bandwidth h_d converges to 0 with increasing sample size m. It was pointed out by Dette et al. (2005) that compared to the bandwidth h in the initial unconstrained local linear estimate the effect of bandwidth h_d on the resulting estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ is negligible. Note that the local linear estimate $\hat{p}_{LL}$ has to be calculated only for the points $\frac{i}{N}$ for $i = 1, \ldots, N$. The basic heuristic idea of this method is that the function

$$\frac{1}{Nh_d} \sum_{i=1}^{N} K_d \left(\frac{p(\frac{i}{N}) - u}{h_d} \right)$$

can be interpreted as an estimate of the density of the random variable $p(U)$, where U is uniformly distributed on the interval $[0, 1]$. If p is strictly increasing and differentiable the density of this random variable is given by $(p^{-1})'(u)$ and the integral

$$\frac{1}{Nh_d} \sum_{i=1}^{N} \int_{-\infty}^{t} K_d \left(\frac{p(\frac{i}{N}) - u}{h_d} \right) du$$

estimates $p^{-1}(u)$. Because p is not known, it is replaced by the local linear estimate $\hat{p}_{LL}$, which yields the estimate (2.5). The smoothing by the kernel K_d makes sure that the obtained estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ is continuous and strictly increasing for a continuous initial unconstrained estimate $\hat{p}_{LL}$. If p is strictly increasing and the bandwidth h_d is chosen sufficiently small it follows that

$$\frac{1}{h_d} \int_0^1 \int_{-\infty}^{\alpha} K_d \left(\frac{p(x) - u}{h_d} \right) du dx \approx \int_0^1 I\{p(x) \leq \alpha\} dx = p^{-1}(\alpha) = \mathrm{ED}_\alpha.$$

In the literature, the method which uses the inverse of the function $\alpha \to \int_0^1 I\{p(x) \leq \alpha\} dx$ as a monotone rearrangement of the function p is known as monotone or measure preserving rearrangement [see Hardy, Littlewood and Pólya (1952)].

3 A comparison of nonparametric estimates of the effective dose level

In this section, the finite sample properties of the introduced estimates are compared by means of a simulation study. Two simulation studies are performed using two different types of experimental designs. In the first example we consider an equidistant design with non repeated observations, while our second example investigates the case where several (independent) measurements are taken at the same dose level. This case corresponds to situation considered by Bhattacharya, M. Kong (2007).

In the following, we investigate the binary response model (2.1) with 8 different shapes of the success probabilities, that is

$$p_1(x) = \Phi \left(\frac{x - \mu}{\sigma} \right), \quad \mu = .5, \sigma = .5 \tag{3.1}$$

$$p_2(x) = \Phi\left(\frac{x-\mu}{\sigma}\right), \quad \mu = .5, \sigma = .1 \tag{3.2}$$

$$p_3(x) = 1 - \exp\{-x^\gamma\}, \gamma = .52876 \tag{3.3}$$

$$p_4(x) = \eta\Phi\left(\frac{x-\mu_1}{\tau}\right) + (1-\eta)\Phi\left(\frac{x-\mu_2}{\tau}\right), \quad \mu_1 = 0.4, \mu_2 = 1.0, \eta = .64946, \tau = .13546 \tag{3.4}$$

$$p_5(x) = \frac{1}{2} + \frac{1}{\pi}\arctan\left(\frac{x-\mu}{\sigma}\right), \quad \mu = 0.15, \sigma = 0.05 \tag{3.5}$$

$$p_6'(x) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)}(1-x)^{\beta-1}x^{\alpha-1}, \qquad \alpha = 2, \beta = 3. \tag{3.6}$$

$$p_7(x) = (1+\exp(5-15x))^{-1} \tag{3.7}$$

$$p_8(x) = \begin{cases} 2x & \text{if } 0 \le x \le 0.3 \\ 0.4x + 0.48 & \text{if } 0.3 \le x \le 0.8 \\ x & \text{if } 0.8 \le x \le 1 \end{cases} \tag{3.8}$$

Model (3.1)-(3.4) have been also considered by Müller and Schmitt (1988) and Dette et al. (2005). The success probability function (3.5) refers to a Cauchy distribution with parameters $\mu = 0.15$ and $\sigma = 0.05$. In the following model (3.6), we specify the density of the function p as a beta distribution with shape parameters $\alpha = 2$ and $\beta = 3$. Model (3.7) corresponds to the traditional logit model, where the logarithm of the odds is defined by $\text{logit}(p(x)) = -5 + 15x$. The last probability function p_8 is piecewise linear. The last two success probability functions are also discussed by Park and Park (2006). In Figure 1 the inverse functions of the functions defined by (3.1)-(3.8) are displayed.

3.1 Non repeated measurements

In our first example, we investigate the performance of the estimates if an equidistant design is used. The sample size is given by $m = 50$ and the experimental design is defined by $x_i = \frac{i-1}{49}$ for $i = 1, \ldots, 50$. The estimate $\hat{\text{ED}}_\alpha^{(\text{BK})}$ is disregarded for this study, since it requires repeated measurements. Hence, we compare the four estimates $\hat{\text{ED}}_\alpha^{(\text{DNP})}$, $\hat{\text{ED}}_\alpha^{(\text{MS})}$, $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ and $\hat{\text{ED}}_\alpha^{(\text{PP2})}$ in this section. For each scenario, 1000 simulation runs are performed to calculate the mean squared error (mse), bias, and variance of the different estimates.

The local linear estimate for $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ and the quasi-likelihood estimates for $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ and $\hat{\text{ED}}_\alpha^{(\text{PP2})}$ are computed by the function `locfit.raw` from the `locfit` package in `R`, respectively. Similarly, the Gasser-Müller estimate for $\hat{\text{ED}}_\alpha^{(\text{MS})}$ is computed using the function `glkerns` from the `lokern`

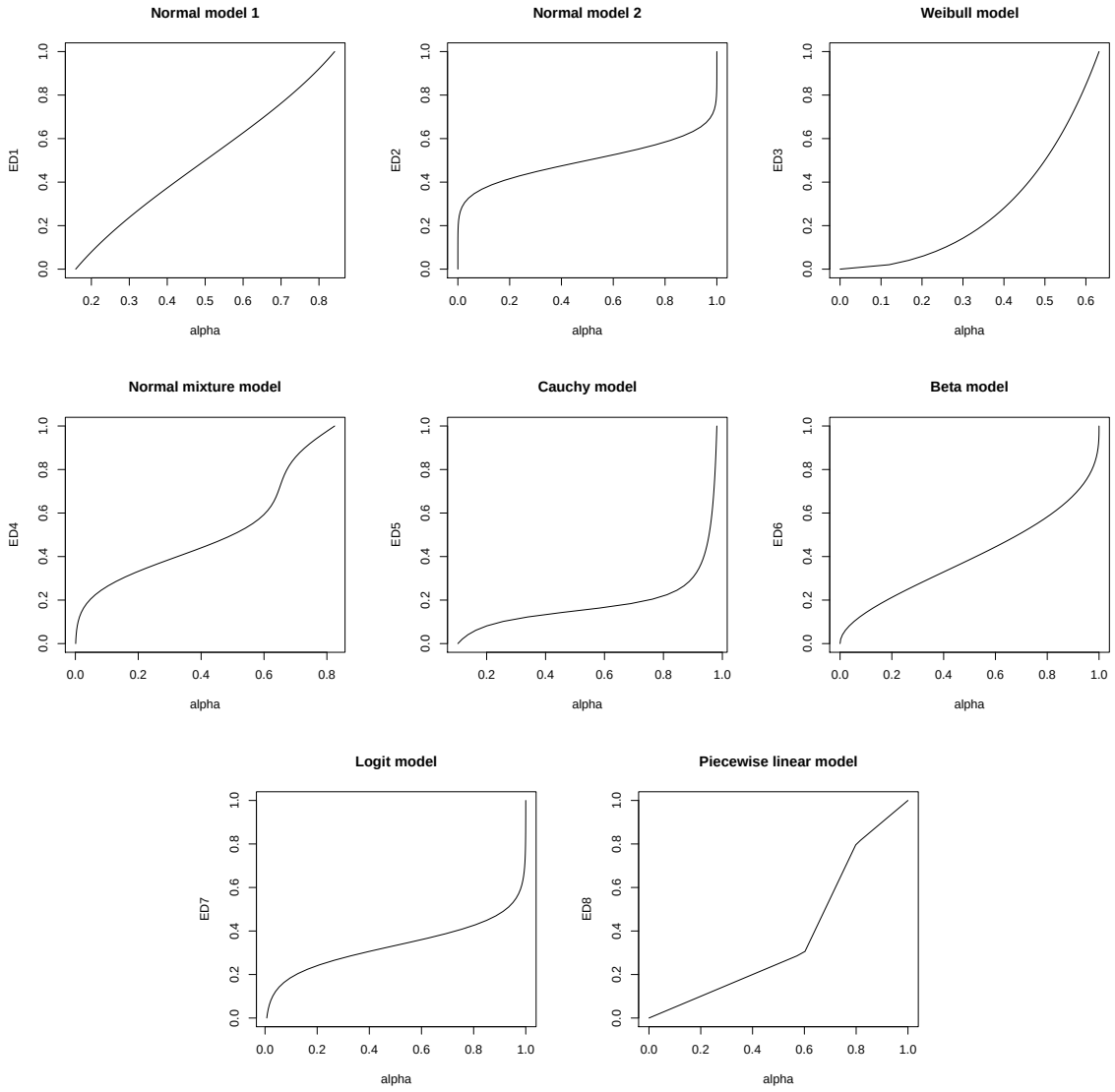

Figure 1: *The effective dose level function for the binary response model with success probability functions $p_1 - p_8$ considered in the simulation study. The functions are defined in (3.1)-(3.8).*

	$\hat{\text{ED}}_\alpha^{(\text{MS})}$	$\hat{\text{ED}}_\alpha^{(\text{PP1})}$	$\hat{\text{ED}}_\alpha^{(\text{PP2})}$	$\hat{\text{ED}}_\alpha^{(\text{DNP})}$
Normal 1 Model (3.1)	0.03103	0.03960	0.02148	0.01916
Normal 2 Model (3.2)	0.00254	0.00334	0.00256	0.00225
Weibull Model (3.3)	0.03565	0.02575	0.02127	0.02039
Normal mixture Model (3.4)	0.01602	0.02298	0.01356	0.01156
Cauchy Model (3.5)	0.00934	0.00396	0.00584	0.00442
Beta Model (3.6)	0.00549	0.00993	0.00598	0.00527
Logit Model (3.7)	0.00314	0.00416	0.00315	0.00278
Piecewise linear Model (3.8)	0.01695	0.02660	0.01387	0.01315

Table 1: *Mean integrated squared error (MISE) of the estimates for the effective dose in the different models. The sample size is* $m = 50$ *and an equidistant design with no replications has been considered.*

package. All bandwidths appearing in the calculations are set to 0.1 to keep things comparable, i.e. $h = h_d = 0.1$, where h defines the size of the local window in each of the three approaches.

First of all, we investigate the global behaviour. In Table 1 we display the mean integrated squared error (MISE) of the four different estimates. In order to avoid boundary effects the MISE was calculated for the slightly smaller interval $[0.1, 0.9]$. Note that the range of the Weibull probability function defined for $x \in [0, 1]$ is smaller than that, and consequently the MISE was calculated for the interval $[0.1, 0.5]$ in this case. In all scenarios but the Cauchy model, the estimate $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ achieves the smallest MISE value. In the Cauchy model, the estimate $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ obtains the best result, although this estimate behaves on average poor in other models compared to the estimates $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ and $\hat{\text{ED}}_\alpha^{(\text{MS})}$. The MISE of the estimate of Müller and Schmitt (1988) is larger than that of $\hat{\text{ED}}_\alpha^{(\text{DNP})}$. In particular, in the Weibull and the Cauchy model, the estimate $\hat{\text{ED}}_\alpha^{(\text{MS})}$ shows some weakness. Summarizing these results we conclude that the estimate $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ shows the best performance with respect to the global measure MISE. In the following discussion we present a more refined analysis and examine the mean squared error, bias and variance from a local perspective for each model.

The results of the detailed analysis for the success probability functions (3.1)-(3.4) are displayed in Figure 2 which shows the squared bias, variance and mse as a function of α. In the Probit model (3.1) all estimates have a similar squared bias behavior. The simulated variance of the estimates $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ and $\hat{\text{ED}}_\alpha^{(\text{PP2})}$ is substantially larger compared to $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ and $\hat{\text{ED}}_\alpha^{(\text{MS})}$. This ordering is also partially reflected in the performance of the mean squared error. Here the estimate $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ yields the worst results, while the method $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ yields the smallest mean squared error over a broad range of the design space. A similar result is obtained for the second Probit model (3.2), where the estimates $\hat{\text{ED}}_\alpha^{(\text{PP2})}$ and $\hat{\text{ED}}_\alpha^{(\text{MS})}$ yield a slightly smaller mean squared error in the interior of the

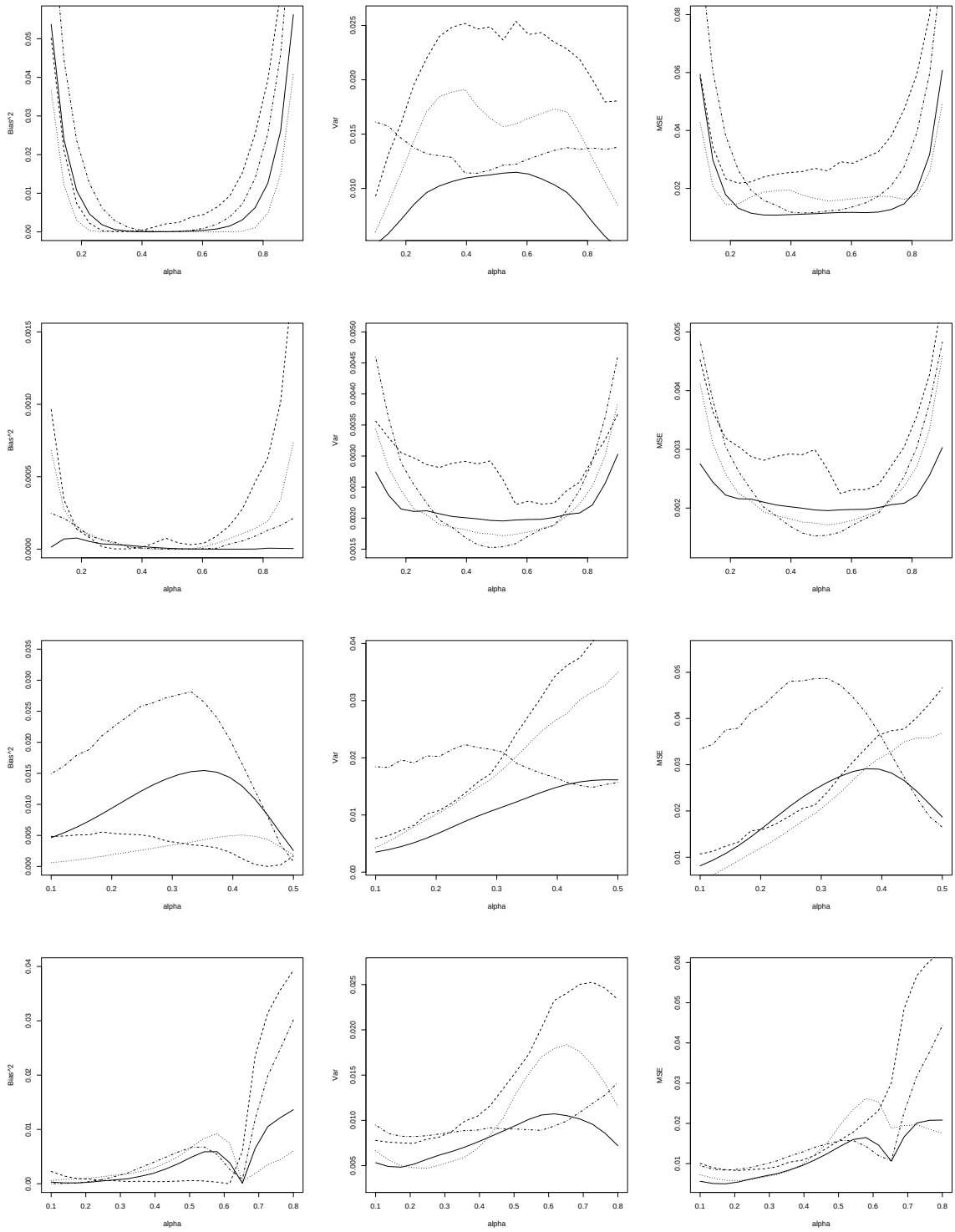

Figure 2: *Simulated bias, variance, and mse of the effective dose level estimates in the binary response models defined by (3.1)-(3.4) under a non repeated measurement design.* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{DNP})}$ *(solid line),* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{PP1})}$ *(dashed line),* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{PP2})}$ *(dotted line), and* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{MS})}$ *(dot-dashed line).*

design space. These advantages are also caused by a smaller variance of the two estimates. If α is smaller than 0.25 or larger than 0.75 the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ shows the best performance. For the Weibull model (3.3), the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ has the largest bias while $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ yield the smallest bias. On the other hand, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ has a substantially smaller variance than the other three estimates. Similarly, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ yields a small variance if $\alpha \in [0.4, 0.5]$. For smaller values of α the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$, $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ yield a substantially smaller mse than $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$, while for larger values of α the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ show the best performance. For the mixed normal model (3.4), a clear peak can be seen in the simulated bias, where the four estimates behave similarly. The estimates exhibit also a similar variance behavior if $\alpha < 0.5$. In the other interval the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ produces the smallest variance and these advantages are also reflected in the mse, where again $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ shows the best performance.

The results for the distribution functions (3.5)-(3.8) are displayed in Figure 3. In the Cauchy model (3.5), the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ behaves for $\alpha > 0.6$ considerably worse in terms of simulated squared bias, variance, and mse than the other estimates [see upper panel of Figure 3]. Here the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ show the best performance, while the last named method is the best for larger values of α. If $\alpha < 0.5$ the four estimates behave similarly. The differences between these two methods are mainly caused by the differences in the squared bias. In the case of the beta distribution function (3.6), the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ is inferior to the other estimates, which becomes especially evident in the simulated variance. The other estimates have a quite similar behavior in terms of bias, variance, and mse. Here the differences are mainly caused by the variance behavior of the estimates. For the logit model (3.7), the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ has again a larger variance in the interval $[0.2, 0.8]$, which results in a larger mse as well. The difference between the other estimates is considerably smaller. The influence of the squared bias on the mse is rather small. The estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ shows the best performance for small and large probability α. If $\alpha \in [0.3, 0.7]$ the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ yield a slightly smaller mse than $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$. In the piecewise linear model (3.8) the bias has distinctive peaks in the knots of the function p_8, where the linear pieces are put together. These peaks can be also clearly identified in the mse. The estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ has the largest variance if α is larger than 0.5, which yields the largest mse for $\alpha > 0.65$. Here the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ yields the smallest mse over the complete range of α.
Summarizing these results we observe that the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ suffers from the fact that the PAV algorithm does not yield a smooth estimate compared to the other estimates, which is expressed in larger mse values in most of the considered cases. On the other hand, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ fails completely in the Weibull and Cauchy model, where the probability function is mostly convex. In these models the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ behaves clearly better than for the other models. In all considered cases the estimate of $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ is always comparable to the best best among the four estimators. In many cases it yields in fact the smallest mse over a broad range for the probability α.

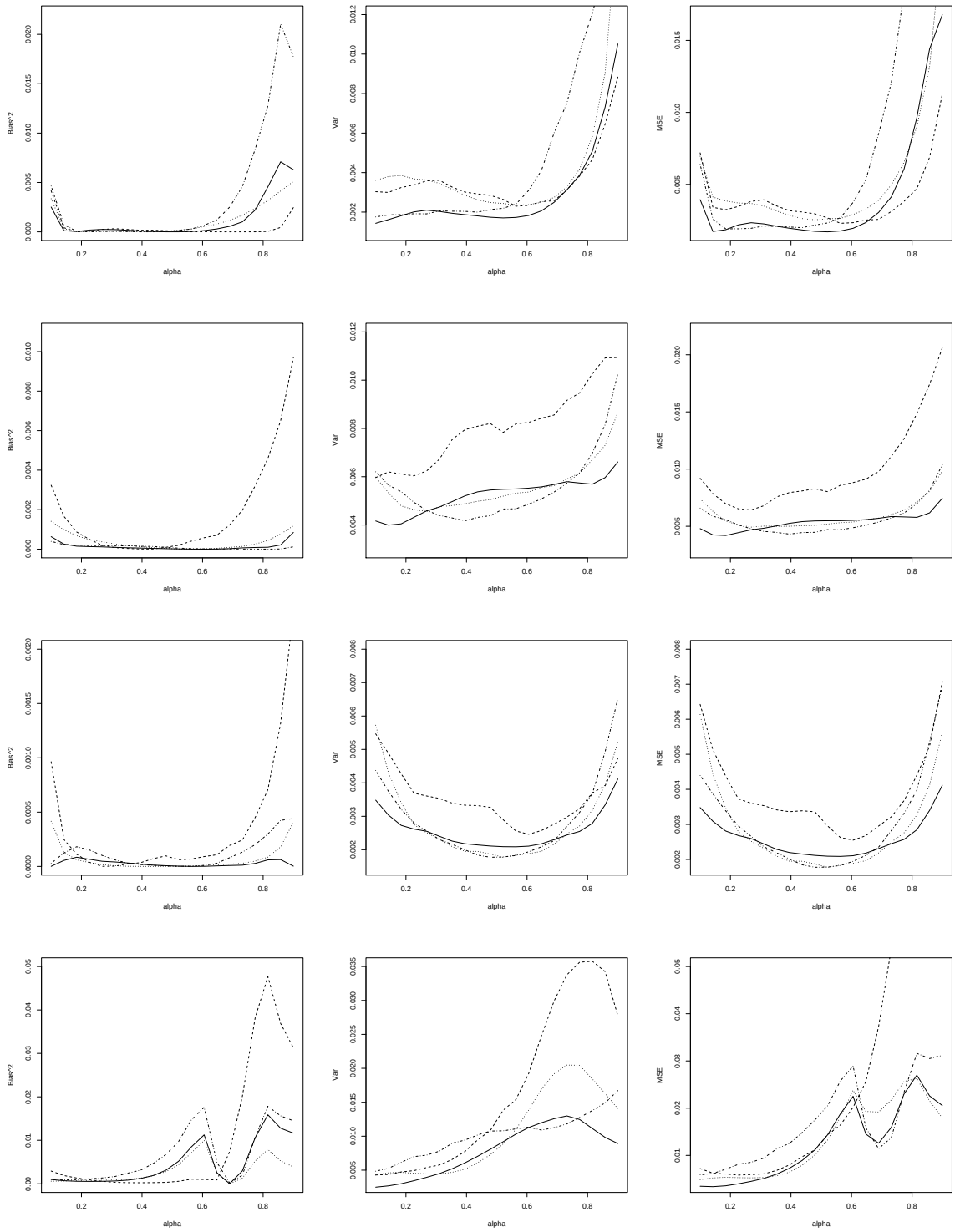

Figure 3: *Simulated bias, variance, and mse of the effective dose level estimates in the binary response models defined by (3.5)-(3.8) under a non repeated measurement design.* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ *(solid line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ *(dashed line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ *(dotted line), and* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ *(dot-dashed line).*

	$\hat{\text{ED}}_\alpha^{(\text{BK})}$	$\hat{\text{ED}}_\alpha^{(\text{MS})}$	$\hat{\text{ED}}_\alpha^{(\text{PP1})}$	$\hat{\text{ED}}_\alpha^{(\text{PP2})}$	$\hat{\text{ED}}_\alpha^{(\text{DNP})}$
Normal 1 Model (3.1)	0.04928	0.02377	0.03429	0.02129	0.01891
Normal 2 Model (3.2)	0.05031	0.00251	0.02139	0.01600	0.00251
Weibull Model (3.3)	0.01432	0.02562	0.02080	0.02450	0.02137
Normal Mixture Model (3.4)	0.04267	0.01364	0.02421	0.01506	0.01191
Cauchy Model (3.5)	0.00451	0.00738	0.00456	0.00633	0.00442
Beta Model (3.6)	0.01604	0.00489	0.00885	0.00719	0.00561
Logit Model (3.7)	0.01659	0.00293	0.00805	0.00689	0.00276
Piecewise linear Model (3.8)	0.02855	0.01268	0.02206	0.01549	0.01360

Table 2: *Mean integrated squared error (MISE) of the estimates for the effective dose in the different models. The sample sizes is m, and a repeated measurement design is considered with 10 equidistant dose levels.*

3.2 Repeated measurements

For the second simulation study, we consider an experimental design, which consists of 10 different equidistant dose levels $0 = x_1 < \ldots < x_{10} = 1$, where for each level 5 subjects are tested. In total, we have again 50 observations as in the simulation study discussed in Section 3.1. 1000 simulation runs are performed to calculate the MISE, mse, bias, and the variance. Basically the same implementation is used as for the equidistant design, but for the estimates $\hat{\text{ED}}_\alpha^{(\text{PP1})}$, $\hat{\text{ED}}_\alpha^{(\text{PP2})}$, $\hat{\text{ED}}_\alpha^{(\text{DNP})}$, and $\hat{\text{ED}}_\alpha^{(\text{MS})}$ we transform the response variable to relative frequencies, which means that $Y_i = \frac{r_i}{5}$, where r_i gives the number of positive responses. In this study the estimate $\hat{\text{ED}}_\alpha^{(\text{BK})}$ is also included.

We begin again with a discussion of the global behaviour of the five estimates. In Table 2, the MISE of the eight different models under consideration is displayed. Except for the Weibull model the estimate $\hat{\text{ED}}_\alpha^{(\text{BK})}$ shows the worst performance followed by the estimate $\hat{\text{ED}}_\alpha^{(\text{PP1})}$ and $\hat{\text{ED}}_\alpha^{(\text{PP2})}$. The estimates $\hat{\text{ED}}_\alpha^{(\text{MS})}$ and $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ exhibit the best performance with respect to the MISE criterion, where there are (slight) advantages for the estimate $\hat{\text{ED}}^{(\text{DNP})}$ in the models (3.1), (3.3) - (3.5) and (3.7). In (3.2) the MISE of both estimates is similar, while in (3.6) and (3.8) the estimate $\hat{\text{ED}}_\alpha^{(\text{MS})}$ has a slightly smaller MISE.
We continue the comparison with a local analysis of the squared bias, variance and mse behaviour of the five estimates. In Fig. 4 we display the results for the estimates (3.1) - (3.4). In the Probit model (3.1), the estimates $\hat{\text{ED}}_\alpha^{(\text{DNP})}$, $\hat{\text{ED}}_\alpha^{(\text{PP1})}$, $\hat{\text{ED}}_\alpha^{(\text{PP2})}$, and $\hat{\text{ED}}_\alpha^{(\text{MS})}$ behave similarly as in the case of an equidistant design with no replications, where there are slight advantages of the estimates $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ [see upper panel of Figure 4]. By contrast, the estimate $\hat{\text{ED}}_\alpha^{(\text{BK})}$ has a substantial larger squared bias and variance for $\alpha > 0.3$, which yields larger mse values as well. For most values of α the estimate $\hat{\text{ED}}_\alpha^{(\text{DNP})}$ and $\hat{\text{ED}}_\alpha^{(\text{MS})}$ yield the smallest mse, and these advantages are mainly

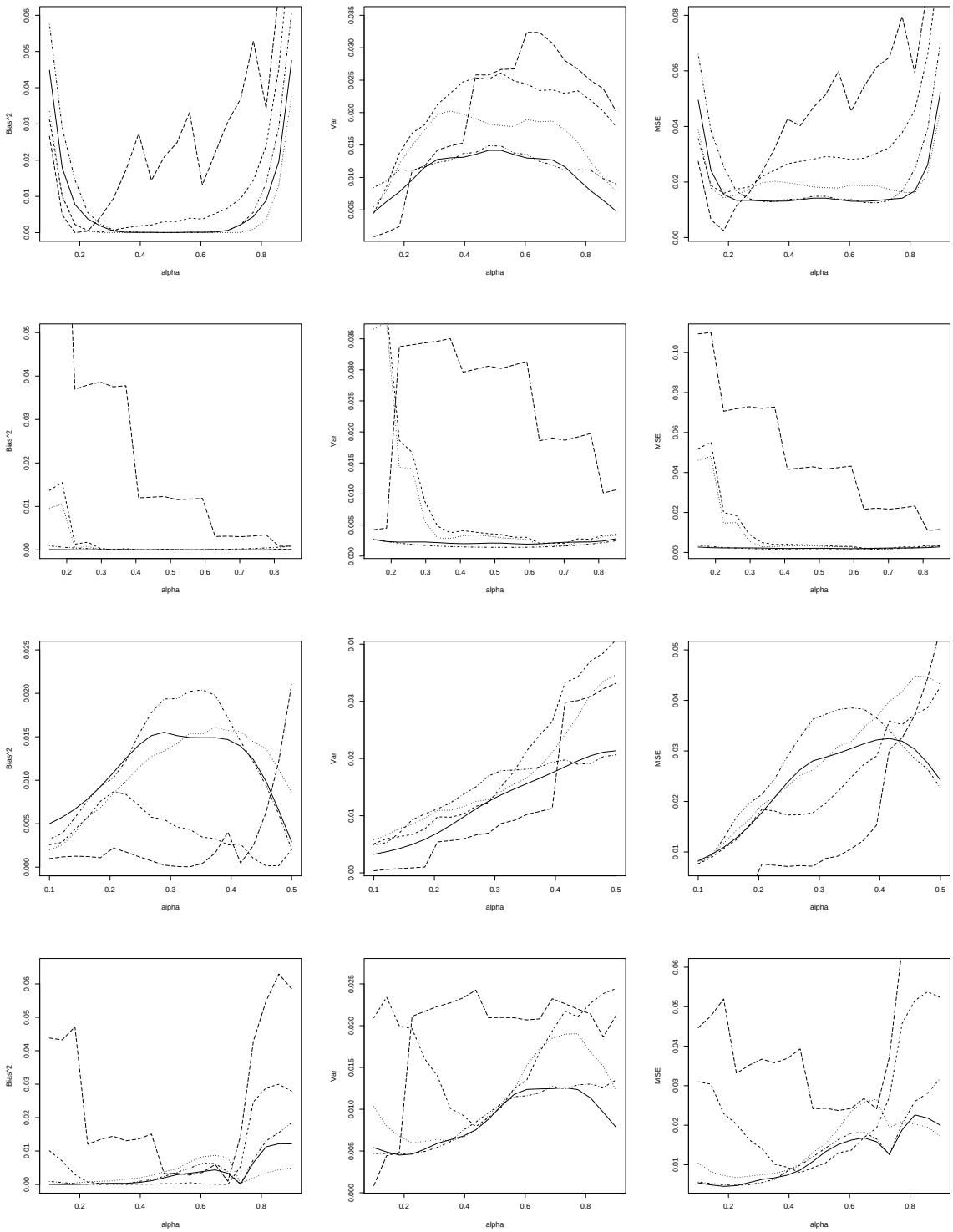

Figure 4: *Simulated bias, variance, and mse of the effective dose level estimates in the binary response models defined by (3.1)-(3.4) under a repeated measurement design.* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ *(solid line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ *(short dashed line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ *(dotted line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ *(dot dashed line), and* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ *(long dashed line).*

caused by the variance. For the second Probit model (3.2) the performance of the five estimates is very similar. Again, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ has larger values in terms of squared bias, variance and mse. The other four estimates can be divided into two classes. The estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ have large variance and mse values for $\alpha < 0.3$, whereas $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ have uniformly small values for the variance and mse. In the Weibull model (3.3), the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ shows again an unsatisfactory behavior for $\alpha > 0.4$. On the other side, for small α values this estimate has the best performance, whereas the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ fails. The other three estimate show a rather similar behavior. In the normal mixture model (3.4), the situation is different and the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ shows the best performance, while the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ yields the largest mse. The performance of the last named estimate and the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ are not reliable, if $\alpha > 0.6$ and the difference in the mse values are mainly caused by a different variance performance.

In Figure 5 we display the corresponding simulation results for the models (3.5)-(3.8). In the Cauchy model (3.5) the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ have a similar behavior in terms of variance and mse [see upper panel of Figure 5], which is smaller than that of the three other estimates if $\alpha > 0.7$. These estimates show better performance for smaller values of α. For $\alpha > 0.7$, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$, $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ drift away and have quite large mse values compared to the other estimates. For the Beta model (3.6) and the Logit model (3.7), the behavior of the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ differs substantially from the others. The simulated bias is very erratic in both cases, which is directly reflected in the mse behavior as well. The other four estimates can be separated into two groups. The estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ behave fairly similarly in terms of bias, variance and mse. Similarly, the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ have the same mse behavior and outperform the three other estimates, where a comparison between the two best estimators shows slight advantages for the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$. In the piecewise linear model (3.8), the simulated bias has visible peaks at the knots $\alpha = 0.6, 0.8$. These peaks can be also observed in the mse. As the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ differs for $\alpha > 0.6$ from the other three estimates in terms of mse values quite substantial. The three other estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$, $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ yield a very similar but substantially smaller mse.

Interestingly, as in the design with no repeated measurements the estimates using the PAV algorithm ($\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$) behave reasonable in the Weibull and the Cauchy model, whereas they fail in the other models. Note also that the difference to the simulation study based on the design with no repeated measurements is rather small. In other words, the particular design does not have a substantial impact on the performance of the estimates. On the other side, the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ seems to be not the perfect choice for estimating the effective dose level, since for most models it shows a substantial larger mse compared to the other estimates.

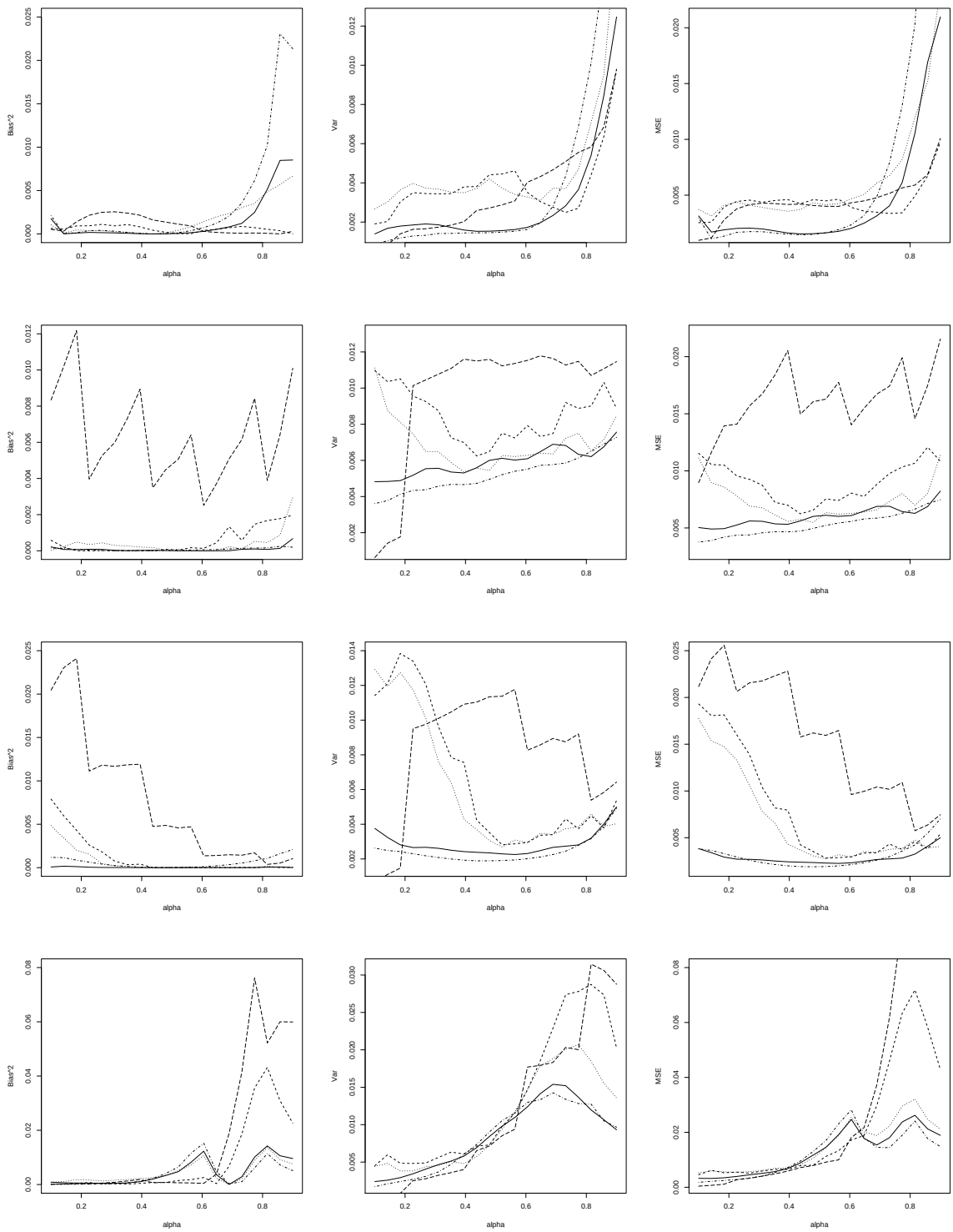

Figure 5: *Simulated bias, variance, and mse for estimator of the effective dose level in the binary response models defined by (3.5)-(3.8) under a repeated measurement design.* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{DNP})}$ *(solid line),* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{PP1})}$ *(short dashed line),* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{PP2})}$ *(dotted line),* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{MS})}$ *(dot dashed line), and* $\hat{\mathrm{ED}}_{\alpha}^{(\mathrm{BK})}$ *(long dashed line).*

$\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$	$\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$	$\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$	$\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$	$\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$
8	25.13321	20	20	20.35229

Table 3: *Values of the different estimates of* $\mathrm{ED}_{.5}$ *for the cancer remission data.*

4 Data examples

In this section, we illustrate the performance of the different estimates by means of a data example. We consider the cancer remission data discussed in Agresti (1990), which contains an explanatory variable *labeling index* (LI) measuring the proliferative activity of cells after receiving an injection of tritiated thymidine. The response variable indicates the remission of the cancer or not. The variable LI varies between 8 and 38 with duplicates. In total, 27 patients were analyzed in this study. Lee (1974) used a logistic regression model to determine $\mathrm{ED}_{.5}$, where for 50% of the patients the cancer responds to the treatment and shrinks. A detailed analysis of this approach can be found in Agresti (1990). Both authors have calculated the maximum likelihood estimates for the parametric logistic regression model, which is given by

$$\mathrm{logit}(p(x)) = -3.777 + 0.145x,$$

and yields $\hat{\mathrm{ED}}_{.5} = 26.05$ as a parametric estimate of the $\mathrm{ED}_{.5}$. In comparison to this result, the nonparametric estimates obtained from the five methods investigated in this paper are displayed in Table 3. The corresponding estimates are depicted in Figure 6 as a function of the probability α. The bandwidth h was chosen as $h = 0.356$ according to the rule of thumb

$$\hat{h} = \left(\frac{\hat{\sigma}^2}{n}\right)^{1/5},$$

where the variance estimate of Rice (1984) is used

$$\hat{\sigma}^2 = \frac{1}{2(n-1)} \sum_{i=1}^{n-1} (Y_{i+1} - Y_i)^2.$$

The bandwidth h_d in the estimate of $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ was chosen as $h_d = h^2$ (but it should be mentioned at this point that difference choices do not lead to substantially different results). We observe again that the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ does not yield reliable results in this example. The estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$, $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ yield very similar results, while the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ gives a similar result as the parametric approach proposed by Lee (1974).

5 Conclusions

In the present paper we have presented a detailed numerical comparison of the finite sample properties of five nonparametric estimates for the effective dose in quantal bioassay. These estimates

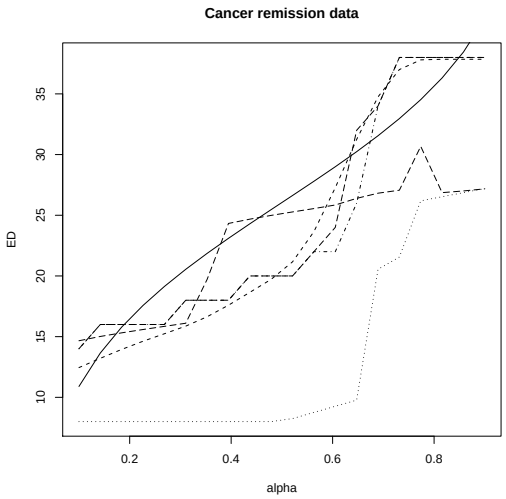

Figure 6: *Different estimates of the effective dose level for the cancer remission data considered by Lee (1974). Logistic regression fit (solid line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ *(short dashed line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ *(dotted line),* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ *(dot-dashed line), and* $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ *(long dashed line).*

can be separated into two groups. The first group consists of the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ proposed by Bhattacharya and Kong (2007) and Park and Park (2006), respectively, who addressed the problem of non-isotone estimate of the probability success curves by applying the pool adjacent violators algorithms. The second group consists of estimates based on different concepts of isotonization, in particular the increasing bandwidth approach suggested by Silverman (1981), denoted by $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$, the method of monotone rearrangements proposed by Dette et al. (2005), denoted by $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$, and an isotonization method proposed by Müller and Schmitt (1988), denoted by $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$. We consider repeated and non-repeated measurement designs, and in both cases the comparison of the estimates yield a similar picture.

It is demonstrated that the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ yields a substantially larger mean squared error in nearly all cases under consideration. The estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP1})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{PP2})}$ show a better mse behaviour than $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ but are worse than the estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$. On the other hand, the monotone rearranged estimate shows the smallest MISE except for the Weibull model, where the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{BK})}$ shows the best performance. The estimates $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ and $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$ have the same asymptotic behaviour, but we observe differences in the finite sample properties of both methods. In some cases (for example in the Cauchy model) the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{MS})}$ yields an mse, which is twice as large as the mse of the estimate $\hat{\mathrm{ED}}_\alpha^{(\mathrm{DNP})}$.

Acknowledgements. The authors are grateful to Martina Stein who typed numerous versions of this paper with considerable technical expertise. The work of the authors was supported by the Sonderforschungsbereich 475, Komplexitätsreduktion in multivariaten Datenstrukturen and by an EU-grant award CCR.IHCP.C433208.X0.

References

A. Agresti (1990). Categorical data analysis. Wiley, N.Y.

M. Ayer, H. D. Brunk, G. M. Ewing, W. T. Reid, E. Silverman (1955). An empirical distribution function for sampling with incomplete information. Ann. Math. Statist., 26, 641–647.

Barlow et al. (1972). Statistical inference under order restrictions. The theory and application of isotonic regression. John Wiley & Sons, London-New York-Sydney.

J. Berkson (1944). Application of the logistic function to bioassay. J. Amer. Statist. Assoc., 39, 357–365.

C.I. Bliss (1934). The method of probits. Science, 79, 38–39.

R. Bhattacharya, M. Kong (2007). Consistency and asymptotic normality of the estimated effective doses in bioassay. J. Statist. Plann. Inference, 137, no. 3, 643–658.

F. Bretz, J.C. Pinheiro, M. Branson (2005). Combining multiple comparisons and modeling techniques in dose-response studies. *Biometrics*, 61(3), 738–748.

J. J. Chen and R. L. Kodell (1989). Quantitative risk assessment for teratological effects. Journal of the American Statistical Association 84, 966–971.

H. Dette, N. Neumeyer, K. Pilz (2005). A note on nonparametric estimation of the effective dose in quantal bioassay. J. Amer. Statist. Assoc., 100, no. 470, 503–510.

T. Gasser, H. G. Müller (1984). Estimating regression functions and their derivatives by the kernel method. Scandinavian Journal of Statistics, 11, 171–185.

M.A. Hamilton (1979). Robust estimates of the ED_{50}. J. Amer. Statist. Assoc., 74, 344–354.

G.H. Hardy, J.E. Littlewood, G. Pólya (1952). Inequalities. Cambridge, University Press.

D.L. Hunt, D. Bowman (2004). A parametric model for detecting hormetic effects in developmental toxicity studies. Risk Analysis 24, 65-72.

R. Kappenman (1987). Nonparametric estimation of dose-response curves with application to ED50 Estimation. J. Statist. Comput. Simul., 28, 1–13.

D. Krewski, R. Smythe, K.Y. Fung (2002). Optimal designs for estimating the effective dose in development toxicity experiments. *Risk Analysis,* 22, 1195-1205.

E. T. Lee (1974). A computer program for linear logistic regression analysis. Computer Prog. Biomed. 4, 80–92.

H. G. Müller, T. Schmitt (1988). Kernel and probit estimates in quantal bioassay. J. Amer. Statist. Assoc., 83, no. 403, 750–759.

D. Park, S. Park (2006). Parametric and nonparametric estimators of $ED100\alpha$. J. Stat. Comput. Simul., 76, no. 8, 661–672.

J. Rice (1984). Bandwidth choice for nonparametric regression. Ann. Statist. 12, 1215–1230.

B.W. Silvermann (1981). Using kernel density estimates to investigate multimodality. J. Roy. Statist. Soc. Ser. B, 43, 97-99.

W. Slob (2001). Dose response modeling of continuous endpoints. Toxicological Science 66, 298–312.

R.A. Woutersen, D. Jonker , H. Stevenson , J.D. te Biesebeek, W. Slob (2001). The benchmark approach applied to a 28-day toxicity study with Rhodorsil Silane in rats: the impact of increasing the number of dose groups