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Evaluation the applicability of the Benchmark approach to existing toxicological data

Framework: Chemical compounds in the working place

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GLOSSARY

CES	=	Critical Effect Size
CED	=	Critical Effect Dose
CED05-ER	=	CED associated with 5% 'extra risk'
L05	=	lower bound of 90% confidence interval
L95	=	upper bound of 90% confidence interval
ED50	=	CED associated with an effect size that demarcates responders from non-responders
NOAEL	=	No Observed Adverse Effect Level
NAEL	=	No Adverse Effect Level
LOAEL	=	Lowest Observed Adverse Effect Level
MOAEL	=	Minimal Observed Adverse Effect Level
DECOS	=	Dutch Expert Committee on Occupational Standards
HBROEL	=	Health Based Recommended Occupational Exposure Limit
Log likelihood	=	Measure for goodness of fit of the dose-response model
PROAST	=	Possible Risk Obtained from Animal Studies
Critical Study	=	study used for deriving a NOAEL/LOAEL
ACNH	=	acetone cyanohydrin
WS	=	White Spirits
BCP	=	*o*-benzyl-*p*-chlorophenol
OPP	=	*o*-phenylphenol
LDH	=	Lactate dehydrogenase
RBC	=	Red Blood Cell Count
MCHC	=	Mean Corpuscular Hemoglobin Concentration
Hb	=	Hemoglobin
BUN	=	Blood Urea Nitrogen
PCV	=	Packed Cell Volume

ABSTRACT

Five chemicals used in workplace, for which a risk assessment had already been carried out, were selected and the relevant critical studies re-analyzed by the Benchmark approach. The endpoints involved included continuous and ordinal data. Dose-response modeling could be reasonably applied to the dose-response data encountered, and Critical Effect Doses (CEDs) could be derived for almost all of the endpoints considered. The overall Benchmark dose for the study was simular to the NOAEL in two cases, and in two other cases where no NOAEL could be derived, this dose was higher than the LOAEL divided by a factor of 10. In the fifth case the dose-response data were considered inconclusive after analysis by the Benchmark approach, making the choice of the study involved as the critical study doubtful.
It is concluded that the Benchmark approach appears applicable to OECD toxicity studies, if at least two dose groups with (different) effects are observed. In situations where only one dose group shows effects the Benchmark approach does not offer much of an improvement over the NOAEL approach. However, the situation that observed effects are not replicated in other dose groups may give unreliable results whatever approach used, including the NOAEL approach. A single significantly different dose group could be the result of some unknown experimental factor other than the applied dose, and therefore replication of effects in different dose groups is a prerequisite, whatever method of analysis is used. The re-analysis of the five compounds selected illustrates that the Benchmark approach helps in getting a more complete view of the toxicity of the compound, if effects at different levels are observed in different dose groups.

PREFACE

In regulatory risk assessments, human exposure limits are frequently based on the No-Observed-Adverse-Effect-Level (NOAEL), which is derived from toxicological studies, usually animal studies. At present, human exposure limits for chemicals are generally established from the NOAEL by applying assessment factors.
Although the NOAEL-approach is generally accepted, concern has risen on the use of NOAELs in risk assessments. The main objections against this approach are the following.

- False-negative results may occur due to masking of the effect by noise: even when the differences between test and control groups are not statistically significant, the presence of real effects cannot be excluded;
- the dose-response relationship curve is not taken into account properly;
- the uncertainty associated with the value of the NOAEL cannot be quantified, i.e. a confidence interval around this value cannot be calculated.

As an alternative to the NOAEL-approach Crump (1984) introduced the Benchmark-approach, which is based on dose-response modeling. In the report 'Toxicology-based recommended exposure limits' (GR 1996/12), the Health Council of the Netherlands recommended to investigate the applicability of the Benchmark approach in regulatory risk assessments. Subsequently, the Health Council requested the Dutch Ministry of Housing, Spatial Planning and Environment (Ministry of VROM) to initiate a project on the Benchmark approach within five legal frameworks. As a result the project "Evaluation of the 'Benchmark dose' approach in practice", project number 601930, started in 1999.

The scope of the project is to evaluate the usefulness and possible restrictions of the Benchmark approach, when applied to results from studies on chemicals for which risk assessments have already been done using the NOAEL-approach. Selected chemicals from the following legal frameworks are studied (in order of priority):

1. New chemicals
2. Existing chemicals
3. Chemicals used in the workplace
4. Pesticides
5. Pharmaceutical compounds

The present report presents the results of this evaluation for framework 3 (chemicals used in the workplace).

Contents

SAMENVATTING

Het doel van dit project is het evalueren van de bruikbaarheid en mogelijke beperkingen van de Benchmark benadering, wanneer deze wordt toegepast op resultaten van studies met chemische stoffen waarvoor een risico-evaluatie al is uitgevoerd m.b.v. de NOAEL benadering. Uit één van de te evalueren kaders (chemische stoffen in de werkomgeving) werden vijf stoffen geselecteerd. De resultaten van de geselecteerde toxiciteitsstudies voor elk van deze stoffen werden opnieuw geanalyseerd met behulp van de Benchmark benadering.

Bij de Benchmark benadering worden effectparameters geanalyseerd met behulp van dosis-effect modellering. Om het aantal effectparameters te beperken, zijn alleen de meest 'kritische' effectparameters van een toxiciteitsstudie geanalyseerd. Hierbij werd het best passende dosis-effect model geselecteerd. Uit het gekozen dosis-effect model werd voor elk van de effect parameters de kritische effect dosis (CED) afgeleid, die behoorde bij een bepaalde kritische effect grootte (CES). Voor de continue variabelen werd een arbitraire CES van 5% gebruikt (een 5% verandering in de gemiddelde respons vergeleken met de controles). Voor ordinale (histopathologische) data werden de categorieën "licht effect" of "minimaal effect" beschouwd als CES. Voor elke CED werd de 5%-ondergrens van het 90% betrouwbaarheids interval (L05) berekend. De laagste van deze L05's werd als de 'overall' Benchmark dosis in de betreffende studie beschouwd.

Butyl acetaat
De Werkgroep van deskundigen van de Gezondheidsraad heeft bij de beoordeling van de toxiciteit van n-butyl acetaat een 13-weken inhalatie toxiciteitsstudie bij ratten benoemd als 'kritische' studie. Gebaseerd op, onder andere, afnemende lichaamsgewichten en een in mate en ernst toenemende necrose van het neus-epitheel, kon in de studie een NOAEL worden afgeleid van 500 ppm (2420 mg/m^3).

De Benchmark benadering werd toegepast op lichaamsgewichten en op necrose van het neus-epitheel. Bij een arbitraire CES van 5% voor afname van lichaamsgewicht was de Benchmark dosis 645 ppm (ongeveer 3122 mg/m^3). De Benchmark dosis voor minimale necrose van het neusepitheel was 1458 ppm (ongeveer 7057 mg/m^3). De 'overall' Benchmark dosis voor deze studie werd vastgesteld op 645 ppm, een waarde die dicht bij de NOAEL ligt. Geconcludeerd kan worden dat de Benchmark benadering is toe te passen op de twee bovengenoemde kritische effectparameters uit de kritische studie. De Benchmark benadering geeft aan dat blootstelling aan doses gelijk aan de NOAEL, een afname van het lichaamsgewicht van ongeveer 3% zou veroorzaken.

Captan
De Werkgroep van deskundigen van de Gezondheidsraad heeft bij de beoordeling van de toxiciteit van captan een 90-dagen inhalatie toxiciteitsstudie bij ratten benoemd als 'kritische' studie. Gebaseerd op het voorkomen van squameuze epitheliale hyperplasie in de larynx bij de laagste dosering, werd in de studie een 'marginal observed adverse effect level' (MOAEL) afgeleid van 0,13 mg/m^3.
De Benchmark benadering werd toegepast op squameuze epitheliale metaplasie en hyperplasie in de larynx. Minimale squameuze epitheliale metaplasie in de larynx werd als een nadelig effect beschouwd en de corresponderende Benchmark dosis was 0,16 mg/m^3. Deze waarde verschilt niet veel van de MOAEL voor captan van 0,13 mg/m^3 uit de kritische studie.

ACNH
De Werkgroep van deskundigen van de Gezondheidsraad heeft bij de beoordeling van de toxiciteit van ACNH een 1- en een 3-maands inhalatie toxiciteitsstudie bij ratten benoemd als 'kritische' studie. Gebaseerd op een toename van de relatieve levergewichten en veranderde bloedparameters, werd een NOAEL afgeleid van 35 mg/m^3.
Bij gebruikmaking van de Benchmark benadering zijn voor een aantal bloedparameters, kritische effect doses afgeleid. Hoewel er significante dosis-effect relaties konden worden aangetoond voor de eindpunten RBC, Hb, MCHC en BUN, werden deze effecten volledig bepaald door één groep, die afweek van de andere 7 groepen. De kans dat deze effecten veroorzaakt worden door andere (onbekende) experimentele factoren, die geassocieerd zijn met de afwijkende groep, is groot. Om deze reden werd de feitelijke aanwezigheid van de effecten als onzeker beschouwd.
Wanneer, echter, de waarden in de afwijkende groep (hoogste dosering; vrouwtjes) het gevolg zouden zijn van de blootstelling aan ACNH, zou de Benchmark dosis ongeveer 4x hoger zijn dan de NOAEL (op basis van een CES van 5%).

Lyorthol
Lyorthol, een veelgebruikt desinfectans in Nederlandse ziekenhuizen, bevat twee actieve ingrediënten: o-benzyl-p-chloorfenol (BCP) en o-fenylfenol (OPP). De kritische toxiciteitsstudies voor BCP en OPP betroffen respectievelijk een 2-jaar orale toxiciteitsstudie bij de rat en een 2-generatie reproductie toxiciteitsstudie bij de rat. Op basis van niereffecten bij de laagste dosering (30 mg/kg lg/d; LOAEL) werd in de chronische toxiciteitsstudie derhalve geen NOAEL afgeleid. In de 2-generatie studie werd op basis van proliferatieve veranderingen van het blaasepitheel (bij 125 mg/kg lg/d; LOAEL) een NOAEL voor parentale effecten afgeleid van 40 mg/kg lg/d (gebaseerd op nier- en urineblaas effecten). Beide stoffen vertonen een carcinogene werking in de nieren en de urineblaas. Met behulp van de Benchmark benadering zijn voor BCP en OPP de dosis-effect curven gemodelleerd van verschillende (nier)effect parameters. Het gaat hier om quantale data, en de CED werd op twee manieren gedefinieerd: als ED50 en als dosis bij een respons niveau van 5% boven de

achtergrond. Wanneer de ED50 als CED beschouwd wordt, dan zou voor BCP en OPP de Benchmark dosis (5%-betrouwbaarheidsondergrens van de CED), respectievelijk, 133 mg/kg lg/dag (hyperplasie van de nierbuisjes) en 366 mg/kg lg/dag (hyperplasie van het urineblaas epitheel) bedragen. Deze waarden zijn duidelijk hoger dan de respectievelijke LOAEL van 30 mg/kg lg/d voor BCP en de NOAEL van 40 mg/kg lg/d voor OPP.
Voor sommige van de quantale eindpunten gerapporteerd in de Lyorthol studies kon de ED50 alleen geschat worden door extrapolatie van het gefitte model naar hogere doses dan experimenteel waren toegepast. In deze gevallen kan de schatting van de ED50 onbetrouwbaar zijn.
Wanneer de CED gedefinieerd wordt als de dosis behorende bij een 5% respons-niveau, dan bedraagt de Benchmark dosis voor BCP 53 mg/kg lg/dag, en voor OPP 69 mg/kg lg/dag.

White Spirits
De Werkgroep van deskundigen van de Gezondheidsraad heeft bij de beoordeling van de toxiciteit van White Spirits een 13-weken inhalatie toxiciteitsstudie bij ratten benoemd als 'kritische' studie. Gebaseerd op, onder andere, toegenomen relatieve levergewichten in beide sexen en milde anemie in mannelijke ratten, werd in de studie een LOAEL afgeleid van 2000 mg/m^3. Op deze LOAEL werd een arbitraire assessment factor van 6 toegepast, resulterend in een 'NOAEL' van 330 mg/m^3. Met behulp van de Benchmark benadering zijn de relatieve gewichten van lever, milt en nieren opnieuw geanalyseerd. De CES werd (arbitrair) op 5% gesteld en de corresponderende Benchmark doseringen werden afgeleid. Deze bedroegen, respectievelijk: 1762/1357 mg/m^3 (lever; m/v), 2363 mg/m^3 (milt) en 1390/2218 mg/m^3 (nieren; m/v). De laagste afgeleide Benchmark dosis (1357 mg/m^3 bij verhoogde relatieve levergewichten bij vrouwtjes) is lager dan de LOAEL van 2000 mg/m^3, maar hoger dan de 'NOAEL' van 330 mg/m^3, die zijn afgeleid uit de kritische studie.

Geconcludeerd kon worden dat de Benchmark benadering zonder grote problemen was toe te passen in alle vijf de studies. Wanneer er twijfel ontstond over de juistheid van het gekozen model, kon dit worden toegeschreven aan het tekort aan dosis groepen, waarin het betreffende effect werd gezien. Dit probleem kan worden opgelost wanneer het aantal dosis groepen wordt uitgebreid, om zo het risico te verkleinen dat effecten slechts in één dosis groep worden waargenomen. Om het aantal proefdieren binnen acceptabele grenzen te houden, kan dit alleen bereikt worden met minder proefdieren per groep. Deze kleinere groepen vormen geen belemmering bij de toepassing van de Benchmark benadering.

Voor twee van de vijf stoffen kon alleen een LOAEL worden afgeleid. In beide gevallen kon de Benchmark benadering zonder problemen worden toegepast. De afgeleide Benchmark doses waren in beide gevallen hoger dan de gecorrigeerde "NOAEL" (LOAEL gedeeld door een veiligheidsfactor). Voor twee van de vijf stoffen was de afgeleide Benchmark dosis vergelijkbaar met de NOAEL. De feitelijke aanwezigheid van effecten van de laatste stof

bleek, na toepassing van de Benchmark benadering, onzeker te zijn. Op basis hiervan zouden de resultaten van de kritische studie waarschijnlijk als ontoereikend worden gekenschetst.

SUMMARY

The scope of the project is to evaluate the usefulness and possible restrictions of the Benchmark approach, when applied to results from studies on chemicals for which risk assessments have already been done using the NOAEL-approach. From one of the frameworks to be evaluated (Chemicals used in the workplace), five chemicals were selected. The results of the selected toxicity studies for each of these chemicals were re-analysed by means of the Benchmark approach.

With the Benchmark approach effect parameters are analyzed by dose-response modeling. To reduce the amount of effect parameters, we analyzed only the most 'critical'effect parameters of a toxicity study. The best fitting dose-response model was selected. From this model the Critical Effect Dose (CED) associated with a particular Critical Effect Size (CES) was derived. For continuous endpoints a CES of 5% was used, i.e. a 5% change in average response level compared to the controls. For ordinal (histopathological) data the categories slight or minimal effect were considered as the CES. For each CED assessed the lower 5%-confidence limit (L05) was calculated. The lowest of these L05s was regarded as the Benchmark dose of the particular study.

Butyl acetate
The Dutch Expert Committee on Occupational Substances of the Health Council of the Netherlands (DECOS), has appointed a 13-week inhalation toxicity study in rats as the 'critical study' with respect to the toxicity of n-butyl acetate. Based on, a.o., decreased terminal body weights and olfactory epithelial necrosis, a NOAEL of 500 ppm (2420 mg/m^3) was derived.

Both terminal body weight and olfactory epithelial necrosis were studied, applying the Benchmark approach. Taking a 5% decrease in terminal body weight as CES, the corresponding CED (L05) was 645 ppm (approx. 3122 mg/m^3). The CED (L05) derived for minimal olfactory epithelial necrosis was 1458 ppm (approx. 7057 mg/m^3). Thus the Benchmark dose for this study was assessed at 645 ppm, which is close to the NOAEL.
It can be concluded that the Benchmark approach is applicable to the critical effects (changed terminal body weights and olfactory epithelial necrosis) in the critical study selected. The Benchmark approach shows that exposure at the NOAEL of 500 ppm (2420 mg/m^3) would cause a decrease in terminal body weight of around 3%.

Captan
The DECOS has appointed a 90-day inhalation toxicity study in rats as critical, with respect to the toxicity of captan. Based on squamous epithelial hyperplasia of the larynx at the lowest dose tested, a MOAEL of 0.13 mg/m^3 was derived in the critical study. Both endpoints were

re-analysed by the Benchmark approach. Minimal squamous epithelial hyperplasia of the larynx was considered adverse, and the corresponding Benchmark dose was 0.16 mg/m^3. For captan, the Benchmark dose is almost equal to the MOAEL of 0.13 mg/m^3, derived in the critical study.

ACNH

The Dutch Expert Committee on Occupational Substances of the Health Council of the Netherlands (DECOS), has appointed a 1- and 3-month inhalation toxicity study in rats as critical, with respect to the toxicity of acetone cyanohydrin (ACNH). Based on increased relative liver weights and changed blood parameters, a NOAEL of 35 mg/m^3 was derived. With use of the Benchmark approach for a number of blood parameters, different CEDs could be derived.

It was concluded that, although significant dose-response relationships were found for the endpoints RBC, Hb, MCHC and BUN, these were all four determined by one treatment group, deviating from the other seven treatment groups. Therefore, the effects were considered uncertain. The probability that the effects may have been caused by some other unknown experimental factor, associated with the deviating treatment group is high.

If, nonetheless, the deviating highest dose-group in females were considered to be an effect of ACNH, the resulting Benchmark dose would be approximately four times higher than the NOAEL (based on a CES of 5%).

Lyorthol

Lyorthol, a commonly used disinfectant in Dutch hospitals contains two active ingredients: *o*-benzyl-*p*-chlorophenol (BCP) and *o*-phenylphenol (OPP). From the two separate toxicological profiles of BCP and OPP, a 2-year chronic toxicity study in rats and a 2-generation reproductive toxicity study in rats were appointed as critical studies, respectively. The LOAEL derived in the critical study was 30 mg/kg bw/d for BCP (based on kidneys effects). For OPP a NOAEL of 40 mg/kg bw/d (based on kidneys and urinary bladder effects) was derived. In order to establish the Benchmark doses for both BCP and OPP, various kidney and urinary bladder effects were evaluated. These are quantal data, and the CED was defined in two ways: as the ED50, and as the dose associated with a 5% response level (above background). When the ED50 is considered to represent the CED, then the Benchmark dose (5% lower confidence limit of CED) for BCP and OPP amounts to 133 and 366 mg/kg bw/day, respectively. These values are both higher than the LOAEL for BCP and the NOAEL for OPP. For some of the quantal endpoints reported in the Lyorthol studies the ED50 could only be estimated by extrapolation of the fitted model to doses higher than applied in the study. In such a situation the estimation of the ED50 may be unreliable. When the CED is defined as the dose associated with a 5% response level the Benchmark doses for BCP and OPP amounted to 53 and 69 mg/kg bw/day, respectively

White Spirits

The Dutch Expert Committee on Occupational Substances (of the Health Council of the Netherlands) has appointed a 13-week inhalation toxicity study in rats as critical, with respect to the toxicity of White Spirits. Based on low-grade anaemia in male rats and on increased relative liver weights in both sexes, a LOAEL of 2000 mg/m^3 and using an arbitrary assessment factor of 6, a NAEL of 330 mg/m^3 was derived. Increased relative liver, spleen, and kidney weights were studied with the Benchmark approach. A 5% change in relative organ weights was arbitrarily chosen as the CES, and the corresponding CEDs (L05) for increased relative liver, spleen, and kidney weights were: 1762/1357 (m/f), 2363, and 1390/2218 (m/f) mg/m^3, respectively. The lowest CED (L05) found (1357 mg/m^3 for increased relative liver weights, females) may be used as the Benchmark dose. This Benchmark dose is lower than the LOAEL of 2000 mg/m^3 derived in the critical study, but higher than the 'NOAEL' of 330 mg/m^3.

It was concluded that the Benchmark approach was applicable in all five studies, without major difficulties. In cases of doubt on the right choice of the dose-response model, this was attributable to the data, usually the scarcity of dose groups. This implies that this doubt equally applies when the NOAEL, or any other approach is used. This situation can only be improved by using more dose groups, to prevent the risk that effects are observed in a single, i.e. unreplicated, dose group. To keep the total number of test animals within acceptable limits, this can only be achieved by smaller dose groups, which is no objection when the Benchmark approach is applied.

In two of the five compounds only a LOAEL could be derived. In both cases the Benchmark approach could be applied without any difficulty, the derived Benchmark doses being higher than the estimated "NOAEL" (i.e. LOAEL divided by some factor. For two of the selected compounds a NOAEL had been derived. The Benchmark dose for these studies was close to the NOAEL.

For the remaining compound the Benchmark approach would probably have dismissed the critical study as inadequate for proper evaluation

1. FRAMEWORK

As described in the preface, five frameworks were nominated in the scope of the RIVM project for evaluating the usefulness of the Benchmark approach in risk assessment. The frameworks selected are; new chemicals, existing chemicals, chemicals used in the workplace, pesticides, and pharmaceutical compounds. In this report a re-analysis of five 'chemicals used in the workplace' is described. The methodology of the statistical analysis of the results is given in Chapter 2. The five substances were selected from 17 candidates. These 17 candidates resulted from a general survey of the information available in the public reports of the Dutch Expert Committee on occupational Standards' (DECOS) and from the public literature from 1992 to 1999. Important criteria for selection were: (i) the 'health based occupational exposure limit' (HBROEL), which was derived by DECOS for the majority of the candidates, (ii) the parameters that showed treatment-related effects in the critical studies and (iii) the number dose-levels that showed toxicologically relevant effects in the critical studies. Genotoxic carcinogens were excluded from the selection. The suitability of each of the candidates was evaluated on the basis of the availability and level of detail of the toxicological data from the critical studies used for driving the HBROEL (using a NOAEL or LOAEL). The five substances that were selected in two selection-rounds from the 17 candidates were: Butyl acetate (n-, iso-, sec-, and tert-Butyl acetate; Chapter 3), Captan (Chapter 4), Acetone cyanohydrin (ACNH; Chapter 5), Lyorthol (*o*-Phenylphenol and *o*-Benzyl-*p*-chlorophenol; Chapter 6) and White spirits (hydrocarbon solvents; Chapter 7). In chapter 8, a discussion and an overall conclusion with respect to the re-analysis of the five selected chemicals used in the workplace are given.

2. STATISTICAL METHODS

The dose-response data were re-analysed using the "Benchmark approach". The analyses were performed using the software PROAST (Possible Risk Obtained from Animal Studies) which has recently been developed at RIVM (Slob, 1999). Using PROAST a dose-response model is fitted to the data, then a Critical Effect Size (CES) is defined, and the corresponding Critical Effect Dose (CED) is derived from the fitted model. The uncertainty in the estimate of the CED is assessed by a bootstrap method (Slob and Pieters, 1998), resulting in an uncertainty distribution from which any desired confidence interval can be derived. In this report the 5% and 95% confidence limits are presented (i.e. 90% confidence intervals). Note that the 5% confidence limit can be considered as the Benchmark dose as originally defined by Crump (1984).

2.1 Model fitting

The dose-response data are described by one of the following mathematical models:

model 1: $y = a$
model 2: $y = a \exp(b\,x)$
model 3: $y = a \exp(b\,x^d)$
model 4: $y = a\,[c - (c - 1) \exp(b\,x)]$
model 5: $y = a\,[c - (c - 1) \exp(b\,x^d)]$.

In these models the parameter *a* represents the background level of the particular endpoint. The parameter *b* reflects the 'slope' or the 'strength' of the response. These models are suitable for describing different (sub)populations by the same model. For example, when males and females are equally sensitive to the compound studied with respect to body weight, male and female body weights can be described by the same model, with only parameter *a* differing between males and females, to account for background body weights differing between sexes. When males and females are not equally sensitive, the parameter *b* differs between sexes.

The selection of the model to be used for deriving the CED follows from a procedure of successively fitting the above models, and applying likelihood ratio tests to see if an increase in the number of parameters leads to a significantly better fit to the data. A model with more parameters is considered better only if this leads to a significantly better fit. The selected model is used for further analysis of the data to see if the fit can be improved by allowing the parameter *a*, the parameter *b,* or the residual variance (or possibly any combination of these

three) to differ between the two sexes. Again, an extension of the number of parameters is only adopted if this results in a significantly better fit. The selected model is also fitted to each of the two sexes separately; the sum of the two associated log-likelihoods may be considered as the maximum achievable log-likelihood value, serving as a reference.

Histopathological data

The above family of models is also used for describing histopathological data (i.e. ordinal or quantal data). This is done in an indirect way, and needs some explanation. The basic idea is thathistopathological scores can be viewed as a discretization of an underlying gradual response. For example, the degree of olfactory epithelial necrosis is in fact a gradual phenomenon, but is classified by the experimental observer in discrete classes as minor, minimal of moderate. The underlying gradual response is assumed to have a dose-response relationship that can be described by the same models 1-5 as used for continuous data. In addition, just as in the case of continuous data this underlying gradual response (also called the latent variable) is subject to experimental error and interindividual variation,assumed to follow a normal distribution. The bounds between the severity categories now determine the fraction of the observations expected to occur at the various doses, by cutting off a certain fraction of the normal distribution (see Fig. 2.1.1.). The expected fractions according to the model is then fitted to the observed fractions using maximum likelihood methods. The bounds between the categories (scores) in terms of the latent variable are estimated by fitting the model to the data.
This same model can also be applied to quantal data, which is in fact only a special case of ordinal data, with only two effect categories. In that case only a single CED can be asssessed, and this CED can also be considered as the ED50.

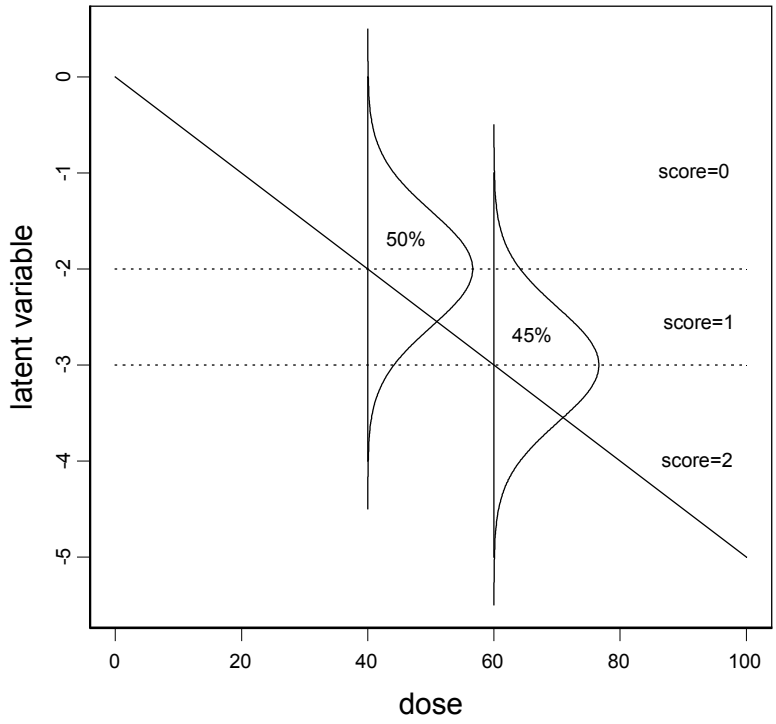

Fig. 2.1.1 Illustration of the basic idea of dose-response modeling of ordinal data. A continuous dose-response relationship is assumed to exist for the underlying gradual response (latent variable), here indicated by the straight, decreasing line. The experimental observer discriminates discrete classes of severity (e.g., score 0: normal; score1: minimal; score 2: moderate). The bounds between these classes cutt off the normal distributions around the underlying continuous dose-response, representing the experimental variation. For instance, at dose 40 half of the observed scores is expected to be 0, and at dose 60 score 1 is expected for 45% of the animals. By fitting the expected fractions of scores to the observed fractions of scores, the underlying dose-response function is estimated, as well as the bounds between the scores. Note that the values at the ordinate have no biological meaning, and can be arbitrarily chosen.

2.2 Critical effect sizes

For continuous endpoints the critical effect size (CES) is defined as a specified change in an effect parameter's level relative to the effect parameter's level at dose zero. The dose at which that specified change occurs is called the critical effect size (CED). For example, considering the effect parameter terminal body weight, the CED05 represents the dose associated with a 5% decrease (i.e., CES of 5%) in the average animal's body weight. The magnitude of the CES may be chosen differently for different effect parameters and should be the subject of discussion among toxicologists, aimed at reaching consensus on their values. Since such a consensus has not yet been reached, we will, as a provisional approach, use a CES of 5% for most of the effect parameters studied.

While for continuous data the CED corresponding to any chosen CES can be assessed, for ordinal data this choice is limited. The magnitude of the effect is in fact defined by the observer, in terms of discrete categories (e.g. a minimal, mild, moderate or severe effect). Only those doses that correspond to the transitions between these categories can be estimated. The CED for each category is defined as the dose at which the average animal's response switches from one category to the next (e.g. from a minimal to a mild effect). Similarly, for quantal data the CED is defined as the dose at which the average animal switches from non-responding to responding. Therefore, in the case of ordinal or quantal data the number of CEDs is determined by the number of categories.

Crump (1984) proposed to use extra (or additional) risk as a measure of effect size for quantal data. However, these measures are difficult to interpret, because of the fact that the slope of a quantal dose-response relationship as estimated from quantal data depends on the experimental error, including measurement errors (see Slob and Pieters, 1998). As a consequence, the extra risk level of 0.05 in a fitted dose-response function cannot be interpreted as 5% of the animals, since it is distorted by noise. In addition to that, variation in response between individual animals in a laboratory setting is not relevant for the human population.
Nonetheless, the CED05 and CED10 are reported for quantal data as well. It should be noted, however, that these CEDs are related to effect sizes that are conceptually distinct from those in the case of continuous data.

3. BUTYL ACETATE

Summary
The Dutch Expert Committee on Occupational Substances of the Health Council of the Netherlands (DECOS), has appointed a 13-week inhalation toxicity study in rats as the 'critical study' with respect to the toxicity of n-butyl acetate. Based on, a.o., decreased terminal body weight and olfactory epithelial necrosis, a NOAEL of 500 ppm (2420 mg/m^3) was derived.
Both terminal body weights and olfactory epithelial necrosis were studied, applying the Benchmark approach. Taking a 5% decrease in terminal body weight as arbitrairy CES, the corresponding CED (L05) was 645 ppm. The CED (L05) derived for minimal olfactory epithelial necrosis was 1458 ppm. Thus the Benchmark dose for this study was assessed at 645 ppm, which is close to the NOAEL.
It can be concluded that the Benchmark approach is applicable to the critical effects (changed terminal body weights and olfactory epithelial necrosis) in the critical study selected. The Benchmark approach shows that the NOAEL of 500 ppm (2420 mg/m^3) corresponds to a decrease of terminal body weight of around 3%.

3.1 Introduction and general toxicity profile

Butyl acetate is a mixture of n-, iso-, sec-, and tert-butyl acetate. Butyl acetates occur in natural and food products and are produced chemically as well. They are used mainly as solvents in paints and lacquers. In the Netherlands the practised inhalatory occupational exposure limits (8-hours time weighted average) for n-butyl acetate, iso-butyl acetate, sec-butyl acetate and tert-butyl acetate are 710 mg/m^3 (150 ppm), 700 mg/m^3 (150 ppm), 950 mg/m^3 (200 ppm) and 950 mg/m^3 (200 ppm), respectively.

In studies with rats, n-butyl acetate was non-irritating and non-sensitising to skin. In studies with rabbits n-butyl acetate was slightly irritating to the eyes. In experimental animals, the acute toxicity of n-butyl acetate and iso-butyl acetate was low after inhalatory, oral, or dermal exposure. Single inhalatory exposure to approximately 3700 - 7300 mg n-butyl acetate/m^3 for 4 - 6 hours resulted in transient effects on the eyes and behaviour. Semichronic inhalatory exposure (13-14 weeks, 6h/d, 5 d/w) of rats to approximately 7260 mg/m^3 resulted in a.o. minimal transient effects on the nervous system (reduced activity) and olfactory epithelial

necrosis (graded minimal to moderate). Exposure (highest dose tested 14520 mg/m^3) did not induce persistent neurotoxic effects. From this study a NOAEL of 2420 mg/m^3 could be derived (Shulman, 1996). The study ("critical study") was used by DECOS for deriving the HBROEL for n-butyl acetate. A developmental toxicity study was considered insufficient and no chronic toxicity or carcinogenicity studies with butyl acetates were available. n-Butyl acetate was not mutagenic or clastogenic. No data on repeated exposures or on mutagenicity were found with respect to other isomers.
In human volunteers exposed for four hours to 700 mg n-butyl acetate/m^3, the substance was only minimally irritating to the eyes and the respiratory tract. N-butyl acetate may occasionally cause allergic contact dermatitis and has probably no skin sensitising properties (DECOS).

3.2 Critical study

13-week inhalation toxicity study in rats

Substance	: n-butyl acetate	Duration	: 13 weeks (6 h/d, 5 d/w)
Route	: inhalation	Dose levels	: 0, 2420, 7260, 14520 mg/m^3 (0, 500, 1500, 3000 ppm)
Recovery per.	: -	Species	: rat
Sex	: m/f	Guideline	: OECD 413
Ref	: Shulman, 1996	GLP	: yes
NOAEL	**: 2420 mg/m^3 (500 ppm)**	**LOAEL**	**: 7260 mg/m^3 (1500 ppm)**

Description:
Method:
Rats (Sprague-Dawley; 10/sex/dose) were exposed inhalatory to 0, 2420, 7260, and 14520 mg/m^3 of n-butyl acetate 6 h/d, 5 d/w for 13 weeks.

Results:
The results are presented in Table 3.2.1.

Table 3.2.1 Observations in a 13-week inhalation toxicity study in rats exposed to n-butyl acetate

Dose (ppm)	0		500		1500		3000		
Effect	m	f	m	f	m	f	m	f	dr
Mortality	none								
Clinical signs	none								
acute transient signs of reduced activity					minimal (both sexes)		minor (both sexes)		m/f
Food consumption					dc	dc	dc	dc	m
Terminal body weight					dc	dc	dc	dc	m
Microscopic findings									
olfactory epithelial necrosis:									
total:	0/10	0/10	0/10	0/10	4/10	6/10	10/10	10/10	
- minimal	0/10	0/10	0/10	0/10	3/10	4/10	0/10	0/10	
- minor	0/10	0/10	0/10	0/10	1/10	2/10	5/10	6/10	m/f
- moderate	0/10	0/10	0/10	0/10	0/10	0/10	5/10	4/10	

m/f : male / female
dc : statistically significantly decreased
dr : dose related

3.3 Benchmark approach

The dose-effect relationships for the effects determining the NOAEL in the 13-weeks study (decreased terminal body weight and olfactory epithelial necrosis) were analysed by the Benchmark approach.

The results of the model-fitting and the relevant associated figures are presented in Appendix 1A en 1B.

The estimated CEDs for terminal body weights and olfactory epithelial necrosis are presented in Table 3.3.2 and in Figure 3.3.1. It should be noted that olfactory epithelial necrosis is a histopathological endpoint (ordinal data), and the CEDs have a special interpretation. For instance, the $CED_{minimal}$ is the estimated dose at which the average animal switches from 'normal' to 'minimal'. For a further explanation of the Benchmark approach in ordinal data, see chapter 2.

Table 3.3.2 Critical effect doses (CED) with 90% confidence intervals for terminal body weight and olfactory epithelial necrosis in a 13-weeks inhalation toxicity study. Note that the CEDs for olfactory epithelial necrosis have a special interpretation (see text).

Endpoint	Model* (sex-dependent parameters)	CES	CED (ppm)	L_{05} (ppm)	L_{95} (ppm)
Terminal body weight	2 (a)	5%	807	645	1060
		10%	1657	1325	2177
		20%	3509	2805	4611
		40%	8032	6422	10555
Olfactory epithelial necrosis	2 (-)	minimal	1520	1458	1609
		minor	1708	1636	2257
		moderate	3020	2939	3111

* see section 2.1

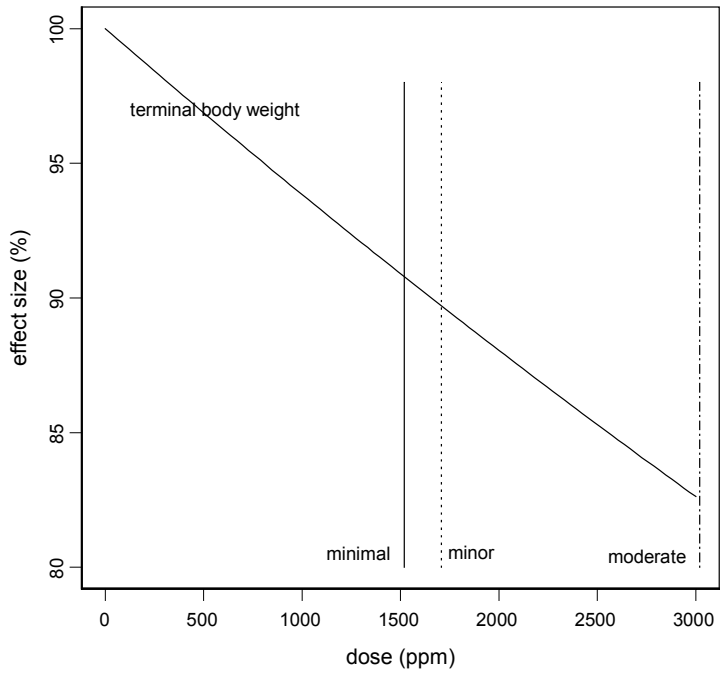

Figure 3.3.1 Standardised dose-response relationship for terminal body weight, and dose levels corresponding with the CEDs for minimal, minor, and moderate olfactory epithelial necrosis

3.4 Interpretation results Benchmark approach

As can be seen in Table 3.3.2 and Figure 3.3.1, the dose levels at which an average rat gets minimal, minor, or moderate olfactory epithelial necrosis correspond with a terminal body weight decrease of about 9%, 10%, and 17.5%, respectively. This indicates that terminal body weight is a more sensitive endpoint to this compound. A CES of 5% for terminal body

weights results in a Benchmark dose (CED-L05) of 645 ppm. For olfactory epithelial necrosis even the minimal effect level would result in a higher Benchmark dose (*viz*. 1458 ppm). Consequently, the overall Benchmark dose of this study would be 645 ppm, in case a 5% decrease in terminal body weight is considered toxicologically relevant.

3.5 Conclusions

- Both for the continuous endpoint decreased terminal body weight and for the ordinal endpoint olfactory epithelial necrosis, the data of the critical study appear suitable for estimating a dose-response relationship. Thus the Benchmark approach may be expected to give reasonable results in this study;
- the NOAEL of 500 ppm (2420 mg/m^3) corresponds with a decrease in terminal body weight of about 3%, and, therefore, appears sufficiently protective in this case;
- For olfactory epithelial necrosis, the NOAEL is also 500 ppm, but the Benchmark dose for this endpoint is about three times higher;
- For terminal body weight, the most sensitive endpoint observed, the NOAEL is close to the Benchmark dose, and, therefore, the NOAEL approach and the Benchmark approach give similar results here.

4. CAPTAN

Summary
The DECOS has appointed a 90-day inhalation toxicity study in rats as critical, with respect to the toxicity of captan. Based on squamous epithelial hyperplasia of the larynx at the lowest dose tested, a MOAEL of 0.13 mg/m^3 was derived in the critical study. With respect to the Benchmark approach, squamous epithelial metaplasia and hyperplasia of the larynx were studied. Minimal squamous epithelial hyperplasia of the larynx was considered adverse, and the corresponding Benchmark dose was 0.16 mg/m^3. For captan, the Benchmark dose is almost equal to the MOAEL of 0.13 mg/m^3, derived in the critical study.

4.1 Introduction and general toxicity profile

Captan is a substance evaluated by the Dutch Expert Committee on Occupational Standards of the Health Council of the Netherlands (DECOS) in order to derive a 'health based recommended occupational exposure limit' (HBROEL). Toxicological evaluation was performed based on publications before April 1, 1995; including seven confidential reports. The toxicological data included kinetics, metabolism, acute toxicity, irritation, sensitisation, sub-acute toxicity, (semi)chronic toxicity, and mutagenicity.
Captan is mainly used as a fungicide in the fruit and bulb growing. At present, the most frequently (also in the Netherlands) applied Threshold Limit Value (TLV) is 5 mg captan/m^3 (8-h time weighted average).
The oral LD50s for rats and mice were well above 5000 mg/kg bw. The LC50 after 4 hours of inhalation exposure in rats was 1160 mg/m^3. This value, however, may be lower in view of the rather large particle-size generated in the test and the unknown purity of the compound tested. Captan was found to be severely irritating to rabbit eyes, mildly irritating to rabbit skin and extremely sensitising to skin in guinea-pigs. The substance was found to be non-mutagenic, non-reprotoxic and non-teratogenic.
Repeated dermal exposure to captan at 1000 mg/kg bw/d resulted in local effects like dermal erythema, oedema and desquamation, and in decreased body weight (gains).
The critical study with respect to the toxicity of captan was a 90-day inhalation study in rats. Inhalation exposure to captan for 90 days at doses up to 13 mg/m^3 caused a.o. metaplasia and hyperplasia of the upper epithelium of the arythenoid projections of the larynx.
In the study, a minimal-observed-adverse-effect-level (MOAEL) of 0.13 mg/m^3 was established, based on minimal laryngial effects (squamous epithelial hyperplasia) at the lowest dose tested. This MOAEL was used by DECOS for deriving a HBROEL.

4.2 Critical Study

Subst.	: Captan	Duration	: 90 days (6h/d, 5 d/w)
Route	: inhalation	Dose levels	: 0, 0.13, 0.6, 5.0, 13.0 mg/m^3
Recov.p.*	: 4 weeks	Species	: rat
Sex	: m/f	Guideline	: OECD 413
GLP	: yes	Ref.	: Hext, 1989
MOAEL	**: 0.13 mg/m^3**	* : only control and high-dose animals	

Description:
Method:
Rats (Alpk:APsfD; 10/sex/dose, high dose and controls: 20/sex/group) were exposed nose-only to actual mean atmospheric concentrations of 0, 0.13, 0.60, 5.0, and 13.0 mg/m^3 of technical captan (88.7%), 6 h/d, 5 d/w for 90 days. Ten animals per sex per group were killed in week 14 while the remaining animals (in control and high-dose group) were killed in week 18 after a four-week exposure-free period.

Results:
The results are presented in Table 4.2.1.

4.3 Benchmark Approach

From the results of the critical study it was concluded that exposure to captan results in, among others, squamous epithelial metaplasia and hyperplasia of the larynx in rats. The critical effects squamous epithelial metaplasia and hyperplasia of the larynx were analyzed by the Benchmark approach.

The results of the model fitting, the selected model and the relevant associated figures are given in Appendix 2A and 2B.
The results of the CEDs for slight or moderate squamous epithelial metaplasia and minimal or slight squamous epithelial hyperplasia in the larynx are presented in Table 4.3.1. It should be noted that squamous epithelial metaplasia is a histopathological endpoint (ordinal data), and the CEDs have a special interpretation. For instance, the $CED_{minimal}$ is the estimated dose at which the average animal switches from ‘normal’ to ‘minimal’. For a further explanation of the Benchmark approach in ordinal data, see chapter 2.

Table 4.2.1 Observations in a 90-days inhalation toxicity study in rats with captan

Dose (mg/m^3)	0		0.13		0.60		5.0		13.0		
Effect	m	f	m	f	m	f	m	f	m	f	dr
Mortality	0/20	0/20	0/10	0/10	0/10	1/10	0/10	1/10	5/20 *	0/20	
Clinical signs	no treatment related findings										
Body weight	no treatment related findings										
Body weight gain	no treatment related findings										
Food consumption	no treatment related findings										
Ophthalmoscopy	no treatment related findings										
Clinical chemistry	no treatment related findings										
Macroscopic findings	no treatment related findings										
Organ weights (absolute/relative)	no treatment related findings										
Histopathological changes											
- larynx; squamous epithelial metaplasia; total	0/0	0/0	0/0	0/0	0/0	0/0	9/9	7/9	6/6	9/9	f
Slight	0/0	0/0	0/0	0/0	0/0	0/0	2	1	4	4	m/f
Moderate	0/0	0/0	0/0	0/0	0/0	0/0	7	6	2	5	
- larynx; squamous epithelial hyperplasia; total	0/0	0/0	1/10	4/9	5/10	7/10	9/9	8/9	6/6	9/9	f
Minimal	0	0	0	2	2	2	4	0	0	1	
Slight	0	0	1	2	3	5	5	8	6	8	m
- nasal rhinitis									+	+	
- nasal focal olfactory epithelial degeneration									+	+	
- minimal parakeratosis	0/0	0/0	0/0	0/0	0/0	0/0	2/9	2/9	2/6	3/9	
- vacuolar degeneration squamous epithelium	0/0	0/0	0/0	0/0	0/0	0/0	3/9	4/9	4/6	7/9	f
- attenuated bronchial epithelium with sub-epithelial cellular necrosis	0/0	0/0	0/0	0/0	0/0	0/0	8/9	9/9	6/6	9/9	

m/f : male / female
dr : dose related
* : due to bronchial / bronchiolar necrosis
+ : effect observed

Table 4.3.1 CEDs for slight and moderate laryngial squamous epithelial metaplasia and minimal and slight laryngial squamous epithelial hyperplasia (with 90% confidence intervals), in a 90-days inhalation toxicity study with captan in rats. Note that the CEDs for histopathological endpoints have a special interpretation (see text).

Endpoint	Model* (sex-dependent parameters)	CES	CED (mg/m^3)	L_{05} (mg/m^3)	L_{95} (mg/m^3)
Squamous epithelial metaplasia in the larynx	2 (-)	slight	6.0	4.7	7.5
		moderate	9.0	7.2	10.7
Squamous epithelial hyperplasia in the larynx	3 (-)	minimal	0.39	0.16	0.76
		slight	1.2	0.73	2.2

* see section 2.1

4.4 Interpretation results Benchmark approach

When minimal squamous epithelial hyperplasia is regarded as a critical effect size, the Benchmark dose is 0.16 mg/m^3 in this study.

4.5 Conclusions

- The data for both ordinal endpoints regarded critical in this study allowed for dose-response modelling, and the Benchmark approach appears to give reasonable results;
- Considering (arbitrarily) minimal squamous epithelial hyperplasia in the larynx as the adverse effect in the critical study, the corresponding Benchmark dose is 0.16 mg/m^3;
- The Benchmark dose (0.16 $mg/m^{3)}$) does not differ much from the MOAEL derived in the critical study (0.13 mg/m^3).

5. ACETONE CYANOHYDRIN

Summary
The Dutch Expert Committee on Occupational Substances of the Health Council of the Netherlands (DECOS), has appointed a 1- and 3-month inhalation toxicity study in rats as critical, with respect to the toxicity of acetone cyanohydrin (ACNH). Based on increased relative liver weights and changed blood parameters, a NOAEL of 35 mg/m^3 was derived. With use of the Benchmark approach for a number of blood parameters, different CEDs could be derived.
It was concluded that, although significant dose-response relationships were found for the endpoints RBC, Hb, MCHC and BUN, these were all four determined by one treatment group, deviating from the other seven treatment groups. Therefore, the effects were considered uncertain. The probability that the effects may have been caused by some other unknown experimental factor, associated with the deviating treatment group is high.
If, nonetheless, the deviating highest dose-group in females were considered to be an effect of ACNH, the resulting Benchmark dose would be approximately four times higher than the NOAEL (based on a CES of 5%).

5.1 Introduction and general toxicity profile

ACNH occurs naturally as its O-glucoside linamarin and is abundantly present in some foods of developing countries like lima beans and cassava roots. The substance is also man-made and mainly used as an intermediate in the manufacture of methyl methacrylate. ACNH is readily absorbed via gastro-intestinal, respiratory, and dermal routes. In animals and man ACNH readily and spontaneously dissociates to yield acetone and HCN. There are similarities with respect to kinetics and metabolism between ACNH and HCN. The formation of cyanide from ACNH determines the toxicity of the compound. Therefore, it was proposed by the Dutch Expert Committee on Occupational Standards, DECOS, to regard and treat ACNH largely as HCN/cyanide.
ACNH vapour is irritating to the eyes and the nose after inhalatory exposure and to the skin after dermal exposure. ACNH is classified as acutely very toxic by all exposure routes. ACNH is not classified as genotoxic, carcinogenic, reprotoxic or teratogenic. A NOAEL for maternal toxicity was established at 1 mg/kg bw/day. The NOAEL for inhalation toxicity of ACNH was placed at 35 mg/m^3, based on a 1-month and a 3-months inhalation toxicity study in rats. Remarkably, the adverse ACNH-related effects were found only in the 1-month study. The observed effects included a significant increase in relative liver weights and changes in haematology and clinical chemistry parameters like T3, LDH, RBC, MCHC, Hb, BUN, and

total protein. In the 3-months study, only significantly decreased glucose levels in mid- and high-dosed females were observed.

In the Netherlands, for cyanides (as CN) and HCN the maximal allowable concentrations in an occupational setting were set at 5 mg/m^3 and 11 mg/m^3 (10 ppm), respectively. For HCN a C-notation is added and for cyanides and HCN a skin-notation.
DECOS has proposed the health based recommended occupational exposure limits (HBROEL) for ACNH to be set at 35 mg/m^3 as a 15 minutes time weighted average (TWA-15 min) and at 3.5 mg/m^3 as TWA-8h based on a 1- and a 3-month inhalation study in rats. A skin notation is also warranted for ACNH.

5.2 Critical Study

Substance	: ACNH	Duration	: 1 month (6 h/d, 5 d/w)
Route	: inhalation	Dose levels	: 0, 35, 104, and 209 mg/m^3
Recovery per.	: -	Species	: rat
Sex	: m/f	Guideline	: OECD 413
GLP	: yes	Ref.	: US-EPA, 1986 (a)
NOAEL	**: 35 mg/m^3**	**LOAEL**	**: 104 mg/m^3**

Description:
Method:
Rats (1-month study: 10/sex/dose) received whole body exposure to concentrations of 0, 10, 30, and 60 ppm ACNH (0, 35, 104, and 209 mg/m^3) in 10 m^3 inhalation chambers, 6h/d, 5d/w for approximately one month. Actual concentrations were 0, 9.2, 29.9, and 59.6 ppm (0, 32.2, 104.7, and 208.6 mg/m^3). The animals were exposed during at least 19 days.
The results are presented in Table 5.2.1.

Table 5.2.1 Observations in a 1-month inhalation toxicity study in rats exposed to ACNH

Dose (mg/m^3)	0		35		104		209		
Effect	m	f	m	f	m	f	m	f	dr
Mortality							3/10		
Clinical signs									
- anoxia / hypoxia							4/10		
- terminal body weight							d		
Organ weights									
- liver (relative)					ic		ic		
Clinical chemistry									
- T3					ic				
- LDH					d			dc	
- RBC								dc	
- MCHC								dc	
- Hb								dc	
- total protein			d		dc		d		
- BUN								i	

m/f : male / female
i/ic : increased / increased statistically significantly
d/dc : decreased / decreased statistically significantly
dr : dose related

5.3 Benchmark Approach

The following endpoints were analysed by the Benchmark approach: relative liver weights, thyroid hormone 3 (T3), lactate dehydrogenase (LDH), red blood cell count (RBC), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb), total protein, and blood urea nitrogen (BUN).

Results of the model fitting, the selected model, and the relevant associated figures are presented in Appendix 3A and 3B.

The results of the CEDs for the different effect parameters are presented in Table 5.3.1 and Figure 5.3.1.

Table 5.3.1 Critical effect doses (CED in mg/m^3 for affected blood parameters (with the 90% confidence intervals) in a 1-month inhalation toxicity study with ACNH in rats

Endpoint	Model* (Sex dependent parameter)	CES (%)	CED (mg/m^3)	L_{05} (mg/m^3)	L_{95} (mg/m^3)
LDH**	4 (var)	5	4	0	15
		10	9	0	33
		20	21	0	106
		40	N.R.	2	-
RBC (males)	3 (b)	5	217	205	235
RBC (females)		5	203	200	209
Hb (males)	3 (b)	5	217	204	240
		10	224	212	248
		20	232	219	257
		40	241	227	266
Hb (females)		5	201	198	205
		10	208	205	212
		20	215	212	219
		40	224	220	228
BUN (males)	3 (b)	5	205	198	230
BUN (females)		5	196	192	200

* : See section 2.1

** : When 3 outliers were omitted from the control and lowest dose group, the significance of the response disappeared

N.R.: effect size not reached due to levelling off

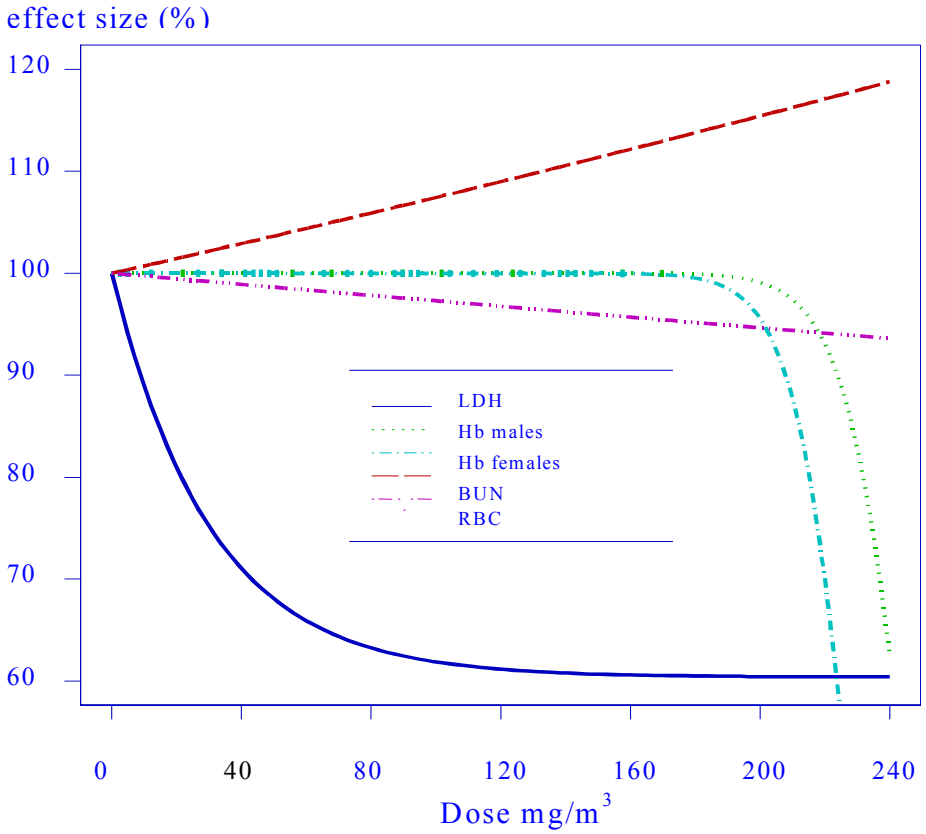

Figure 5.3.1 Percentage of changed blood parameters (LDH, RBC, Hb, and BUN).

5.4 Interpretation results Benchmark approach

The Benchmark dose analysis showed significant dose-effect relationships for LDH, RBC, Hb, and BUN. For changed Hb-levels, females appeared more sensitive. For relative liver weights, the Benchmark approach did not result in a significant dose-response.

Figure 5.3.1 shows, a steeply declining dose-response curve for LDH-levels, indicating that LDH-decrease occurs at low ACNH-exposure levels. More detailed examination of the data, revealed that this dose-effect curve is the result of the presence of three outliers in the control and low-dose groups. Re-analysis after removal of the outliers from the data file, results in a non-significant dose-response for LDH (see Appendix 3A).
The Hb-levels (for both males and females), show a similar decrease at (relative) high dose levels.
The analyses of blood parameters HB, RBC as well as the blood chemistry parameter BUN all result in model 3 with sex-dependent *b* as the best model. In the case of MCHC model 3 with parameters *a,b* was selected. However, closer examination of the associated data, reveals that in all these cases only the highest dose group for females deviates from the other seven dose-groups. It is this single dose-group that determines the significance of the response. It remains unclear if this observed difference is caused by the test substance or by some unknown experimental condition differing in this group compared to the others. Due to the lack of replicated dose-groups in which a response is observed, the conclusion that the effect is caused by ACNH is uncertain, even though it is statistically significant. Overall, the most likely conclusion from this analysis appears to be that none of the parameters observed clearly shows effects from ACNH. This conclusion would be consistent with the findings in the three months study, where no effects were observed as well.
It should be noted that the meaning of the CEDs as shown in Table 5.3.1 depends on the assumption that the effects in the female highest dose-group, deviating from the other seven dose-groups is caused by ACNH.

5.5 Conclusions

- Whereas the NOAEL-approach led to the conclusion that ACNH caused effects on several endpoints in the one-month inhalation study, the analysis of the Benchmark approach shows that these effects are uncertain. This is in agreement with the lack of effects in a three-months inhalation study.
- Although significant dose-response relationships were found for endpoints RBC, HB, MCHC, and BUN, these were all four determined by a single treatment group (highest dose-group for females), deviating from the other seven treatment groups.

- This one-month inhalation study for ACNH clearly illustrates the risk of using few dose-groups: such a study may result in only one treatment group deviating from the others, which makes it impossible to decide whether this is an effect caused by the test compound or by some other unknown experimental factor, associated with that treatment group.
- If, nonetheless, the deviating highest dose-group in females were considered to be an effect of ACNH, the resulting Benchmark dose would be approximately four times higher than the NOAEL.

6. LYORTHOL

Summary
Lyorthol, a commonly used disinfectant in Dutch hospitals contains two active ingredients: *o*-benzyl-*p*-chlorophenol (BCP) and *o*-phenylphenol (OPP). From the two separate toxicological profiles of BCP and OPP, a 2-year chronic toxicity study in rats and a 2-generation reproductive toxicity study in rats were appointed as critical studies, respectively. The NOAELs derived in the critical studies were less than 30 mg/kg bw/d for BCP (based on kidneys effects) and 40 mg/kg bw/d for OPP (based on kidneys and urinary bladder effects), respectively. In order to establish the Benchmark doses for both BCP and OPP, different kidney and urinary bladder effects were evaluated. The lowest ED50s (and corresponding 5%-lower confidence limits) for BCP and OPP were 175 (133) mg/kg bw/d and 441 (366) mg/kg bwt/d. These ED50s for BCP and OPP were found for renal tubule hyperplasia and transitional cell hyperplasia in the urinary bladder (males), respectively. When these ED50s are considered the CESs, the CEDs (L05) derived for the different effects of BCP or OPP are at all times higher than the respective LOAEL of 30 mg/kg bw/d or the NOAEL of 40 mg/kg bw/d.
For some of the quantal endpoints reported in the Lyorthol studies, the ED50 could only be estimated by extrapolating the fitted dose-response model to higher doses. In those cases the estimate of the ED50 may be unreliable.
For BCP kidney weight was used as the endpoint for estimating the Benchmark dose and the fitted dose-response model resulted in a Benchmark dose of 53 mg/kg bw (CES of 5%), which is close to the LOAEL of 30 mg/kg bw.
For OPP the Benchmark dose was based on transitional hyperplasia in the kidney. When the ED50 is used for deriving the Benchmark dose, it results in 335.7 mg/kg. The Benchmark dose based on an extra risk level of 5% amounts to 69 mg/kg, which is very close to the NOAEL of 40 mg/kg for this compound.

6.1 Introduction and general toxicity profile

Lyorthol is a disinfectant used in hospitals. For assessing a health risk for workers handling lyorthol, available toxicological information on the active ingredients in lyorthol (*o*-phenyl-phenol (OPP) and *o*-benzyl-*p*-chlorophenol (BCP)) was evaluated. Consequently, two separate toxicological profiles considering the active ingredients were prepared and published by Stouten (1998, a,b). A short summary of the toxicity profiles is presented below.

<u>*o*-Benzyl-*p*-chlorophenol (BCP)</u>
BCP is irritating and corrosive to skin. Based on acute toxicity studies BCP is considered to be of low acute toxicity after single oral, dermal, or inhalatory exposure. Based on two

2-week oral toxicity studies in rats and mice, NOAELs of 62.5 mg/kg bw/d (kidney effects in males) and 125 mg/kg bw/d (liver effects in females) were derived in the respective studies. In 13-weeks oral toxicity studies in which BCP was administration by daily gavage or continuous via the diet, NOAELs of 180 mg/kg bw/d or less than 30 (males) mg/kg bw/d were found, respectively. In a comparable toxicity study in mice (gavage) the kidneys and the liver were also the target organs for BCP. The NOAEL derived for BCP was 480 mg/kg bw/d. In 2-year chronic oral toxicity studies in mice (doses 0, 120, 240, and 480 mg/kg bw/d) and in rats (doses 0, 30, 60, 120 for males, and 0, 60, 120, 240 for females, respectively) the kidney was shown to be the target organ as well. In rats mortality was not influenced, whereas in mice survival in the highest dose group was decreased. In mice body weights of all dosed males and mid- and high-dosed females were decreased. In both rats and mice the incidence and severity of nephropathy increased with dose and length of treatment. Male mice showed an increased incidence of renal tubule adenomas and carcinomas in the mid- and high-dose groups. For BCP the lowest effect level in the 2-year oral toxicity study in rats was found at 30 mg/kg bw/d (LOAEL). At this level, the kidneys (nephropathy in males and increased relative kidney weights) and the parathyroid gland (hyperplasia in males) were affected. As the (male) rat appeared to be the most sensitive animal with respect to exposure to BCP, the 2-year study in rats is appointed the ‘critical study’ (see section 6.2).

o-Phenylphenol (OPP)

OPP is irritating to skin and eyes. The sodium salt of OPP (OPP-Na) is irritating to skin also and may cause serious damage to the eyes. OPP and its sodium salt have slight sensitising properties. Acute inhalatory toxicity is low for both OPP and OPP-Na. Acute oral toxicity is low for OPP. OPP-Na though, can be harmful when acutely ingested. In a 13-week oral toxicity study in rats with OPP, a NOAEL of 0.62% (410 and 432 mg/kg bw/d for males and females, respectively) was derived, based on decreased body weights and proliferative urinary bladder lesions. In a similar oral toxicity study with OPP-Na, a NOAEL of 0.5% (353 mg/kg bw/d) was derived, based on induced papillomas of the urinary bladder. Both OPP and OPP-Na are considered to be non-genotoxic, based on *in vivo* and *in vitro* experiments that were negative. In chronic toxicity/carcinogenicity studies in rats, both OPP-Na and OPP (to a lesser extent) induce tumours via a non-genotoxic mechanism. For OPP a NOAEL of 40 mg/kg bw/d (based on proliferative changes in bladder epithelium at 240 mg/kg bw/d; LOAEL) was derived in a 2-generation toxicity study in rats. In a 2-year chronic toxicity study in rats for OPP-Na, a NOAEL of 0.25% (100 mg/kg bw/d) was derived, based on urinary bladder papillomas. From teratogenicity studies it was concluded that OPP induces no irreversible developmental effects when animals are dosed below maternally toxic doses. In the toxicity profile, the lowest observed NOAEL for OPP was 40 mg/kg bw/d, based on the 2-generation toxicity study in rats. The 2-generation study in rats is appointed the ‘critical study’ (see section 6.2).

6.2 Critical Studies

6.2.1 Critical study with o-Benzyl-p-chlorophenol (BCP)

Substance	: *o*-Benzyl-*p*-chlorophenol	Duration	: 2 years
Route	: oral (gavage)	Dose levels	: 0, 30/60, 60/120, 120/240 mg/kg bw/d for males/females
Recovery per.	: -	Species	: rat
Sex	: m/f	Guideline	: OECD guideline 452
GLP	: yes	Reference	: Marsman et al. ,1995
NOAEL	**: <30 mg/kg bw/d**	**LOAEL**	**: 30 mg/kg bw/d**

Description:

Method:

Rats (80/sex/dose and 50/sex/dose at end of study) were dosed 0, 30/60, 60/120, 120/240 mg *o*-Benzyl-*p*-chlorophenol in corn oil/kg bw/d for males/females respectively, orally (gavage) for two years. Interim evaluations (clinical chemistry) were made at 3 and 15 months. At 15 months haematology was performed also.

Results:

The results are presented in Table 6.2.1.

Table 6.2.1 Observations in a 2-year oral toxicity study in rats exposed to BCP

Dose (mg/kg bw/d)	0		30	60	60	120	120	240	
Effect	m	f	m	f	m	f	m	f	dr
Mortality	not treatment-related								
Clinical signs - staining urigenital area	9/80		66/80		69/80		77/80		f
Feed consumption	no treatment-related findings								
Haematology	no treatment-related findings								
Clinical chemistry	no treatment-related findings								
Body weight gain	no treatment-related findings								
Organ weights - kidney (relative)			i	i	ic	ic	ic	ic	m/f
Histopathology *kidney* - nephropathy severity[1] - renal tubule hyperplasia			ic		ic	ic	ic ic	ic	m/f

Dose (mg/kg bw/d)	0		30	60	60	120	120	240	
Effect	m	f	m	f	m	f	m	f	dr
- renal tubule adenoma & carcinoma	1/50	0/50	1/49	-	2/50	1/50	2/50	2/50	
parathyroid gland									
- hyperplasia	0/47	nr	2/47	nr	5/45	nr	8/46	nr	m
skeletal; fibrous osteodystrophy									
- cranial	0/50	nr	0/50	nr	2/50	nr	4/51	nr	m
- femoral	0/50	nr	0/50	nr	2/50	nr	6/51	nr	m

m/f : male / female
i/ic : increased / increased statistically significantly
d/dc : decreased / decreased statistically significantly
dr : dose related
1 : characterised by tubule dilatation, flattening of tubule epithelium, presence of regenerative tubules surrounded by a thickened basement membrane
nr : figures not recorded in study Marsman (1995)

Remark:
The critical effects observed for BCP included increased kidney weights, nephropathy, hyperplasia of the parathyroid gland, and skeletal findings.

6.2.2 Critical Study with o-Phenylphenol (OPP)

Substance	: o-phenylphenol	Duration	: two generations
Route	: oral	Dose levels	: 0, 40, 140, 490 mg/kg bw/d
		Actual doses	: 0, 36,125, 437 mg/kg bw/d
Recovery per.	: -	Species	: rat (Sprague-Dawley)
Sex	: m/f	Guideline	: OECD guideline 416
GLP	: yes	Reference	: Eigenberg, 1989
$NOAEL_{parental}$	**: 40 mg/kg bw/d**	**$LOAEL_{parental}$**	**: 140 mg/kg bw/d**
$NOAEL_{developmental}$	**: ≥490 mg/kg bw/d**	**$LOAEL_{developmental}$**	**: -**

Description:
Method:
OPP was administered to rats (35 pairs/dose, 27 males and 29 females for controls) in concentrations of 0, 40, 140, and 490 mg/kg bw/d for 15 weeks (10 weeks post-weaning). Adult animals were evaluated for body weight gain, food consumption, clinical signs, oestrus cycle, fertility, gestation period, and litter size. The offspring was evaluated for sex ratio, pup viability, body weight gain, and clinical signs.

Results:
The results are presented in Table 6.2.2.

Table 6.2.2 Observations in a 2-generation reproduction toxicity study in rats exposed to OPP

Dose (mg/kg bw/d)	0		40		140		490		
Effect	m	f	m	f	m	f	m	f	dr
F0 animals									
Mortality	not treatment related[1]								
Clinical signs	no treatment-related findings								
Body weight gain							dc	dc	
Organ weights									
- kidney (relative)							ic	ic	
Histopathology									
kidney									
- transitional cell hyperplasia							ic		
- incidence calculi					i		i		
- hemorrhage							ic		
- pyclonephritis							i		
urinary bladder									
- incidence calculi					i		i		
- transitional cell hyperplasia					i	i	ic	ic	m/f
Clinical chemistry	no treatment-related findings								
F1 pups									
Litter size	no treatment-related findings								
Survival index	no treatment-related findings								
Sex ratio	no treatment-related findings								
Body weight	no treatment-related findings								
Pathology	no treatment-related findings								
F1 animals									
urinary bladder									
- incidence calculi					i		i		
- transitional cell hyperplasia					i		ic		m

m/f : male / female
i/ic : increased / increased statistically significantly
d/dc : decreased / decreased statistically significantly
dr : dose related
1 : possibly one animal in dose group 140 mg/kg bw/d died due to dose related effects: uremia, kidney failure

6.3 Benchmark Approach

In the critical study for BCP it was concluded that the target organs for BCP exposure are the thyroid, the kidneys, and the skeleton. For OPP the urinary system (kidneys, urinary bladder) could be identified as the target system for exposure to OPP.
The critical effects observed in the 2-year oral toxicity study with BCP included changed kidney weights, hyperparathyroidy, cranial and femoral fibrous osteodystrophy, and renal tubule hyperplasia, adenomas, and carcinomas. The critical effects observed in the 2-generation reproduction toxicity study with OPP included transitional cell hyperplasia in urinary bladder and in kidneys, urinary bladder and renal calculi, renal hemorrhage, and pyclonephritis. These critical effects were analysed by the Benchmark approach.

The results of the model fitting, the selected model and the relevant associated figures are given in Appendix 4A and 4B.

For BBP significant dose-response relationships were found for all the critical end points re-analysed, except for observed adenomas and carcinomas in the renal tubule (see Appendix 4A). For OPP, six of the eight re-analysed endpoints were found to show significant dose response relationships (see Appendix 4A). For each of these endpoints the CED defined as an ED50 was assessed, as well as the CEDs associated with 5% or 10% extra risk (see Tables 6.3.1 and 6.3.2).

Table 6.3.1 BCP: critical effect doses ($ED50_{,animal}$ in mg/kg bw/d) for different effects. In addition the CED05-ER and CED10-ER are calculated

Parameter	Model* (sex dependent parameter)	ED_{50} (L_{05}, L_{95})		CED05-ER (L_{05}, L_{95})	CED10-ER (L_{05}, L_{95})
Hyperplasia parathyroid gland	2 (-)	200 (151, 317)		57 (47, 86)	87 (71, 130)
Cranial fibrous osteodystrophy	2 (-)	235 (134, 540)		97.4 (77.8, 174)	127.4 (102, 249.6)
Femoral fibrous osteodystrophy	2 (-)	200 (131, 309)		87 (70, 121)	111 (93, 156)
Renal tubule hyperplasia	2 (-)	176 (133, 286)		35 (28, 55	61 (48, 98)
		CES (%)	CED	L_{05}	L_{95}
Relative kidney weight**	2 (var, a)	5 10 20 40	65 130 240 450	53 104 198 366	83 162 310 572

* : See section 2.1
** : continuous variable for which CES of 5, 10, 20, and 40% were calculated.

Table 6.3.2 OPP: critical effect doses ($ED50_{,animal}$ in mg/kg bw/d) for different effects. In addition the CED05-ER and CED10-ER are calculated

Parameter	Model* (sex dependent parameter)	ED_{50} (L_{05}, L_{95})	CED05-ER (L_{05}, L_{95})	CED10-ER (L_{05}, L_{95})
Transitional cell hyper-plasia urinary bladder, F0 males and females	2 (a)	441.1 (m) (366, 535) 591.2 (f) (509, 708)	94.3 (76, 128) 173 (137, 236)	160.1 (131, 203) 212 (173, 277)
Transitional cell hyperplasia kidney, F0 males	2 (-)	490.1 (335.7, 1182.8)	102 (69, 288)	195 (130, 562)
Renal calculi, F0 males	2 (-)	630.2 (482.2, 932.3)	131 (100, 191)	225 (173, 330)
Renal hemorrhage , F0 males	2 (-)	749.7 (529.4, 1220)	299.5 (236, 471)	397 (324, 559)
Pyclonephritis, F0 males	2 (-)	1193.8 (704.8, 23777)	297 (198, 5108)	474 (322, 8654)
Transitional cell hyperplasia urinary bladder, F1 males	2 (-)	536 (442, 695)	133 (104, 193)	

* : See section 2.1

6.4 Interpretation results Benchmark approach

The CED defined in terms of the ED50 can be interpreted as the dose at which the average animal changes from a non-responder into a responder. For instance, the ED50 for hyperplasia of the parathyroid gland indicates the dose at which the average animal starts to show hyperplasia at a certain level of seriousness as determined by the observer. If this level of seriousness were considered acceptable, the associated ED50 can be regarded as the Benchmark dose for that endpoint. If this level is considered to be higher than the CES, the associated ED50 would be higher than the CED for that endpoint.

Another approach is to derive the dose associated with an extra risk level (as proposed by Crump, 1984). However, the interpretation of an extra risk level is problematic: it strongly depends on the magnitude of the experimental noise in the underlying experiment. The reason is that the 'slope' of a quantal dose-response relationship not only reflects the slope of the underlying relationship with the experimental dose, it also reflects the variability of the strain of animals used, as well as the observation errors and the experimental error of the experiment. The latter results from all circumstances and conditions being experienced differently by the individual animals during (and before) the experiment. As a result, the

Benchmark dose associated with a certain extra risk level suffers from the same problem as the NOAEL: its value depends on the quality of the experiment.
Of course, this problem not only holds for the two Lyorthol studies, but for quantal data in general. Slob and Pieters (1998) proposed to use the ED50 as a starting point for risk assessment, based on two arguments: 1) in quantal data the ED50 is the only stable dose parameter, i.e. its value does not depend on the magnitude of the experimental noise, and 2) the variation in laboratory animal population is completely irrelevant for the variation in the human population.
The drawback of the ED50 is that the level of response constituting the borderline between a responder and a non-responder is too high to accept it as a critical effect size.
The re-analysis of the Lyorthol studies shows an additional problem: some of the observed (quantal) endpoints do not reach the 50% response level. Therefore, the ED50 may not be adequately estimated from these endpoints, since they are obtained by extrapolation. The CEDs associated with a 5% or 10% extra risk level do not suffer from that, and may be regarded as more reliable estimates.

For BCP the difficulties of quantal data are irrelevant because the CED for kidney weight is lower than most of the values found for the L05 of the CEDs for the quantal endpoints. Choosing kidney weights as the critical endpoint results in a Benchmark dose of 53 mg/kg bw/d (L05 of CED05).
For OPP all critical endpoints are quantal. Transitional cell hyperplasia in the kidney of F0 males appears the most sensitive endpoint. Here the L05 of the ED50 amounts to 336 mg/kg bw/d. For this endpoint the ED50 happens to be within the range of observation. Therefore, the Benchmark dose for OPP may be assessed at 336 mg/kg bw/d. If, however, an extra risk level of 5% were chosen, the Benchmark dose would be much lower, *viz*. 69 mg/kg bw/d.

6.5 Conclusions

- Most of the endpoints reported to be affected by BCP or OPP concerned quantal dose-response data. For quantal data the ED50 is the only parameter that does not depend on the experimental noise. The ED50 can be interpreted as the CED associated with a CES equal to the response level demarcating responding and non-responding subjects;
- When the CES associated with the ED50 is considered too high to be acceptable, one may estimate the Benchmark dose associated with an extra risk level of 5%, as a conservative

approach. Although the choice of the 5% extra risk level is equally arbitrary as the 5% significance level in the NOAEL approach, the Benchmark approach should be preferred: the Benchmark dose decreases when an experiment is carried out less carefully, while a NOAEL approach tends to result in higher values.

- For some of the quantal endpoints reported in the Lyorthol studies, the ED50 could only be estimated by extrapolating the fitted dose-response model to higher doses. In those cases the estimate of the ED50 may be unreliable.
- For BCP kidney weight could be used as the endpoint for estimating the CED. The kidney weights were reported as means and standard errors per dose-group, and the fitted dose-response model resulted in a Benchmark dose of 53 mg/kg bw/d, associated with a CES of 5%. The NOAEL approach resulted in a LOAEL of 30 mg/kg bw/d.
- For OPP the CED was based on transitional hyperplasia in the kidney. When the ED50 is used for deriving the Benchmark dose results in 335.7 mg/kg bw/d. The Benchmark dose based on an extra risk level of 5% amounts to 69 mg/kg bw/d. The NOAEL was assessed at 40 mg/kg bw/d for this compound, which is very close to the Benchmark dose based on the 5% extra risk level.

7. WHITE SPIRITS

Summary

The Dutch Expert Committee on Occupational Substances (of the Health Council of the Netherlands) has appointed a 13-week inhalation toxicity study in rats as critical, with respect to the toxicity of White Spirits. Based on low-grade anaemia in male rats and on increased relative liver weights in both sexes, a LOAEL of 2000 mg/m^3 and using an arbitrary assessment factor of 6, a NAEL of 330 mg/m^3 was derived. Increased relative liver, spleen, and kidney weights were studied with the Benchmark approach. A 5% change in relative organ weights was arbitrarily chosen as the CES, and the corresponding CEDs (L05) for increased relative liver, spleen, and kidney weights were: 1762/1357 (m/f), 2363, and 1390/2218 (m/f) mg/m^3, respectively. The lowest CED (L05) found (1357 mg/m^3 for increased relative liver weights, females) may be used as the Benchmark dose. This Benchmark dose is lower than the LOAEL of 2000 mg/m^3 derived in the critical study, but higher than the 'NAEL' of 330 mg/m^3.

7.1 Introduction and general toxicity profile

White spirits (WS) is a substance evaluated by the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands in order to derive a 'health based recommended occupational exposure limit' (HBROEL). Toxicological data are based on both animal studies and studies with human volunteers.

WS is a generic name covering a broad section within the wide range of hydrocarbon solvents. In this chapter the WS dealt with, is the hydro-desulphurised type 1 White Spirits, grade "regular" or "Stoddard solvent". This WS consists of C7-C14 hydrocarbons; 80-100% are aliphatic hydrocarbon: n-, iso-, monocyclicalkanes, 0% up to maximal 20% are aromatic hydrocarbons, primarily C9 and C10. WS is produced from petroleum and used in printing inks, glues, paints, maintenance products, for cleaning and degreasing in garages, in the production of resin mixtures, paint solvent, and wood preservatives. The present occupational exposure limit for WS in the Netherlands is 575 mg/m^3 (100 ppm; 8-h time weighted average; TWA).

From animal irritation and sensitisation toxicity studies, WS appeared to be (at most) slightly irritating to the eyes and skin of rabbits and they irritate the respiratory tract of mice, rats, and guinea pigs. Cats died within 7.5 hours inhalatory exposure to 10,000 mg/m^3, while rats survived an 8 hours inhalatory exposure to 8200 mg/m^3. Impaired performance in a neurobehavioral test in rats was observed to be dose dependent at acute exposure levels of 1200 - 4800 mg/m^3. Rats exposed for 8 weeks to 1900 mg/m^3 showed no changes in haematological parameters or histopathological changes. Signs of minor lung irritation in guinea pigs were shown after exposure to 593 mg/m^3 for 6 weeks. There were no exposure

related deaths at this level. Guinea pigs exposed to 238 mg/m^3 or 313 mg/m^3 for 90 days resulted in 0% and 29% mortality, respectively. In male rats, exposure up to 4800 mg/m^3 for 26 weeks did not result in histopathological or chronic neurobehavioral effects. Inhalatory exposure to 2000 mg/m^3 during 13 weeks resulted in very low-grade anaemia and increased kidney weights (absolute/relative) in male rats and in increased liver weights (absolute/relative) in female rats. WS was found to be non-mutagenic. There were no adequate data on carcinogenicity of WS. According to abstracts on potential teratogenic or fetotoxic effects, WS appeared to be non-teratogenic and non-fetotoxic.

In human studies WS is irritating to skin, causing ulcers and erythemas, especially at occlusion. Volunteers reported symptoms of eye and upper respiratory tract irritation after exposure to 580 mg/m^3 for 7 hours (not previously exposed volunteers showed effects at higher exposure levels). Exposure to increasing levels (625 - 2500 mg/m^3) for four 30 minute periods did not affect performance, while exposure to 4000 mg/m^3 (50 minutes) resulted in adversely influenced reaction time and short-term memory. Seven hours exposure of long-term exposed painters, to 290 mg/m^3 resulted in affected short-term memory, and seven hours exposure of not previously exposed students, to 580 mg/m^3, resulted in affected vigilance and attention function. In one study some indications on impairment of nervous system functioning due to long-term exposure to approximately 500 mg/m^3 have been found. From the limited data available, WS seems to be non-carcinogenic to man.
In deriving an HBROEL for WS, DECOS used the LOAEL of 2000 mg/m^3 (from the 13-week inhalation toxicity study in rats). Since a LOAEL instead of a NOAEL was used in deriving the HBROEL, an (arbitrarily chosen) extra assessment factor of 6 was introduced.

7.2 Critical Study

Substance	: low aromatic White Spirits (WS)	Duration	: 13 weeks (6 h/d, 5 d/w)
Route	: inhalation	Dose levels	: 0, 2000, 4000, and 8000 mg/m^3
Recovery per.	: -	Species	: rat
Sex	: m/f	Guideline	: OECD 413
Ref.	: Blair *et al*., 1979	GLP	: not mentioned
LOAEL	**: 2000 mg/m^3**		

Description:
Method:
Rats (Albino Wistar strain, Shell laboratory; n=18/sex/group) were exposed to vapour atmospheres of WS of 0, 2000, 4000, and 8000 mg/m^3 for 6 h/d, 5 d/w during 13 weeks.

Results:
The results are presented in Table 7.2.1.

Table 7.2.1 Observations in a 13-week inhalation toxicity study in rats exposed to White Spirits

Dose (mg/m^3)	0		2000		4000		8000		
Effect	m	f	m	f	m	f	m	f	dr
Mortality	none								
Clinical signs									
- slightly lethargic							+	+	
Water intake							i	i	
Body weight gain					d		d	d	
Organ weights									
- heart							ic^{r}		
- spleen					ic^{a,r}		ic^{a,r}		
- kidney			ic^{a,r}		ic^{a,r}	ic^{a,r}	ic^{a,r}	ic^{a,r}	m
- liver				ic^{a,r}	ic^{r}	ic^{a,r}	ic^{r}	ic^{a,r}	f
Clinical chemistry									
- ALP							i	i	
- ASAT							i		
- total protein								i	
- RBC[1]			d		d		d		
- PCV[1]			d		d		d		
- mean red cell volume[1]			i		i		i		
- mean cell haemoglobin[1]			i		i		i		
- albumin factor								i	
Histopathology									
- hyalic intracyto-plasmic inclusions			+		+		+		
- tubular degenerative change			+		+		+		
- accelerated splenic extramedullary haemo-poiesis					+		+	+	
- hemosiderin deposition					+		+	+	

m/f : male / female
dr : dose related
i/ic : increased/increased statistically significantly
d : decreased
1 : slight effect
+ : effect observed
a/r : absolute/relative

7.3 Benchmark Approach

For analysis by the Benchmark approach the critical effects studied (in the 13-week inhalation toxicity study), included increased relative liver, spleen, and kidney weights, and decreased RBC and PCV levels. These effects were re-analysed by the Benchmark approach.

The results of the model-fitting, the selected model and the relevant associated figures are given in Appendix 5A and 5B.

The results of the estimated CEDs for increased relative liver, spleen, and kidney weights are presented in Table 7.3.1 and Figure 7.3.1.

Table 7.3.1 Critical effect doses (CED) with 90% confidence intervals for changed relative liver, spleen, and kidney weights in a 13-week inhalation toxicity study with White Spirits

Endpoint	Model (sex-dependent parameter)	CES (%)	CED (mg/m^3)	L_{05} (mg/m^3)	L_{95} (mg/m^3)
Relative liver weight (males)	2 (b)	5	2112	1762	2614
		10	4126	3442	5107
		20	7892	6584	9770
		40	14565	12150	18030
Relative liver weight (females)		5	1559	1357	1839
		10	3046	2652	3593
		20	5826	5073	6874
		40	10852	9361	12685
Relative spleen weight	2 (a, var)	5	3462	2342	6500
		10	6764	4575	12698
		20	12939	8751	24290
		40	23879	16150	44826
Relative kidney weight (males)	2 (a, b)	5	1704	1390	2218
		10	3328	2715	4333
		20	6366	5193	8289
		40	11749	9583	15298
Relative kidney weight (males)		5	3781	2607	7811
		10	7386	5092	15259
		20	14129	9741	28190
		40	26075	17977	53869
RBC	4 (a, var)	1[1]	233	-	-
		3[1]	1162	-	-

[1] Critical Effect Sizes of 1% and 3% were determined instead of 5, 10, 20, and 40%, because higher response levels were not reached

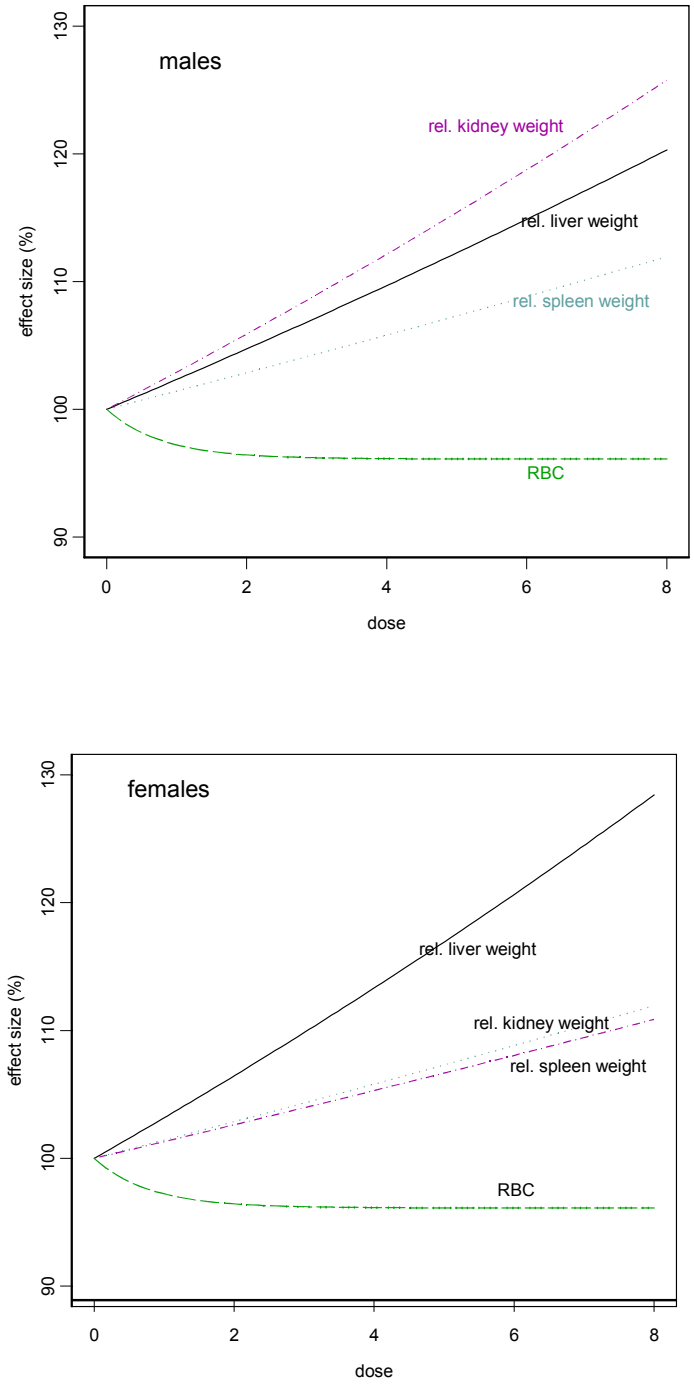

Figure 7.3.1 Standardised dose-response relationships for increased relative liver, spleen and kidney weights for males (upper panel) and females (lower panel).

7.4 Interpretation results Benchmark approach

As Table 7.3.1 shows, the lowest value for L05 of the CED05 is found for female relative liver weight. The Benchmark dose resulting from this study thus amounts to 1357 mg/m^3. Females appear more sensitive with respect to relative kidney weight, whereas males appear more sensitive with respect to relative liver weight. With respect to relative spleen weight, the sexes are equally sensitive. For RBC and relative spleen weight the parameter *var* appeared to be dependent on sex, indicating that the variation between the individual animals was different between the two sexes.

7.5 Conclusions

- The effect parameters from the critical study (increased relative liver, spleen, kidney weights, and RBC) were all suitable for dose-response modelling;
- In this study the lowest dose applied was considered a LOAEL, and the NOAEL approach fails here. The Benchmark approach does not suffer from this problem;
- The LOAEL of 2000 mg/m^3 used for deriving an HBROEL, was transferred into a 'NAEL' of 330 mg/m^3 by the DECOS by applying an arbitrarily chosen safety factor of 6 through which the effects observed at the LOAEL (slight effects on relative liver weights and haematology parameters) are taken into account. The Benchmark dose of 1357 mg/m^3 for increased relative liver weights in females is lower than the LOAEL and higher than the NAEL.

8. OVERALL DISCUSSION AND CONCLUSIONS

The purpose of the present study was to explore the applicability of the Benchmark approach to the results of toxicity studies as they are currently performed according to OECD guidelines. These toxicity studies have been designed to result in a NOAEL. Conducting a study according to this NOAEL approach, requires a sufficient number of animals per dose group at the expense of the number of dose groups. A study design based on the Benchmark approach (i.e. dose-response modeling) would be better off with more dose groups, even at the expense of the number of animals per dose group. This raises the question to what extent the Benchmark approach is applicable to existing toxicological data sets, the underlying study design being sub-optimal for that approach. Thus, the main question addressed in this report is whether or not dose-response modelling is appropriate and applicable using results from OECD studies.

8.1. Applicability to OECD studies

Five compounds, already assessed by DECOS using the NOAEL approach, were selected. The critical endpoints included continuous, quantal, and ordinal (histopathological) data, and these were re-analyzed by dose-response modelling.

For most continuous endpoints analysed it was concluded that the available dose-response data were generally suitable for dose-response modeling, with a more or less straightforward selection of the model for deriving the Benchmark dose. This includes one case where the data were reported as means and standard errors per dose group. In all other cases the data from the individual animals could be obtained. Only in the case of ACNH the selected dose-response model (for RBC, HB and BUN) was dubious, as it was completely determined by a single dose group (out of eight, i.e. four dose groups for both sexes). This case illustrates the problem of a low number of dose groups: a single, unreplicated, dose group showing effects may as well be caused by systematic differences between dose groups other than the applied dose of the test compound (e.g. housing, location, time of dosing, time of section). It should be noted, however, that this problem equally holds for the NOAEL approach: a single significant (top)dose group may be considered as a LOAEL, without any guarantee that this was due to the applied dose or some other (unknown) experimental condition deviating in that group. The solution of using more dose groups, hence creating a more reliable study design, would imply a dramatic increase of test animals when using the NOAEL approach. In

the Benchmark approach an increase in the number of animals is not necessary when more dose-groups are used.

For all continuous endpoints considered in this report a CES of 5% was arbitrarily chosen. The associated Benchmark dose (L05 of CED05) could be assessed without further difficulties. Given the assumption that a 5% effect level for these continuous endpoints is acceptable, the Benchmark dose appeared to give an appropriate estimate of an acceptable dose level in all cases, even though the study design was sub-optimal.

For the ordinal data considered in this report the Benchmark approach could be applied as well, and a model could be straightforwardly selected in all cases. For these histopathological data the choice of CES strongly depends on the experimental observer and how he defined the various categories of severity. Therefore, a description of the categories in terms of observed histopathological lesions, accompanied by a toxicological judgment of these lesions, would be helpful in choosing the category to be considered as the CES for these endpoints.

For quantal data the application of the Benchmark approach is confronted with some difficulties. Here the Benchmark dose as proposed by Crump (1984) is defined as the dose associated with a particular “extra risk” level. The problem is that this dose depends on the experimental noise and the variation between the animals used in the experiment. Minimizing the experimental noise in an experiment causes the dose associated with a particular extra-risk level to move towards the ED50. Indeed, the ED50 is the only stable dose parameter, i.e. it does not change with the quality of the experiment. However, the effect-size associated with the ED50, i.e. the borderline between a responder and non-responder as defined by the experimental observer, may be too high to be acceptable as a CES. A second problem may arise when, as in one of the cases in this report, the ED50 lies outside the range of observation (as in the case of lyorthol, see chapter 6). Then it must be estimated by extrapolating the fitted model, which is an unreliable method. In situations that the estimation of the ED50 is not appropriate, one may estimate the Benchmark dose associated with a 5% extra risk level as a conservative approach. Because of the reasons indicated above, a biological basis for choosing a 5% extra risk level cannot be given. It is just a pragmatic choice, and equally arbitrary as the 5 % significance level in the NOAEL approach. However, there is an important argument to prefer the Benchmark approach over the NOAEL, also in the case of quantal data. The Benchmark dose associated with a specified extra risk level decreases when the experimental error increases, as opposed to the NOAEL approach that will result in a higher NOAEL when the experiment is carried out less carefully.
Regarding the 5 compounds analyzed in this report only for lyorthol quantal data were encountered. The dose-response data of these quantal endpoints were suitable for dose-response modeling, and the Benchmark dose, both in terms of an ED50 and/or in terms of an associated 5% extra risk, could be derived.

We concluded that in all cases investigated the Benchmark approach can be applied, and a Benchmark dose could be derived. In the case of ACNH there was some doubt on which model to choose, but this concerned doubt if the change in response was really caused by the applied dose. Clearly, such doubt is also present when using the NOAEL approach. Rather, we concluded that the Benchmark approach provided more detailed information compared to the NOAEL approach. The Benchmark approach resulted in a significant, although weak, dose-response, indicating that effects were either absent (significance caused by experimental artifact) or small (around 5%). The fact that in a similar study (with three months of exposure instead of one month) no effects were found, corroborates the conclusion that the effect may not be caused by the applied dose. Therefore, the Benchmark approach would probably have resulted in dismissing the one-month inhalation study results as insufficient and hence not acceptable as the critical study.

In conclusion, in the case of ACNH the NOAEL approach and the Benchmark approach differed in their conclusions. In the other critical studies where a NOAEL could be derived, the Benchmark dose as assessed in this report did not deviate much from the NOAEL. In two studies no NOAEL could be derived, and the LOAEL was divided by some arbitrary factor. Obviously, this problem does not occur in the Benchmark approach, and the Benchmark dose could indeed be derived straightforwardly. In both cases the Benchmark dose was higher (about 4 times) than the NOAEL assessed by correcting the LOAEL.

8.2 Critical effect size

A crucial issue in the Benchmark approach is the choice of the critical effect size. Current toxicological and biological knowledge is insufficient to make strong statements on the CES of each individual endpoint. Extensive discussions among toxicologists, clinical chemists, toxicological pathologists and all other scientists involved will be needed to further clarify this issue. However, application of the Benchmark approach does not have to wait for that. As a pragmatic approach we propose to use a CES of 5% for most continuous endpoints, unless there are good reasons to do otherwise. In fact, the choice of the value of the CES should then be regarded as *ad hoc* expert judgement by the toxicologist. Future empirical evaluation of the total of such decisions made by toxicologists for each different endpoint, would help the development of consensus on this issue.
Furthermore, the statistical power associated with the usual OECD studies is most likely not large enough to detect effects smaller than 5%.
For ordinal (histopathological) data, one can only choose one of the categories as defined by the experimental observer as a CES, and therefore this choice largely depends on how the histopathogolists interprets the observed lesions. Again, this constitutes expert judgement by the pathologist.

For quantal data only one effect size is defined: the borderline between responder and nonresponder. If this is deemed acceptable as a CES, the CED is given by the ED50 (the dose at which the average animal crosses the borderline between responder and nonresponder). If however the borderline between responder and nonresponder is unacceptable as a CES, one can only resort to the "extra risk" approach. A discussion on a reasonable choice for the extra risk level does not help much here, since the extra risk level cannot be interpreted as the extra percentage of subjects suffering from the particular lesion: it is polluted by experimental error of unknown magnitude. Furthermore, the percentage of responding subjects in test animals is completely irrelevant for the human situation. The variation between human subjects should be (and is) taken into account by the intraspecies extrapolation factor.

8.3 Comparing endpoints

In deriving a Benchmark dose we have followed the approach of choosing the lowest L05 of the CEDs assessed. Nonetheless, a 5% effect size in one (continuous) endpoint might be more relevant than in another. Therefore, it is important to consider all relevant dose-response relationships simultaneously. This was done by standardizing the (continuous) dose-response relationships, and plot them together. Histopathological effects were plotted in these figures as CEDs (their underlying dose-response relationships are not directly comparable to those of continuous endpoints). In this way an overall picture is obtained, and the reasonableness of the derived Benchmark dose can be judged in retrospect.

8.4 Toxicological / mathematical judgment

Although the procedure for fitting dose-response models and selecting the model for deriving the CED is straightforward, it should be kept in mind that it is only meant as a tool in analyzing and interpreting the dose-response data. The interpretation of dose-response data is a scientific undertaking, and one should always carefully examine the data together with the fitted models. The reasonableness of the fit is not merely a matter of a significantly better log-likelihood. For example, poor data sets, e.g. few dose groups showing effects, may result in perfect model fits, and yet be unreliable. Or a few outliers may dictate the model in a way that results in a dose-response relationship that is unlikely from a toxicological point of view (as exemplified by LDH in the ACNH study, see chapter 5). The analysis of dose-response data and the interpretation of the results from the study as a whole should always be judged from a statistical and biological point of view at the same time.

8.5 Conclusions

Although the choice of the Critical Effect Size should still receive much attention from all experts involved in chemical risk assessment, application of the Benchmark approach need not be postponed until full agreement has been reached. As a pragmatic approach we propose:

- to use a default CES of 5% for continuous endpoints, unless there are good reasons to do otherwise;
- to choose one of the lower categories scored in histopathological endpoints as a critical effect level, e.g. slight or minimal;
- to estimate the ED50 for quantal endpoints, if the borderline between responders and nonresponders is considered as an acceptable effect level, and take the 5% extra risk level as an arbitrary Benchmark response otherwise.

After re-analyzing the critical studies for five selected chemical compounds (used in the working place) by the Benchmark approach we conclude:

1. No major difficulties were encountered hampering the applicability of this alternative approach. Overall, this approach has worked satisfactorily in each of the five critical studies that were re-analyzed in this report. However, it should be noted that the studies were selected for showing effects at two (or more) dose levels. Indeed, as some of the endpoints analyzed in this report illustrate, when effects are observed in a single dose group only, the Benchmark approach does not appear to offer much of an improvement over the NOAEL approach.
2. In one critical study the Benchmark approach resulted in a qualitatively different conclusion: the effects in this study (used for deriving a NOAEL), were considered to be absent or small. Thus, by applying the Benchmark approach the pertinent study would probably not have been selected as the critical study. The results from this study would probably have been dismissed as inadequate for proper evaluation.
3. In two critical studies the derived Benchmark dose (based on an arbitrary CES of 5%) was close to the NOAEL.
4. In the remaining two studies no NOAEL could be assessed, and the LOAEL was divided by an arbitrary factor. In the Benchmark approach this problem does not occur, and the Benchmark dose could be assessed without difficulties. It was in both cases higher than the corrected LOAEL.

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APPENDICES

Appendix 1A. Butyl acetate: selection of dose-response models

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

Butyl acetate											
		sex-dependent parameter									
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male + female
Olfactory epithelial necrosis												
y=a	(1)	-83.30										
y=a·exp(bx)	(2)	**-32.29**	-32.27	-32.27						-15.91	-16.16	-32.07
$y=a\cdot\exp(bx^d)$	(3)	-32.29										
y=a·[c-(c-1)exp(bx)]	(4)	N.C.										
$y=a\cdot[c-(c-1)\exp(bx^d)]$	(5)											
Terminal body weight												
y=a	(1)	58.65	128.68									
y=a·exp(bx)	(2)	61.23	**146.54**			148.35	147.53			70.21	79.13	149.34
$y=a\cdot\exp(bx^d)$	(3)		N.C.									
y=a·[c-(c-1)exp(bx)]	(4)		136.2									
$y=a\cdot[c-(c-1)\exp(bx^d)]$	(5)		N.C.									

N.C. : no convergion reached

Appendix 1B. Butyl acetate: dose-response data and fitted models

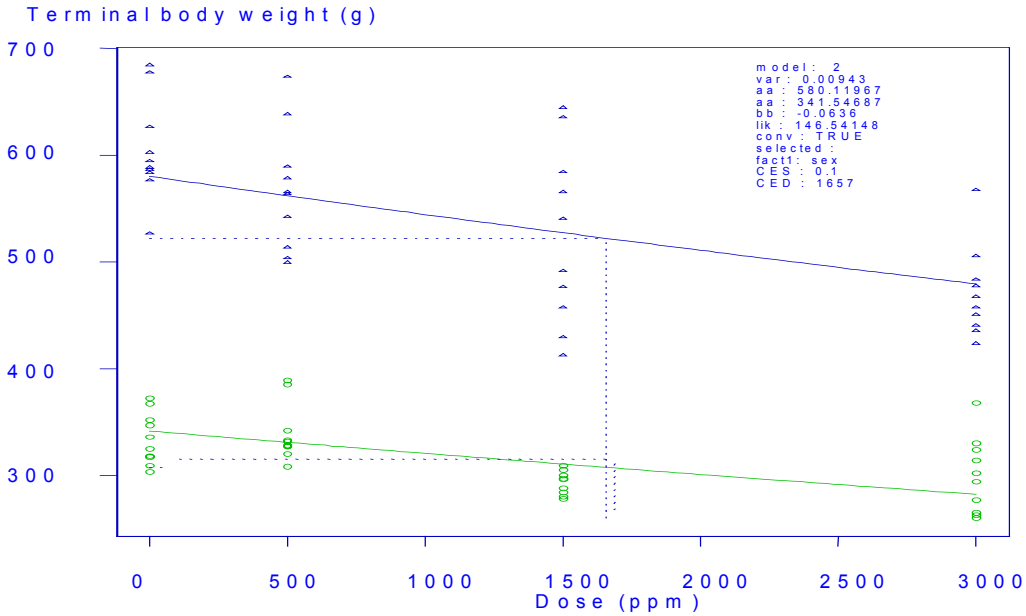

Figure 1B.1 Terminal body weight for males (triangles) and females (circles)

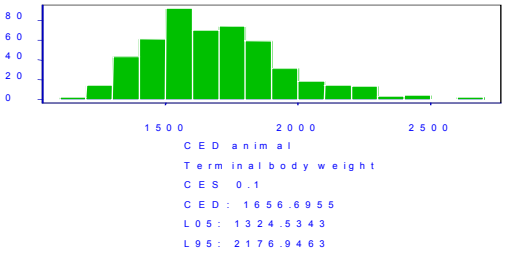

Figure 1B.2 Uncertainty distribution around the CED10 for terminal body weight obtained by 500 bootstrap runs

Appendix 1B. (Butyl acetate, continued)

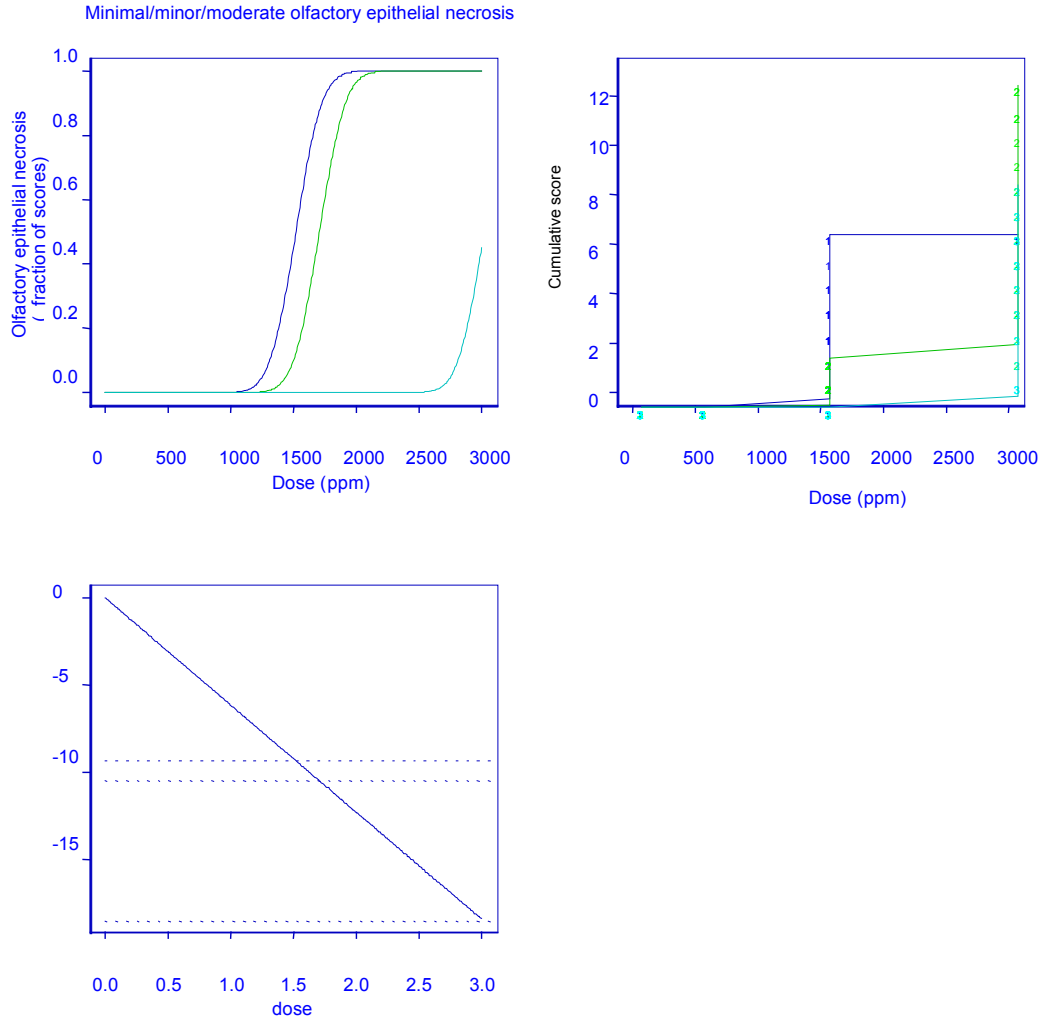

Figure 1B.3 Olfactory epithelial necrosis, all animals

Upper left panel: Dose-response curves for each category (from left to right: minimal, minor, moderate)
Upper right panel: Observed scores (1=minimal, 2=minor, 3=moderate) and fitted model plotted cumulatively, illustrating the goodness of fit
lower left panel: Dose-response relationship of the underlying latent variable (on log scale). The dotted lines indicate the transitions from one category to the next. The points of intersection are the CEDs for the respective categories.

Appendix 2A. Captan: selection of dose-response models

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

Captan		sex-dependent parameter								male	female	male + female
		total	a	b	var	a, b	a, var	b, var	a, b, var			
Squamous epithelial metaplasia in the larynx												
y=a	(1)	-78.95										
y=a·exp(bx)	(2)	**-51.27**								-26.36	-24.81	-51.17
y=a·exp(b·x^c)	(3)	N.C.										
y=a·[c-(c-1)exp(bx)]	(4)	N.C.										
y=a·[c-(c-1)exp(bx^d)]	(5)											
Squamous epithelial hyperplasia in the larynx												
y=a	(1)	-89.67										
y=a·exp(bx)	(2)	-65.53										
y=a·exp(b·x^c)	(3)	**-53.65**	-52.04	-52.04						-24.29	-27.03	-51.32
y=a·[c-(c-1)exp(bx)]	(4)	-57.31										
y=a·[c-(c-1)exp(bx^d)]	(5)	-53.65										

N.C. : no convergion reached

Appendix 2B. Captan: dose-response data and fitted models

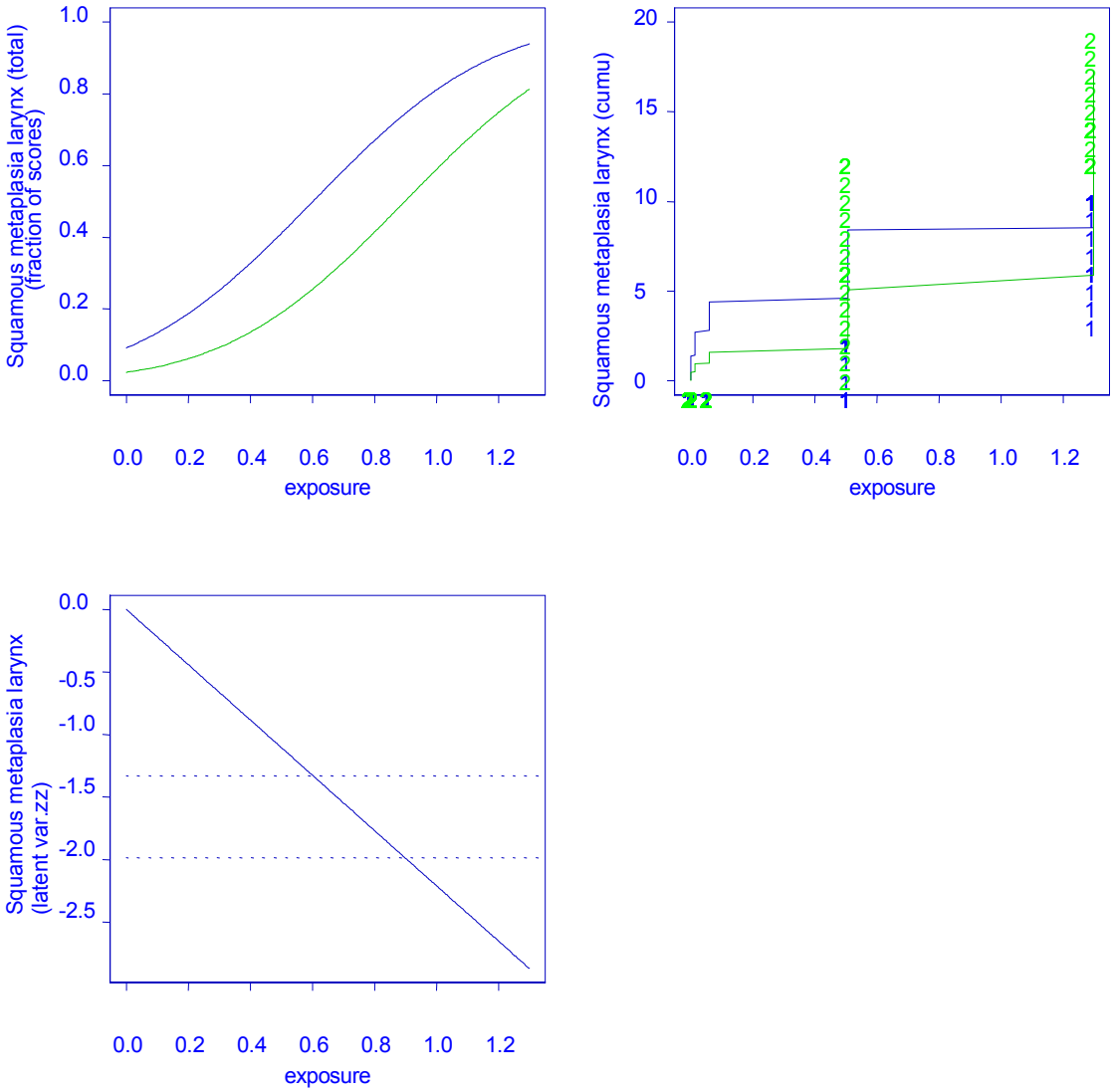

Figure 2B.1 Squamous epithelial metaplasia of the larynx.

Upper left panel: Dose-response curves for each category (from left to right: minimal, minor, moderate)
Upper right panel: Observed scores (1=slight, 2=moderate) and fitted model plotted cumulatively, illustrating the goodness of fit
Lower left panel: Dose-response relationship of the underlying latent variable (on log scale). The dotted lines indicate the transitions from one category to the next. The points of intersection are the CEDs for the respective categories.

Appendix 2B. (Captan, continued)

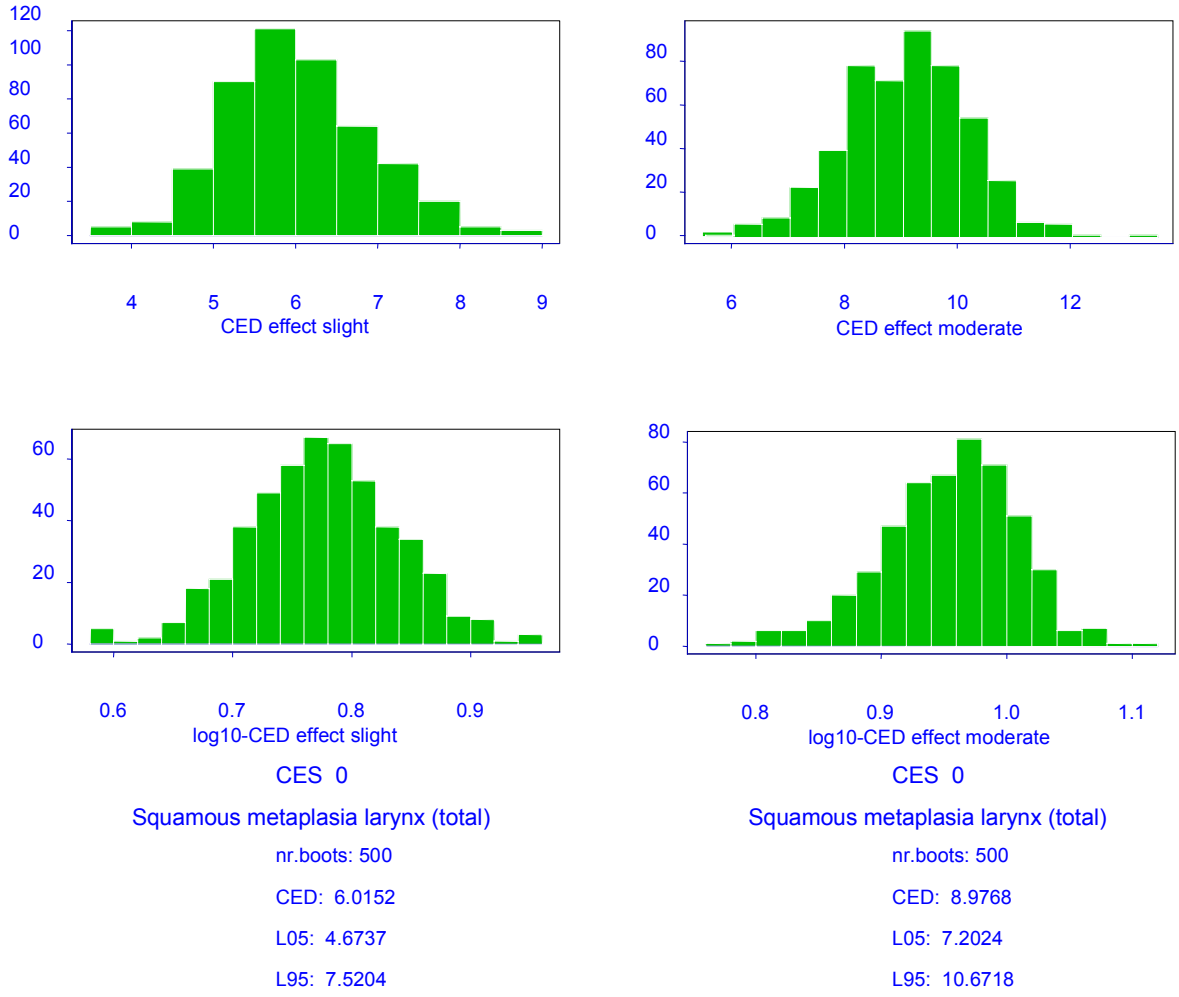

Figure 2B.2 Uncertainty distribution around slight and moderate squamous epithelial metaplasia of the larynx obtained by 500 bootstrap runs.

Appendix 2B. (Captan, continued)

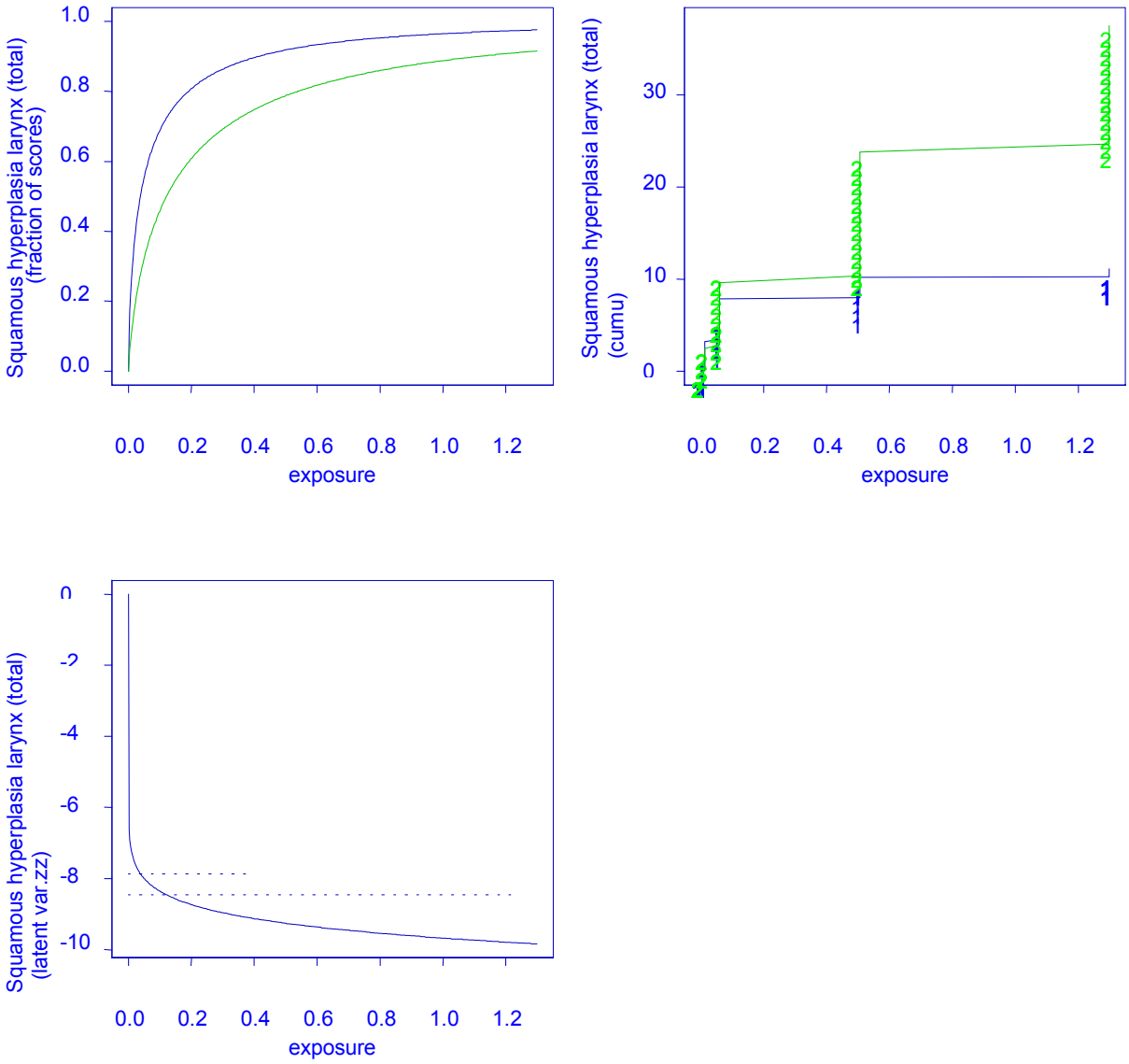

Figure 2B.3 Squamous epithelial hyperplasia of the larynx. 1 = minimal, 2 = slight

Upper left panel: Dose-response curves for each category (from left to right: minimal, minor, moderate)

Upper right panel: Observed scores (1=minimal, 2=slight) and fitted model plotted cumulatively, illustrating the goodness of fit

lower left panel: Dose-response relationship of the underlying latent variable (on log scale). The dotted lines indicate the transitions from one category to the next. The points of intersection are the CEDs for the respective categories.

Appendix 2B. (Captan, continued)

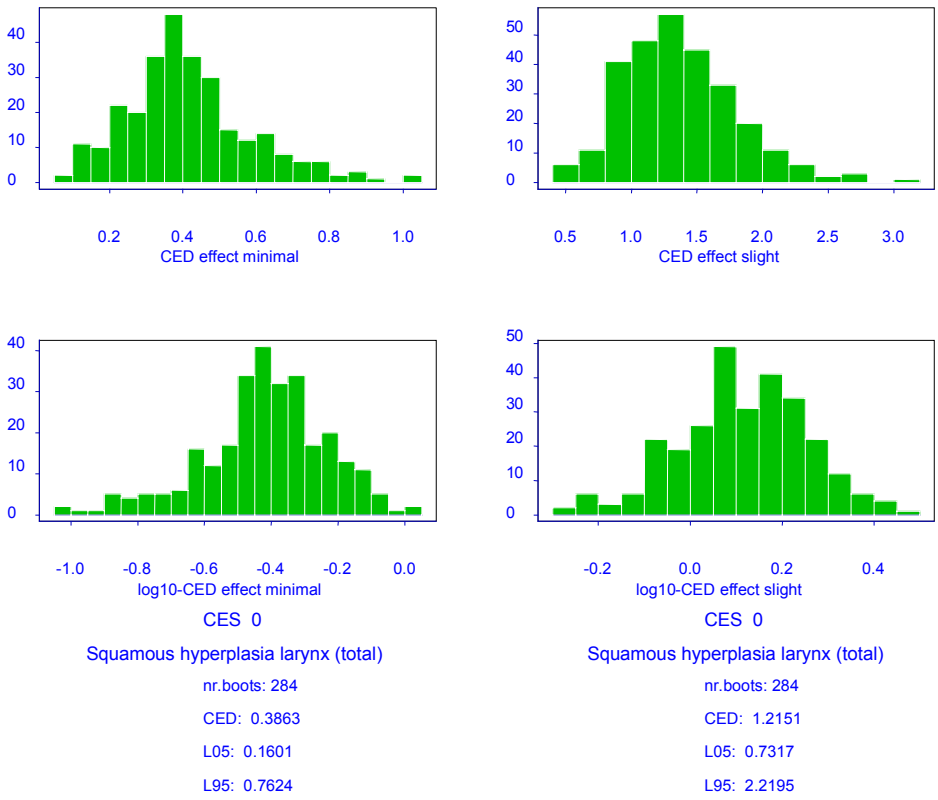

Figure 2B.4 Uncertainty distribution around minimal and slight squamous epithelial hyperplasia of the larynx obtained by 500 bootstrap runs.

Appendix 3A. Acetone cyanohydrin: selection of dose-response models

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

Acetone cyanohydrin		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male + female
Relative liver weight												
y=a	(1)	144.4	**146.16**									
y=a·exp(bx)	(2)	145.01	146.98							72.88	74.63	147.51
y=a·exp(b·x^c)	(3)	146.07	148.04									
y=a·[c-(c-1)exp(bx)]	(4)	146.07	148.04									
y=a·[c-(c-1)exp(bx^d)]	(5)		148.04									
T3												
y=a	(1)	66.57	**77.69**									
y=a·exp(bx)	(2)	67.12	78.08							33.39	46.08	79.47
y=a·exp(b·x^c)	(3)	68.07	79.40									
y=a·[c-(c-1)exp(bx)]	(4)	68.39	79.76									
y=a·[c-(c-1)exp(bx^d)]	(5)	68.63										
LDH												
y=a	(1)	4.0			8.26							
y=a·exp(bx)	(2)	5.7										
y=a·exp(b·x^c)	(3)	7.11										
y=a·[c-(c-1)exp(bx)]	(4)	8.14	8.92	8.14	**14.75**					19.39	-3.45	15.94
y=a·[c-(c-1)exp(bx^d)]	(5)	9.26										

Appendix 3A. (Acetone cyanohydrin, continued)

Acetone cyanohydrin		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	Male + female
LDH*												
y=a	(1)	**8.84**	10.20									
y=a·exp(bx)	(2)	9.65	10.89									
y=a·exp(b·x^c)	(3)											
y=a·[c-(c-1)exp(bx)]	(4)											
y=a·[c-(c-1)exp(bx^d)]	(5)											
RBC												
y=a	(1)	155.37										
y=a·exp(bx)	(2)	158.17								79.32	80.57	159.89
y=a·exp(b·x^c)	(3)	159.99		**162.07**						79.6 (N.C.)	82.81	(162.41)
y=a·[c-(c-1)exp(bx)]	(4)	158.17										
y=a·[c-(c-1)exp(bx^d)]	(5)	159.99										
MCHC												
y=a	(1)	N.C.	281.63									
y=a·exp(bx)	(2)	256.05	283.12									
y=a·exp(b·x^c)	(3)	279.90	287.22	286.68	279.94	**289.89**				141.86	148.95 (N.C)	(290.81)
y=a·[c-(c-1)exp(bx)]	(4)	N.C.										
y=a·[c-(c-1)exp(bx^d)]	(5)	279.90										

N.C. : no convergion reached

* : with three outliers omitted from the control and lower dose group

Appendix 3A. (Acetone cyanohydrin, continued)

Acetone cyanohydrin		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male + female
Hb												
y=a	(1)	147.90										
y=a·exp(bx)	(2)	151.57										
y=a·exp(b·x^c)	(3)	153.53	155.13	**156.30**						74.41 (N.C)	82.53	(156.94)
y=a·[c-(c-1)exp(bx)]	(4)	151.57										
y=a·[c-(c-1)exp(bx^d)]	(5)	153.53										
Total protein												
y=a	(1)	185.60	202.57									
y=a·exp(bx)	(2)	185.60	202.68	200.93	187.34	205.19	204.48	202.80	**207.1**	105.65	101.45	207.10
y=a·exp(b·x^c)	(3)	N.C.										
y=a·[c-(c-1)exp(bx)]	(4)	186.34										
y=a·[c-(c-1)exp(bx^d)]	(5)	N.C.										
BUN												
y=a	(1)	110.49										
y=a·exp(bx)	(2)	116.66	116.71	117.45						58.75	59.29	118.04
y=a·exp(b·x^c)	(3)	117.84		**119.72**						59.44	61.61	121.05
y=a·[c-(c-1)exp(bx)]	(4)	N.C.										
y=a·[c-(c-1)exp(bx^d)]	(5)	117.84										

N.C. : no convergion reached

Appendix 3B. Acetone cyanohydrin: dose-response data and fitted models

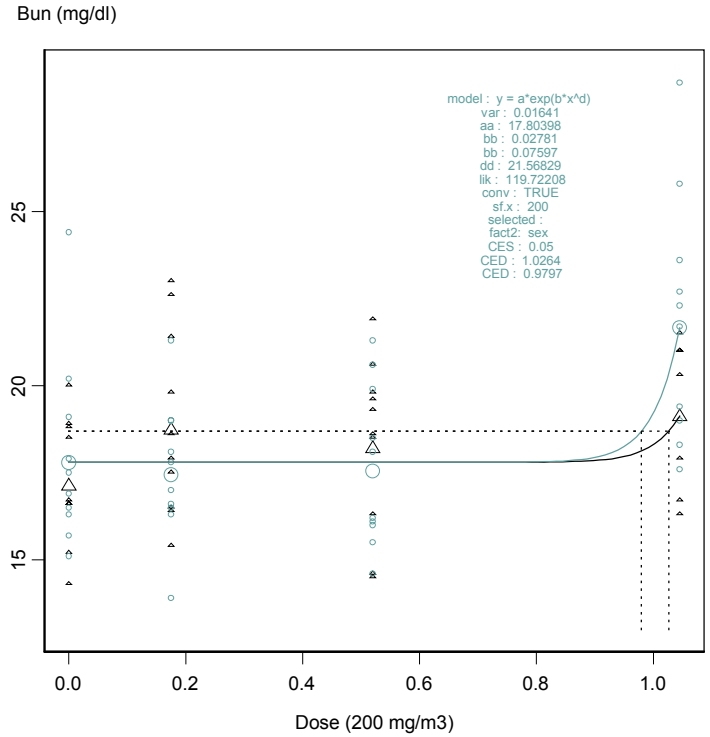

Figure 3B.1 Blood Urea Nitrogen (BUN) as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 3B. (Acetone cyanohydrin, continued)

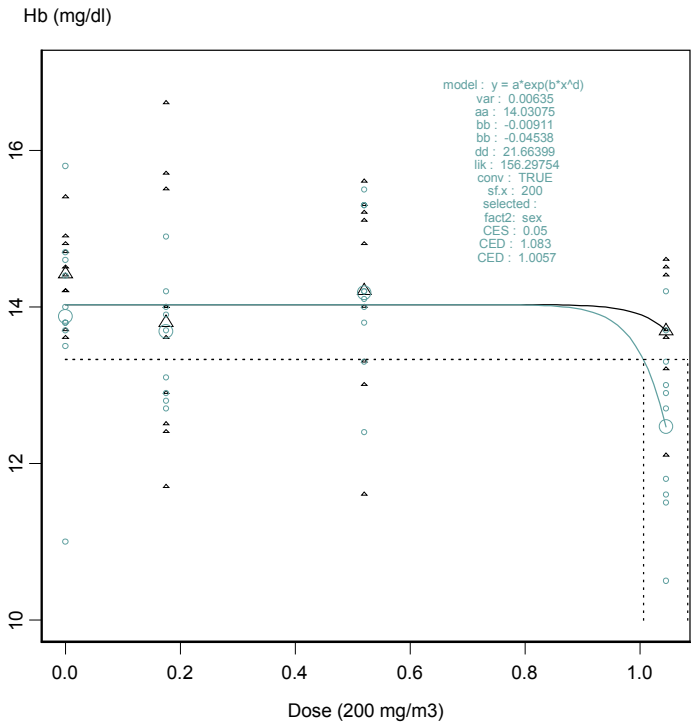

Figure 3B.2 Hemoglobin as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 3B. (Acetone cyanohydrin, continued)

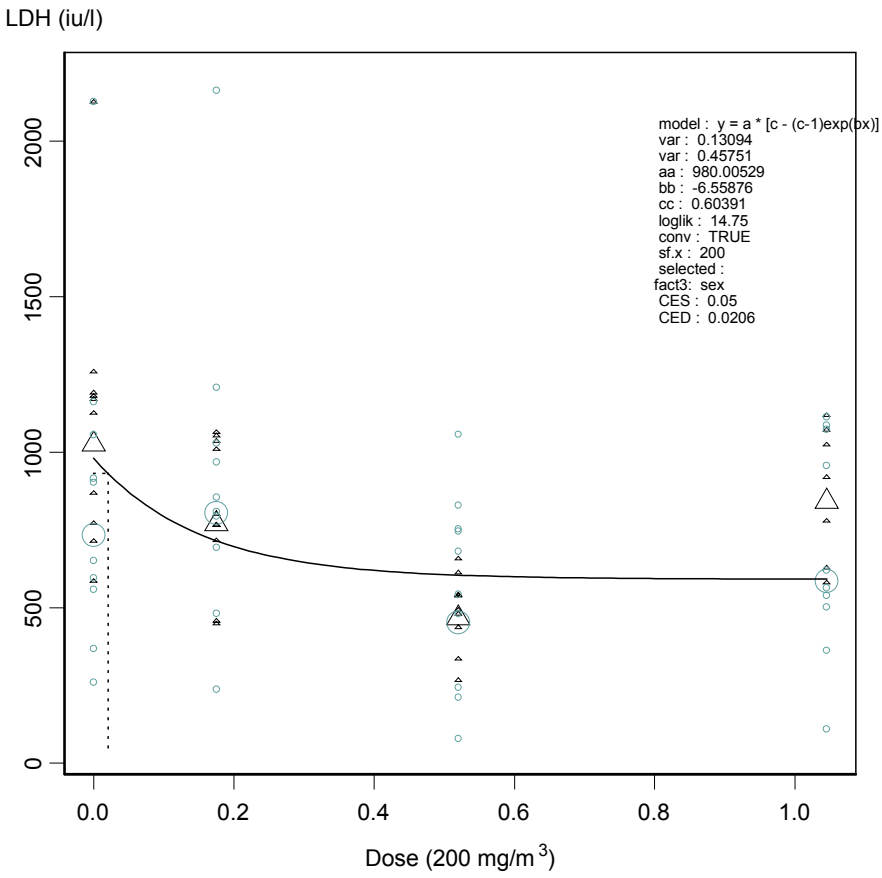

Figure 3B.3 Lactate dehydrogenase (LDH) as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means. After omitting the (high) outliers in the control and lowest dose group the dose-response was not significant anymore.

Appendix 3B. (Acetone cyanohydrin, continued)

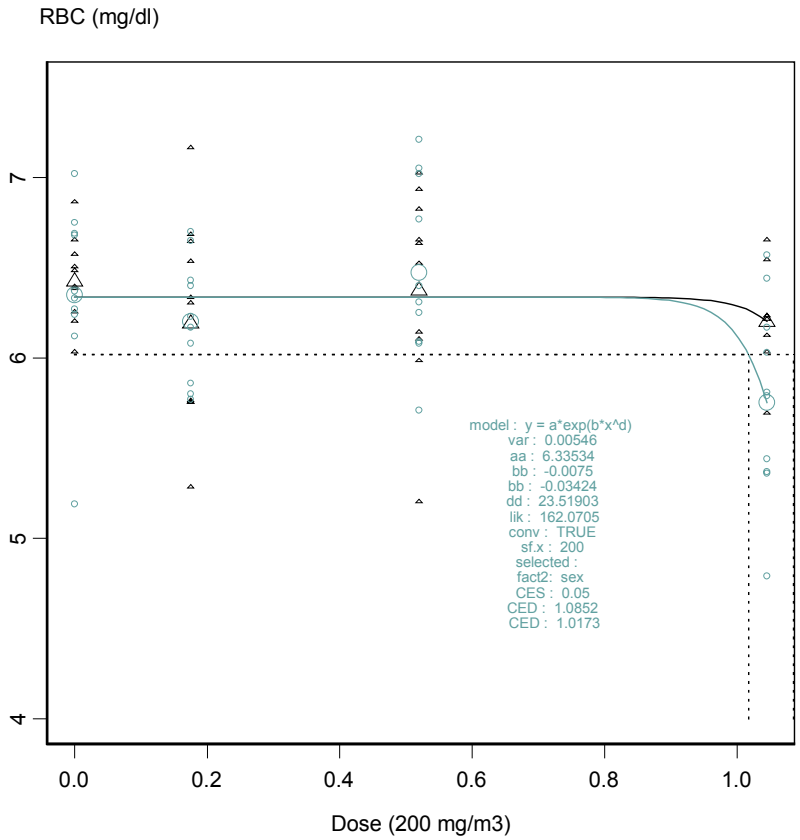

Figure 3B.4 Red blood cell count (RBC) as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 3B. (Acetone cyanohydrin, continued)

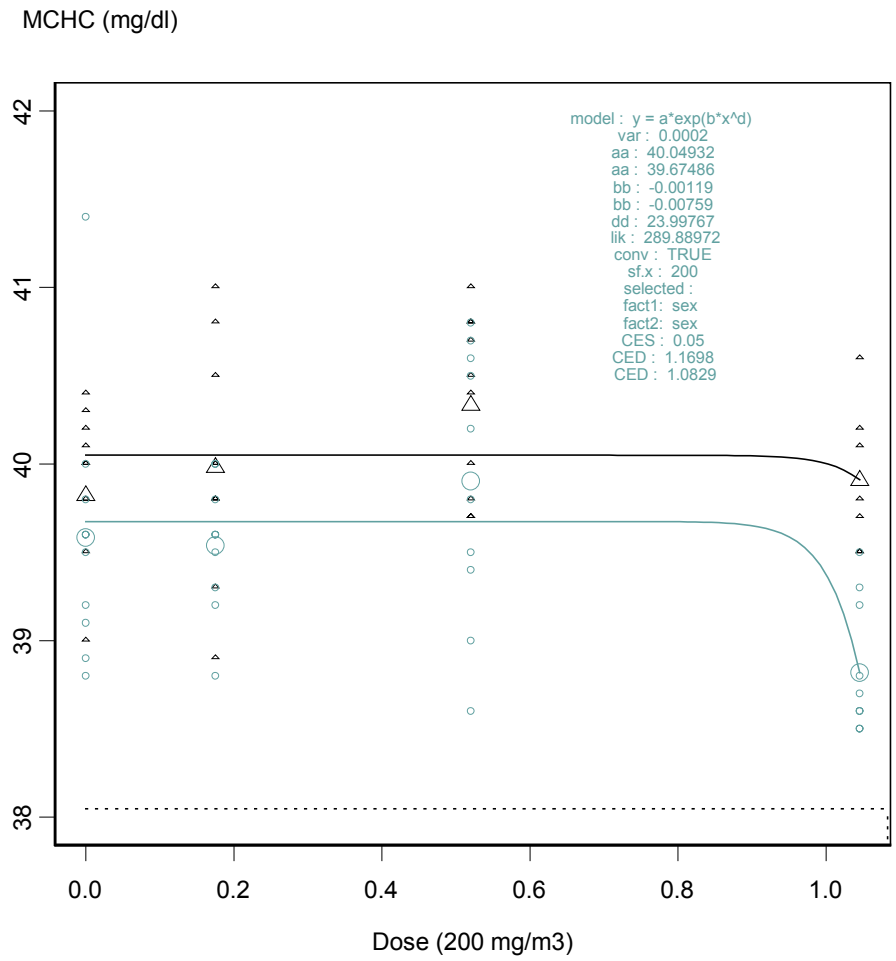

Figure 3B.5 Mean Corpuscular Hemoglobin Concentration (MCHC) as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 4A. Lyorthol: selection of dose-response models

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

o-benzyl-p-chlorophenol (BCP)		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male & female
Hyperparathyroidy												
y=a	(1)									-51.97		
y=a·exp(bx)	(2)									**-46.45**		
y=a·exp(b·x^c)	(3)									-45.08		
y=a·[c-(c-1)exp(bx)]	(4)									-45.15		
y=a·[c-(c-1)exp(bx^d)]	(5)									-45.03		
Cranial fibrous osteodystrophy												
y=a	(1)									-26.95		
y=a·exp(bx)	(2)									**-23.38**		
y=a·exp(b·x^c)	(3)									N.C		
y=a·[c-(c-1)exp(bx)]	(4)											
y=a·[c-(c-1)exp(bx^d)]	(5)											
Femoral fibrous osteodystrophy												
y=a	(1)									-33.59		
y=a·exp(bx)	(2)									**-27.63**		
y=a·exp(b·x^c)	(3)									N.C.		
y=a·[c-(c-1)exp(bx)]	(4)											
y=a·[c-(c-1)exp(bx^d)]	(5)											

N.C.: no convergion reached

Appendix 4A. (Lyorthol, continued)

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

o-benzyl-p-chlorophenol (BCP)		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male & female
Renal tubule hyperplasia												
y=a	(1)									-92.55		
y=a·exp(bx)	(2)									**-86.87**		
y=a·exp(b·x^c)	(3)									-86.63		
y=a·[c-(c-1)exp(bx)]	(4)									-86.67		
y=a·[c-(c-1)exp(bx^d)]	(5)									-86.63		
Renal tubule adenoma & carcinoma												
y=a	(1)									**-26.92**		
y=a·exp(bx)	(2)									-26.68		
y=a·exp(b·x^c)	(3)									-26.67		
y=a·[c-(c-1)exp(bx)]	(4)									-26.65		
y=a·[c-(c-1)exp(bx^d)]	(5)									-26.58		
Mean kidney weights*												
y=a	(1)		450.2									
y=a·exp(bx)	(2)		506.4			508.1	**529.1**		530.3	217.1	313.3	530.3
y=a·exp(b·x^c)	(3)		506.8									
y=a·[c-(c-1)exp(bx)]	(4)		506.9									
y=a·[c-(c-1)exp(bx^d)]	(5)											

N.C. : no convergion reached

* : log-likelihoods calculations based on group mean kidney weights and their standard error

Appendix 4A. (Lyorthol, continued)

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

o-phenylphenol (OPP)		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male &female
Transitional cell hyperplasia urinary bladder F0												
y=a	(1)	-123.4										
y=a·exp(bx)	(2)	-94.7	**-91.4**							-48.0	-41.5	-89.5
y=a·exp(b·x^c)	(3)	-94.7										
y=a·[c-(c-1)exp(bx)]	(4)	-94.6										
y=a·[c-(c-1)exp(bx^d)]	(5)	N.C.										
Transitional cell hyperplasia urinary bladder F1 males												
y=a	(1)									-59.2		
y=a·exp(bx)	(2)									**-45.7**		
y=a·exp(b·x^c)	(3)									-45.6		
y=a·[c-(c-1)exp(bx)]	(4)									-45.5		
y=a·[c-(c-1)exp(bx^d)]	(5)									N.C.		
Transitional cell hyperplasia kidney F0 males												
y=a	(1)									-87.2		
y=a·exp(bx)	(2)									**-83.3**		
y=a·exp(b·x^c)	(3)									-83.0		
y=a·[c-(c-1)exp(bx)]	(4)									-83.3		
y=a·[c-(c-1)exp(bx^d)]	(5)									N.C.		

N.C. : no convergion reached

Appendix 4A. (Lyorthol, continued)

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

o-phenylphenol (OPP)		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male & female
Renal calculi F0 males												
y=a	(1)									-60.9		
y=a·exp(bx)	(2)									**-53.7**		
y=a·exp(b·x^c)	(3)									-53.6		
y=a·[c-(c-1)exp(bx)]	(4)									-53.7		
y=a·[c-(c-1)exp(bx^d)]	(5)									N.C.		
Renal hemorrhage F0 males												
y=a	(1)									-29.8		
y=a·exp(bx)	(2)									**-21.1**		
y=a·exp(b·x^c)	(3)									N.C.		
y=a·[c-(c-1)exp(bx)]	(4)											
y=a·[c-(c-1)exp(bx^d)]	(5)											
Pyclonephritis F0 males												
y=a	(1)									-33.4		
y=a·exp(bx)	(2)									**-31.5**		
y=a·exp(b·x^c)	(3)									N.C.		
y=a·[c-(c-1)exp(bx)]	(4)											
y=a·[c-(c-1)exp(bx^d)]	(5)											

N.C. : no convergion reached

Appendix 4A. (Lyorthol, continued)

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

o-phenylphenol (OPP)		sex-dependent parameter										
		none	a	b	var	a, b	a, var	b, var	a, b, var	male	female	male & female
Urinary bladder calculi F0 males												
y=a	(1)									**-89.34**		
y=a·exp(bx)	(2)									-87.8		
y=a·exp(b·x^c)	(3)									-87.5		
y=a·[c-(c-1)exp(bx)]	(4)									-87.1		
y=a·[c-(c-1)exp(bx^d)]	(5)									-86.6		
Urinary bladder calculi F1 males												
y=a	(1)									**-49.8**		
y=a·exp(bx)	(2)									-48.1		
y=a·exp(b·x^c)	(3)									-48.1		
y=a·[c-(c-1)exp(bx)]	(4)									-48.1		
y=a·[c-(c-1)exp(bx^d)]	(5)											

Appendix 4B. Lyorthol (BCP): dose-response data and fitted models

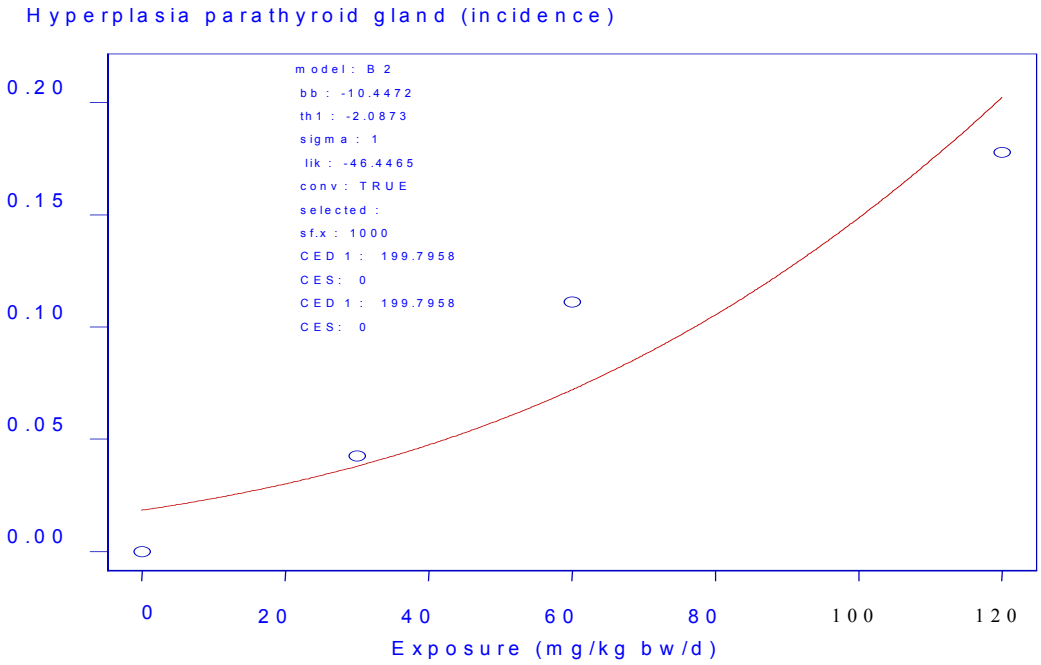

Figure 4B.1 Hyperplasia of the parathyriod gland

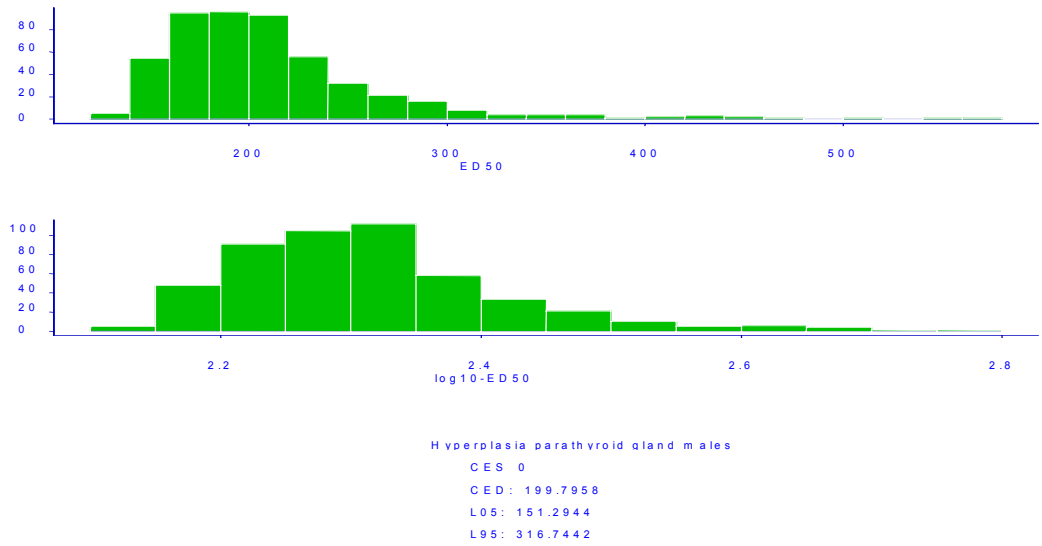

Figure 4B.2 Uncertainty distribution around the CED (ED50) for hyperplasia of the parathyriod gland after 500 bootstrap runs

Appendix 4B. (Lyorthol - BCP, continued)

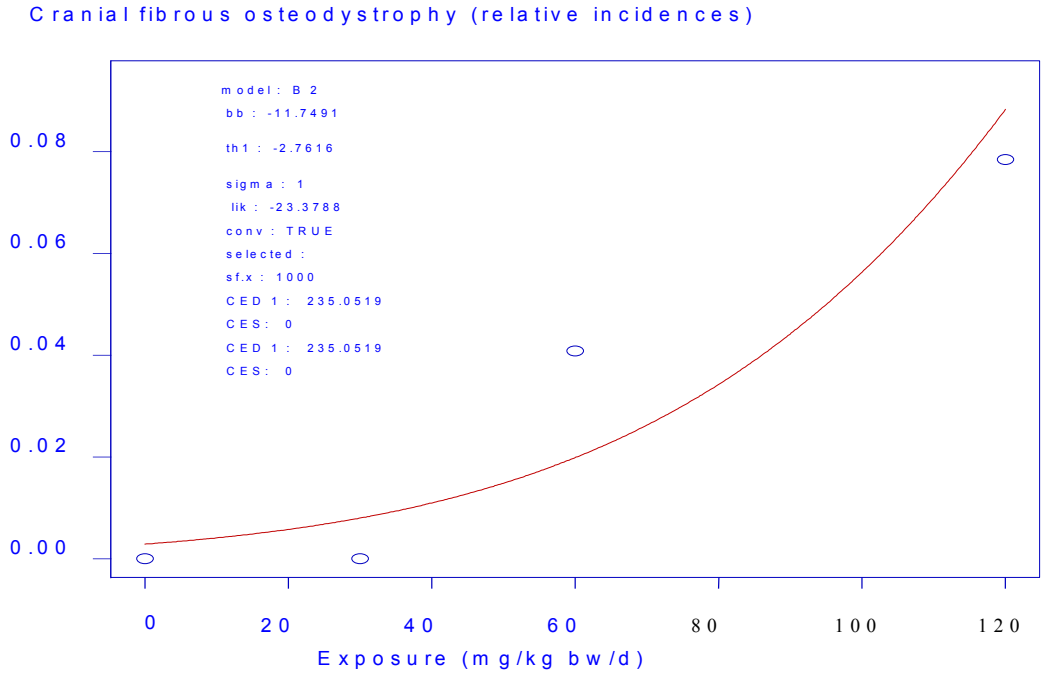

Figure 4B.3 Cranial fibrous osteodystrophy

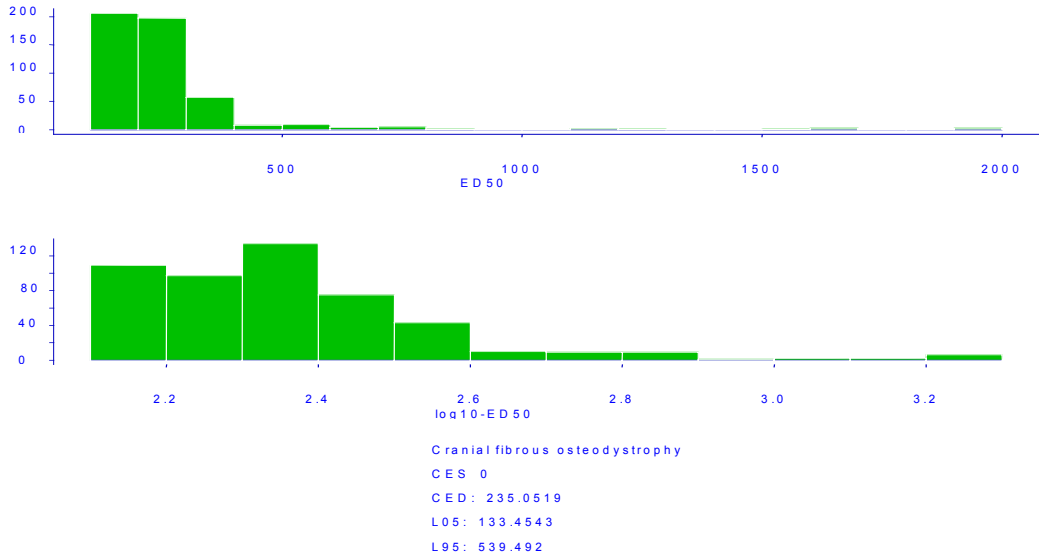

Figure 4B.4 Uncertainty distribution around the CED (ED50) for cranial fibrous osteodystrophy after 500 bootstrap runs

Appendix 4B. (Lyorthol - BCP, continued)

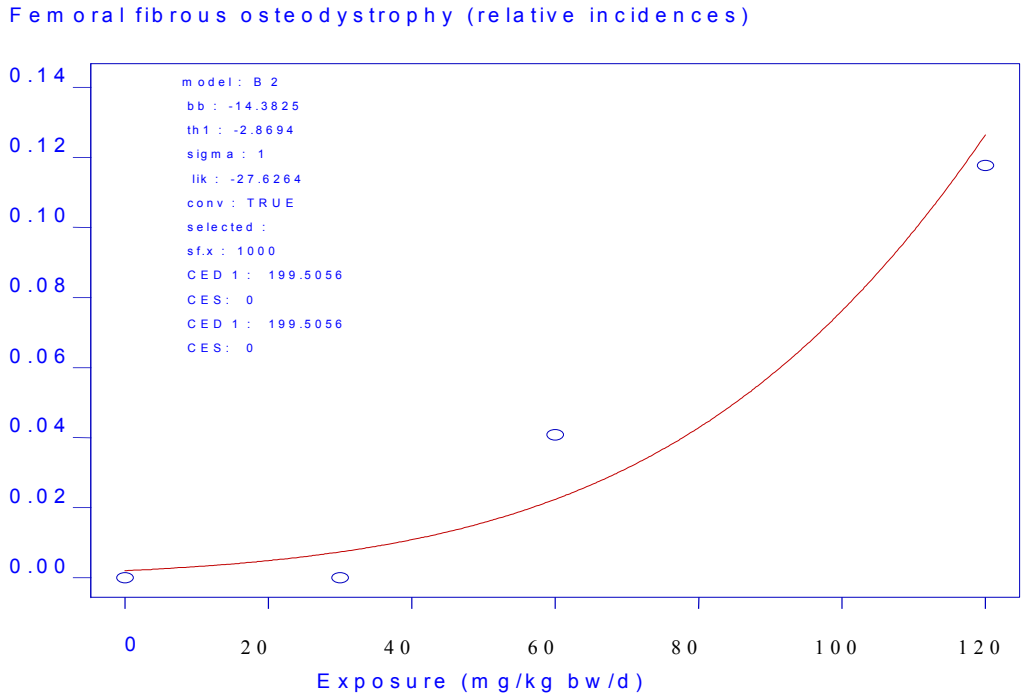

Figure 4B.5 Femoral fibrous osteodystrophy

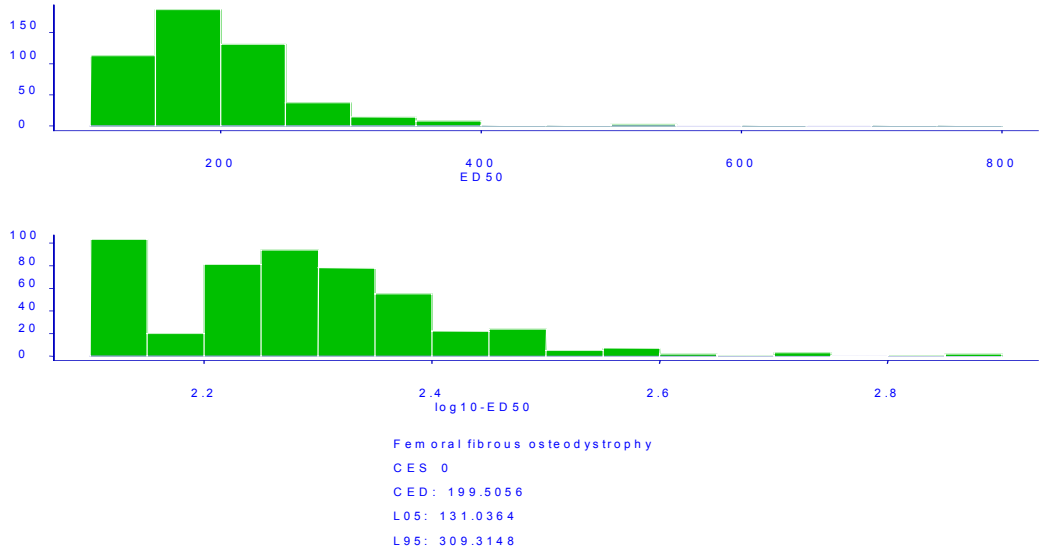

Figure 4B.6 Uncertainty distribution around CED (ED50) for femoral fibrous osteodystrophy after 500 bootstrap runs

Appendix 4B. (Lyorthol - BCP, continued)

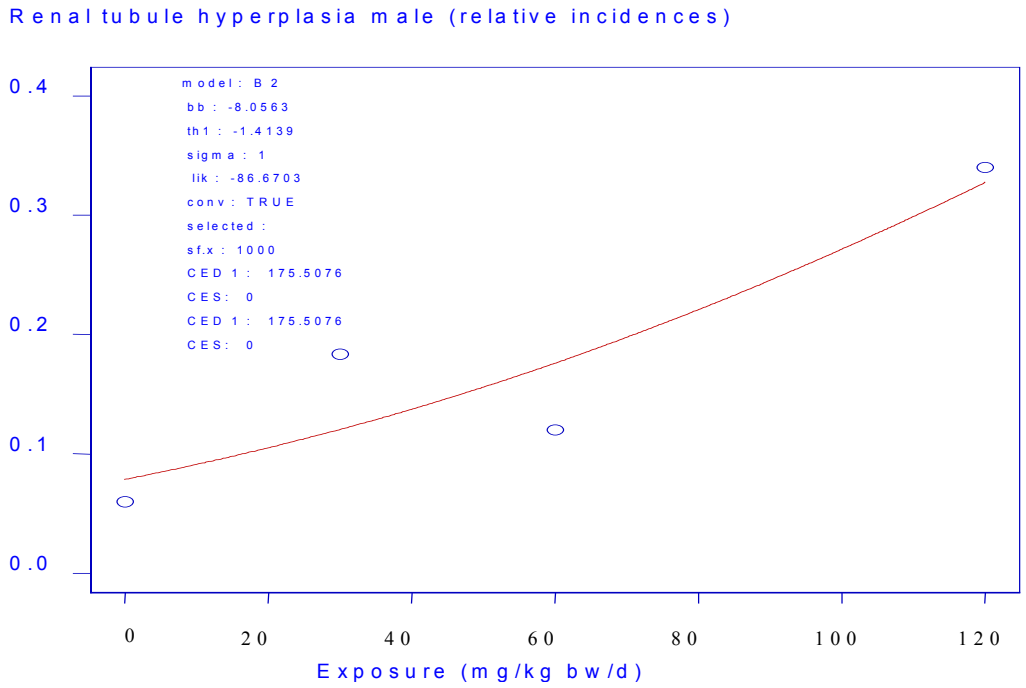

Figure 4B.7 Renal tubule hyperplasia (males)

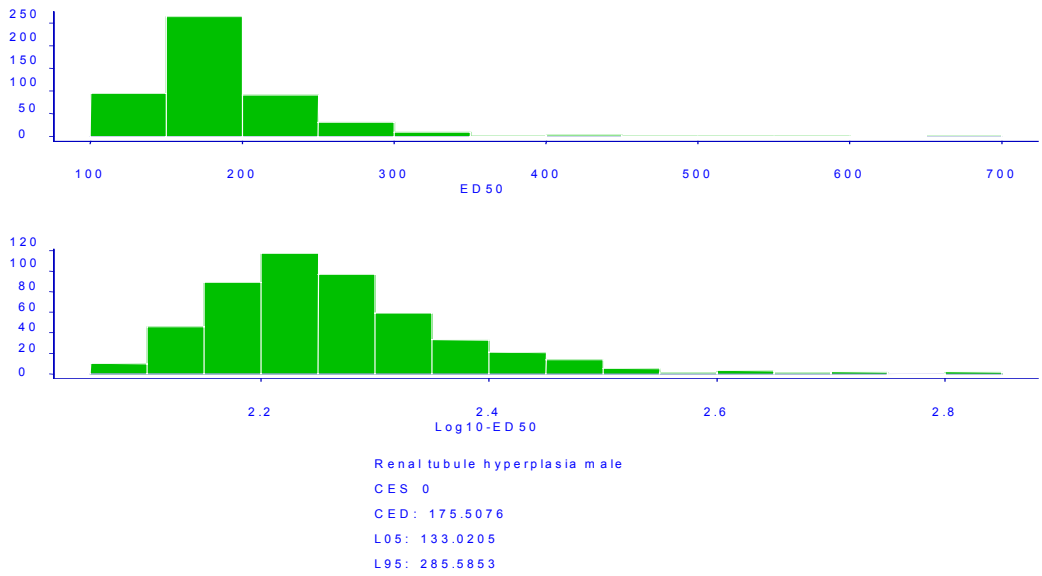

Figure 4B.8 Uncertainty distribution around CED (ED50) for renal tubule hyperplasia after 500 bootstrap runs

Appendix 4B. (Lyorthol - BCP, continued)

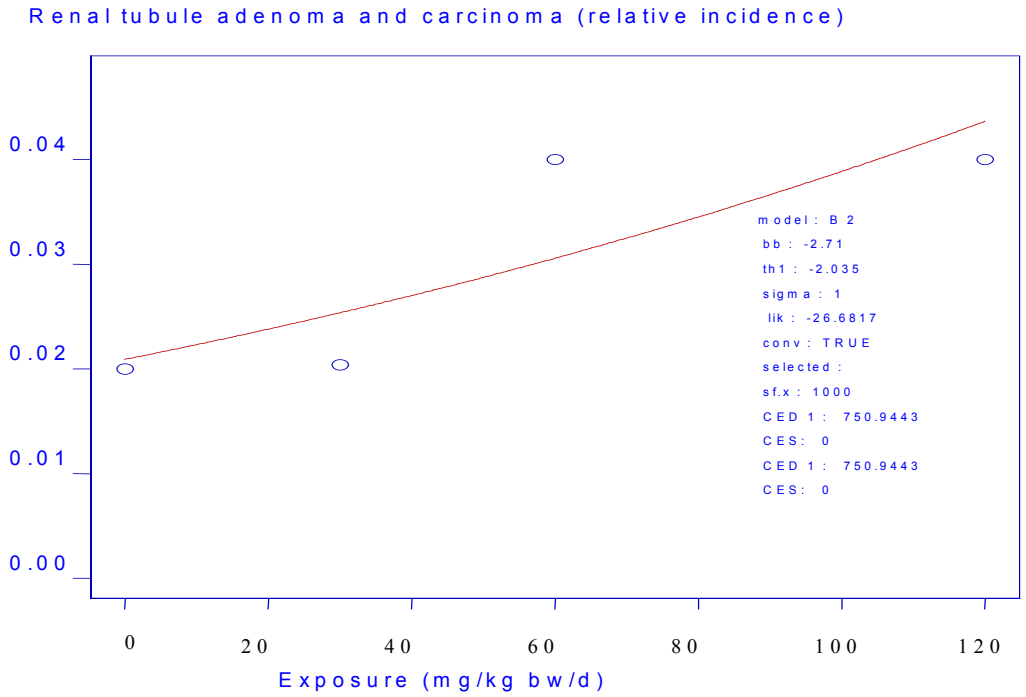

Figure 4B.9 Renal tubule adenoma and carcinoma

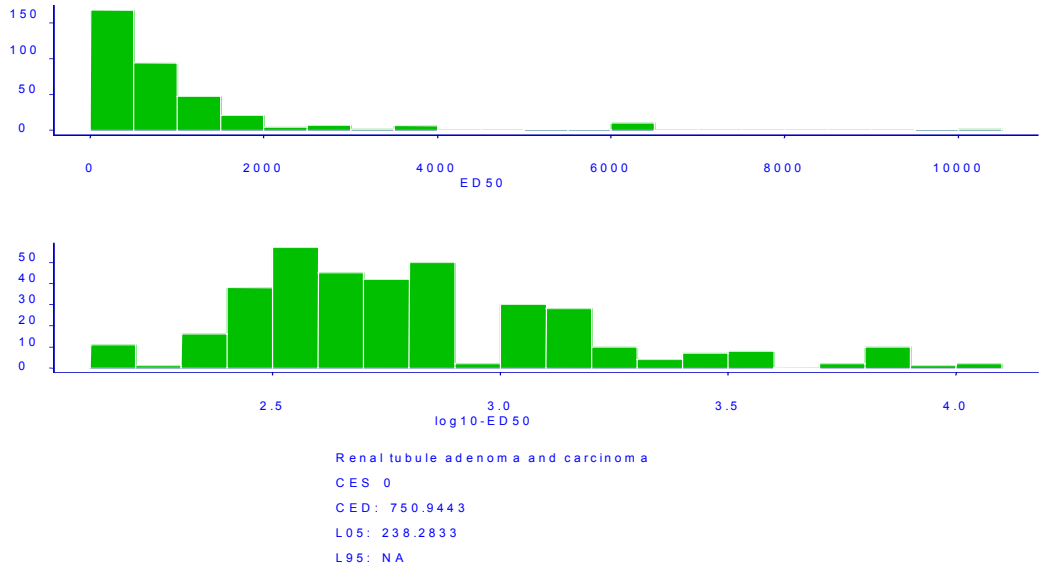

Figure 4B.10 Uncertainty distribution around CED (ED50) for renal tubule adenoma and carcinoma after 500 bootstrap runs

Appendix 4C. Lyorthol (OPP): dose-response data and fitted models

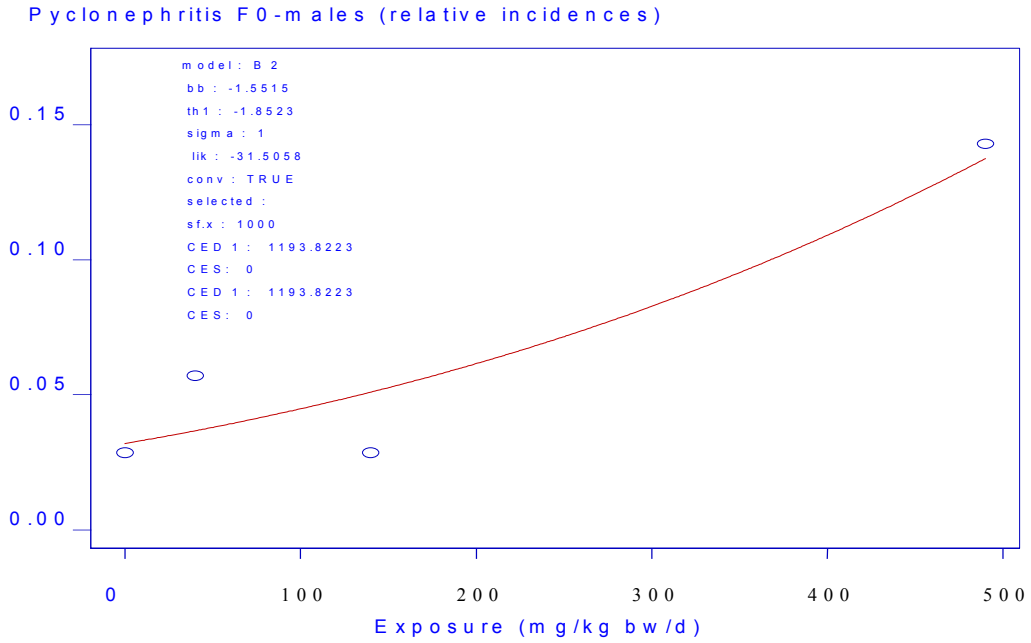

Figure 4C.1 Pyclonephritis

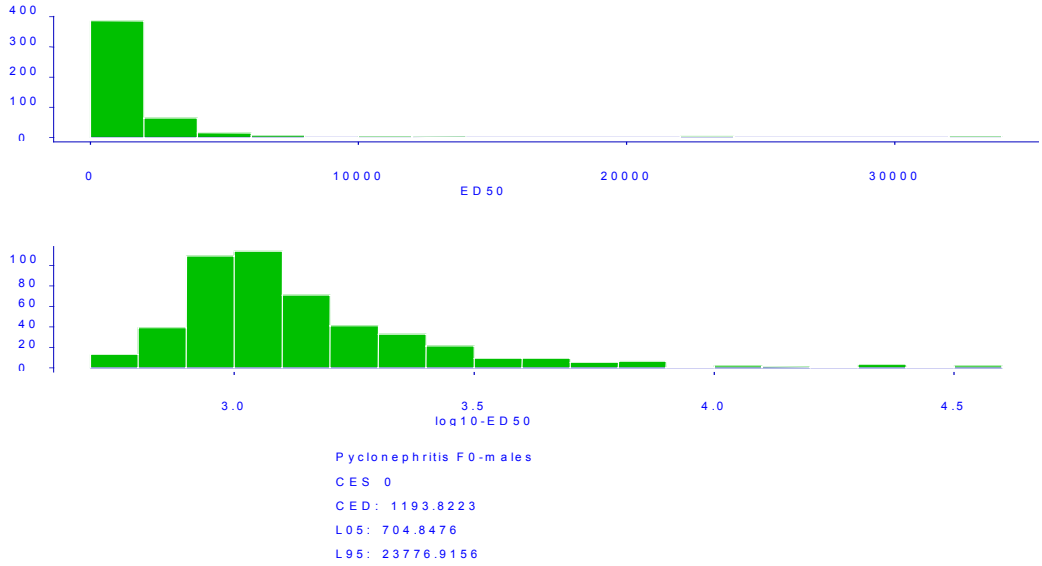

Figure 4C.2 Uncertainty distribution around CED (ED50) for pyclonephritis after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

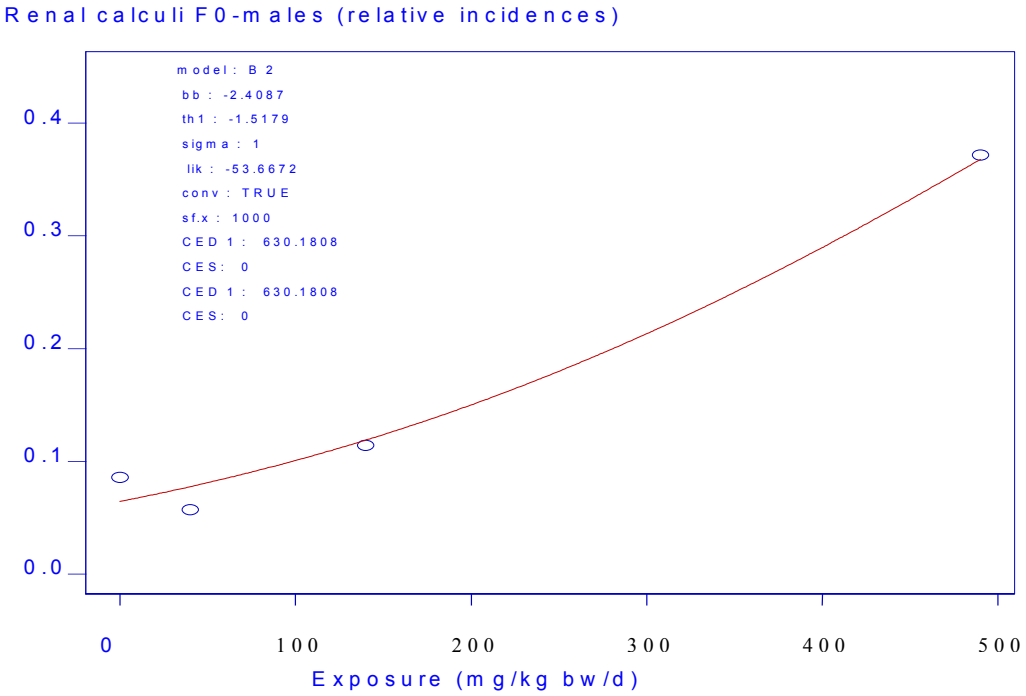

Figure 4C.3 Renal calculi in F0 males

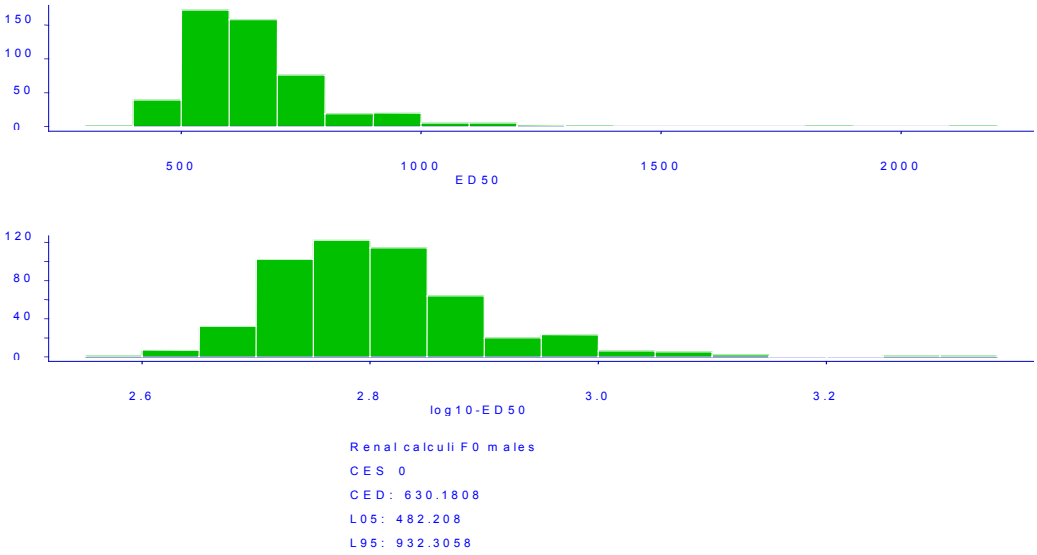

Figure 4C.4 Uncertainty distribution around CED (ED50) for renal calculi in F0 males after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

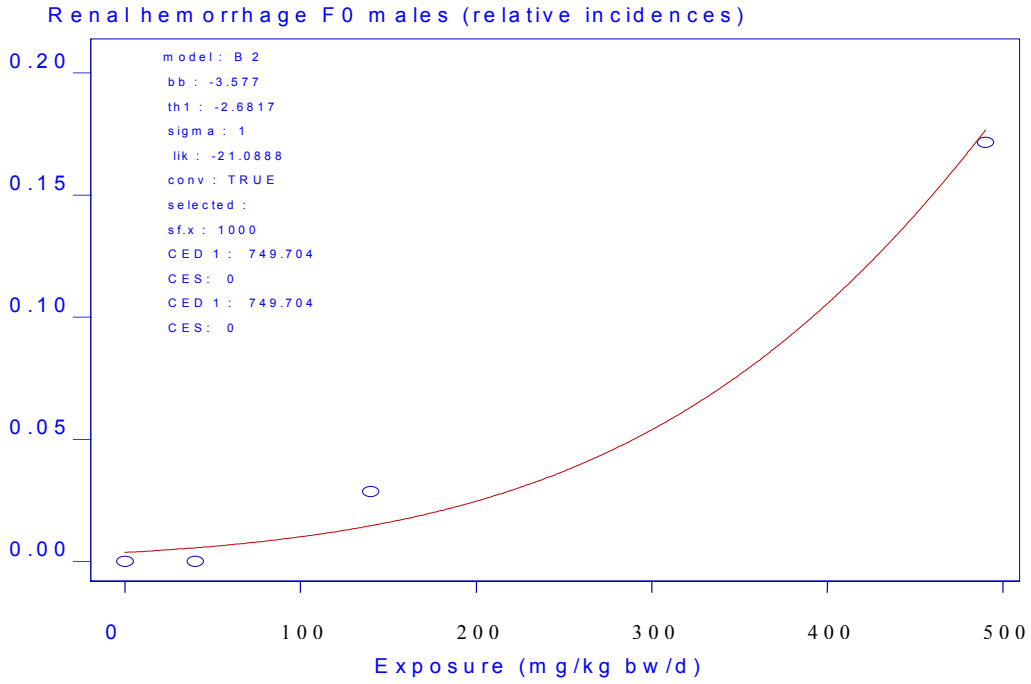

Figure 4C.5 Renal hemorrhage in F0 males

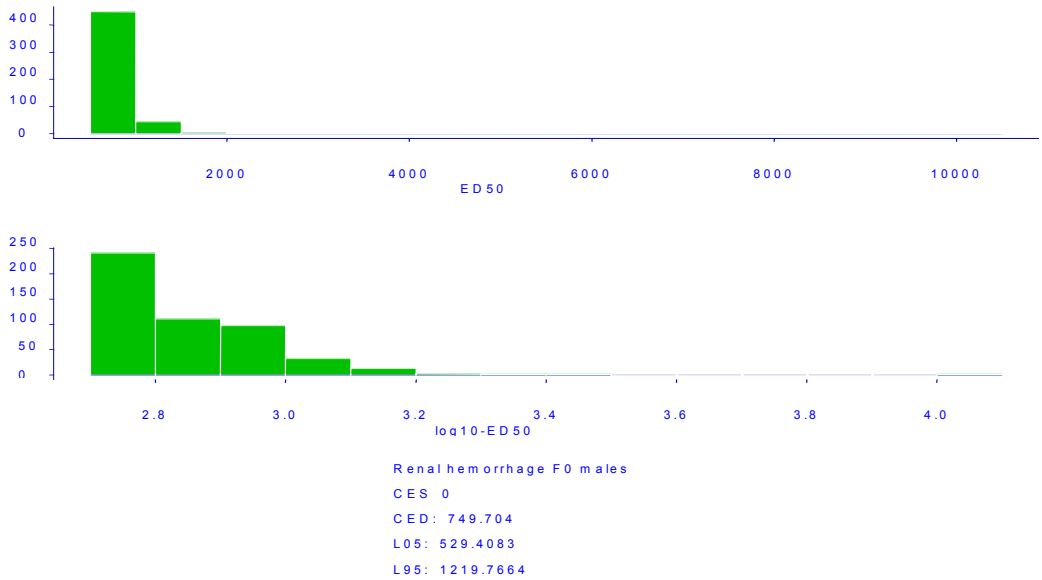

Figure 4C.6 Uncertainty distribution around CED (ED50) for renal hemorrhage after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

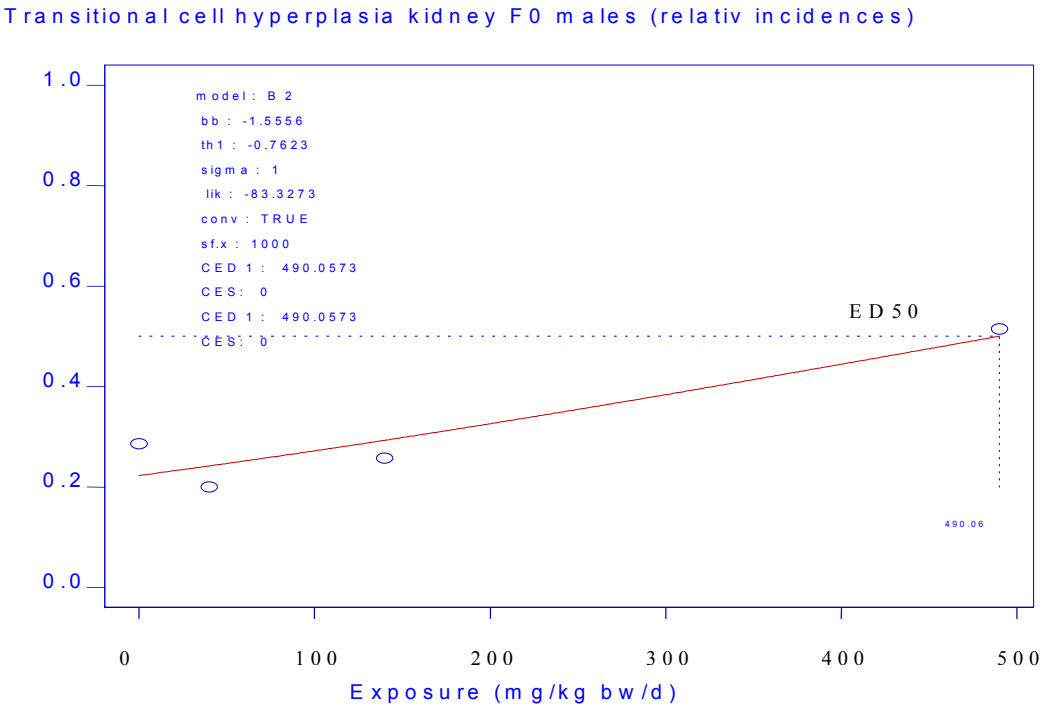

Figure 4C.7 Transitional cell hyperplasia in kidney of F0 males

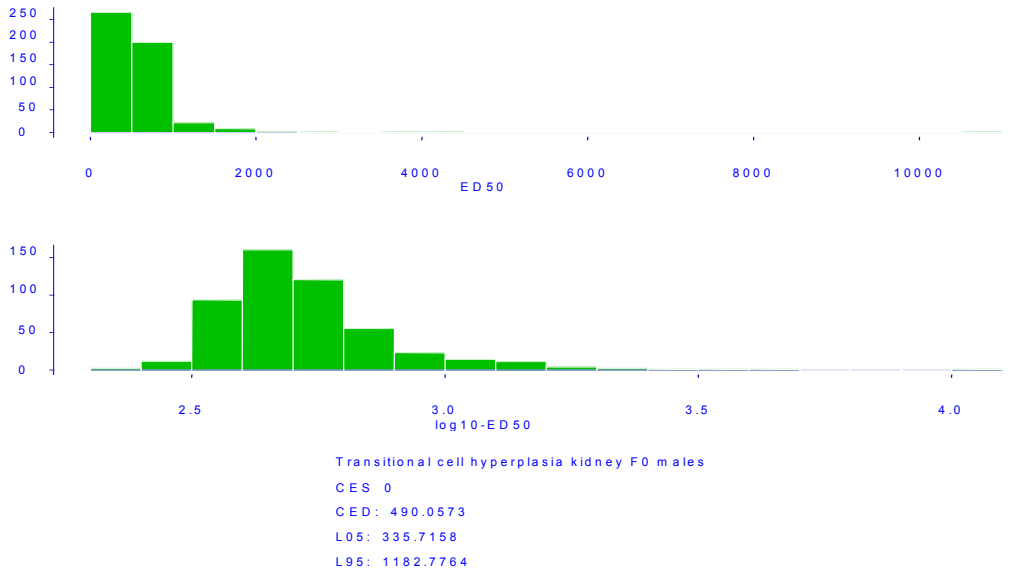

Figure 4C.8 Uncertainty distribution around CED (ED50) for transitional cell hyperplasia in the kidney after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

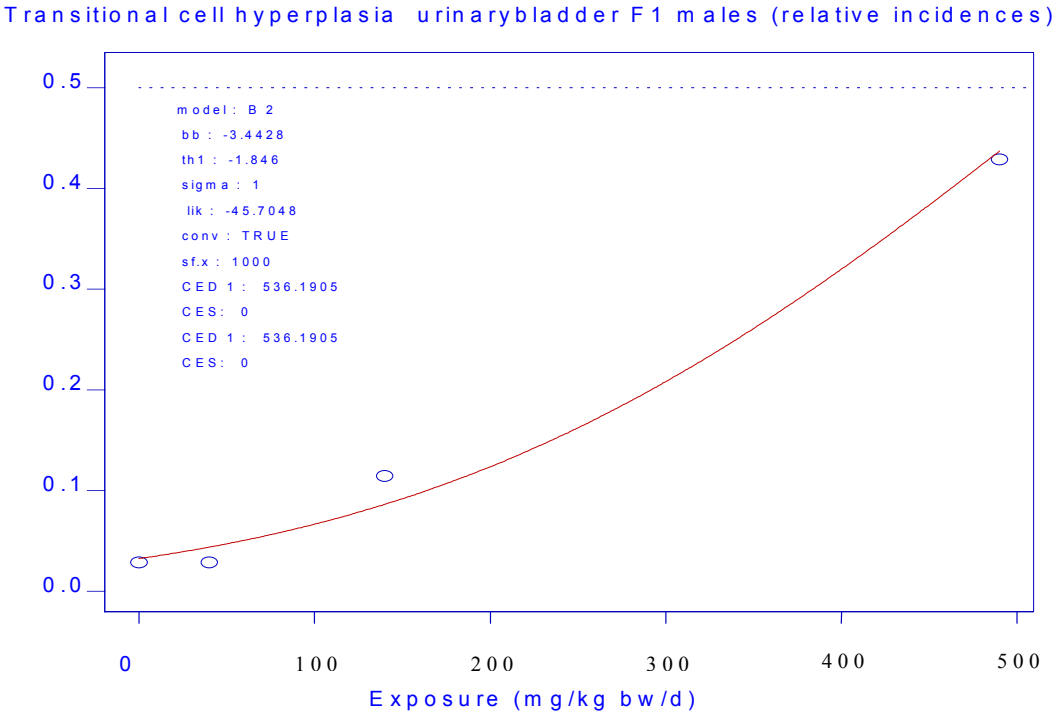

Figure 4C.9 Transitional cell hyperplasia in the urinary bladder of F1 males

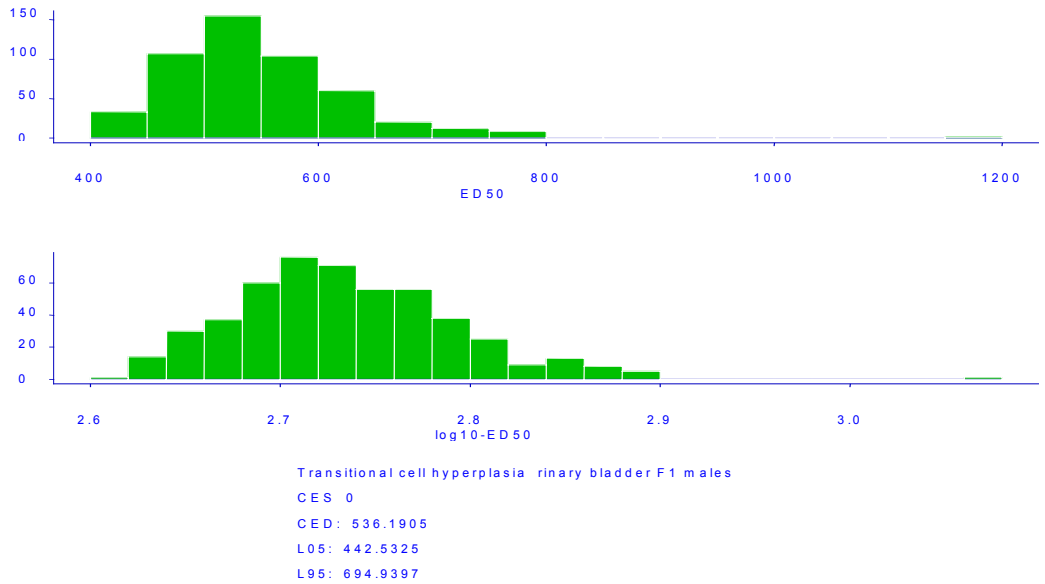

Figure 4C.10 Uncertainty distribution around CED (ED50) for transitional cell hyperplasia in the urinary bladder after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

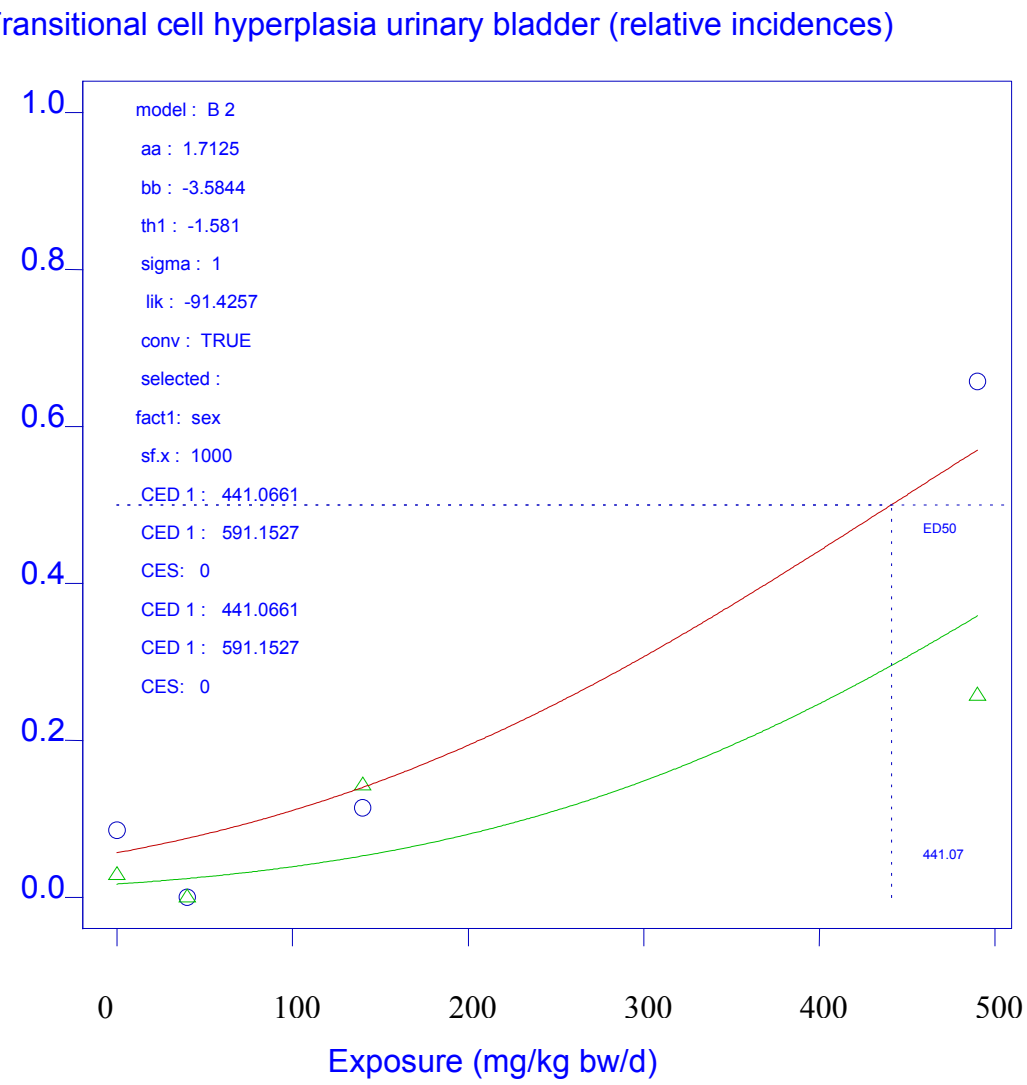

Figure 4C.11 Transitional cell hyperplasia in urinary bladder of F0 males (triangles) and females (circles).

Appendix 4C. (Lyorthol - OPP, continued)

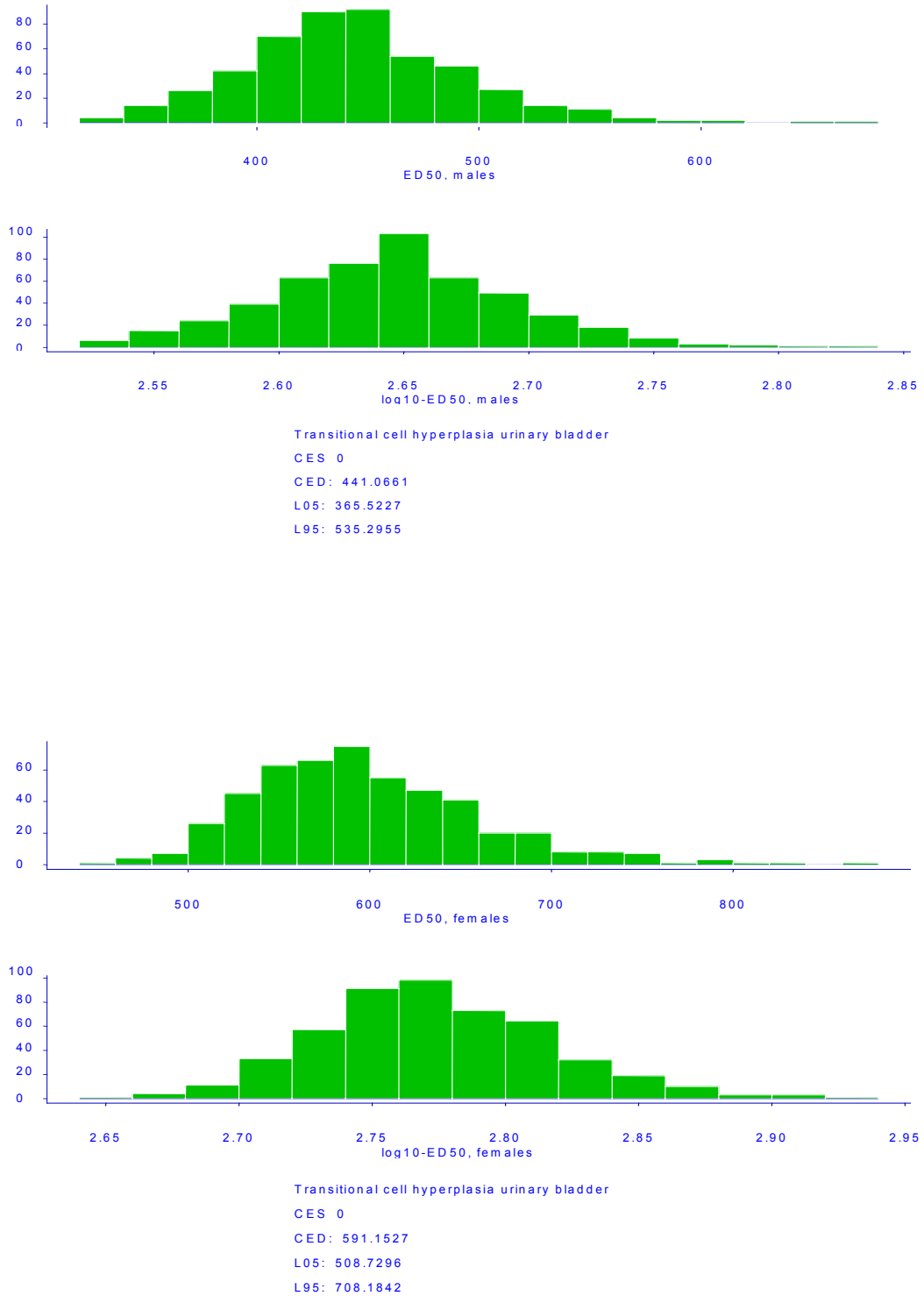

Figure 4C.12 Uncertainty distribution around CED (ED50) for transitional cell hyperplasia in urinary bladder of F0 males and females after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

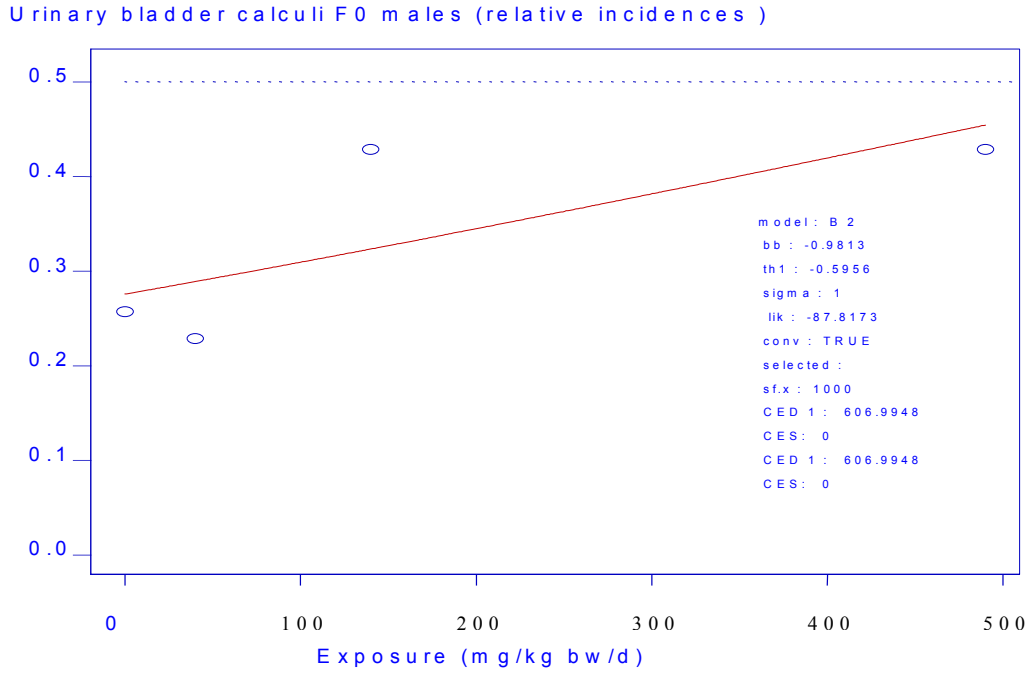

Figure 4C.13 Urinary bladder calculi in F0 males

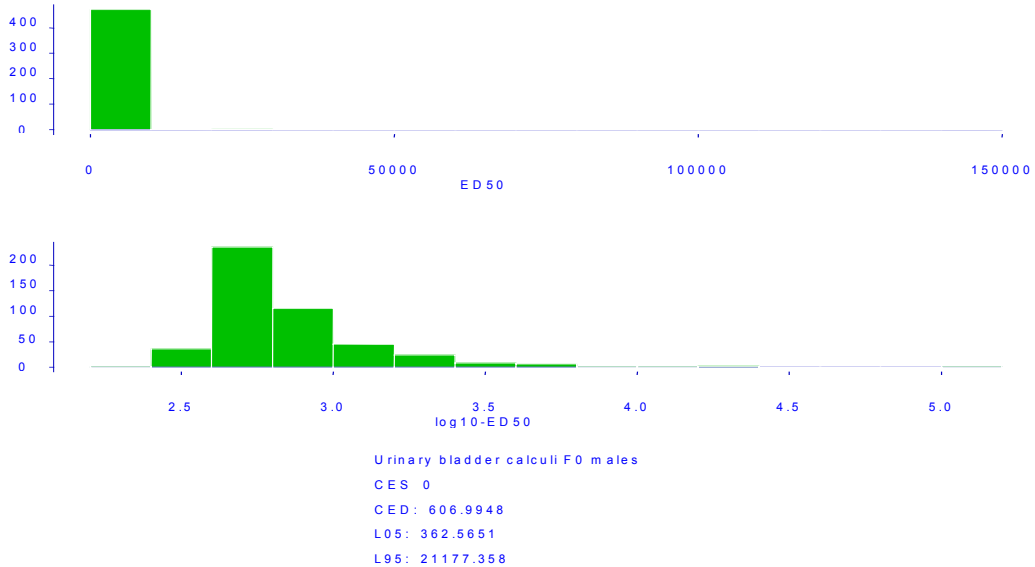

Figure 4C.14 Uncertainty distribution around CED (ED50) for urinary bladder calculi in F0 males after 500 bootstrap runs

Appendix 4C. (Lyorthol - OPP, continued)

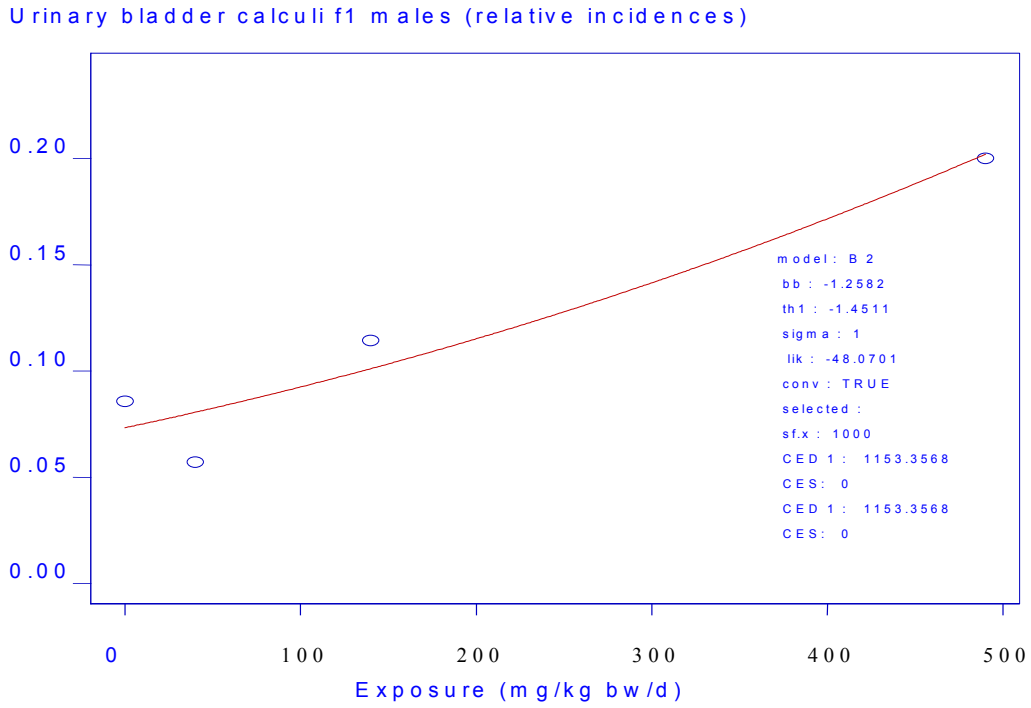

Figure 4C.15 Urinary bladder calculi in F1 males

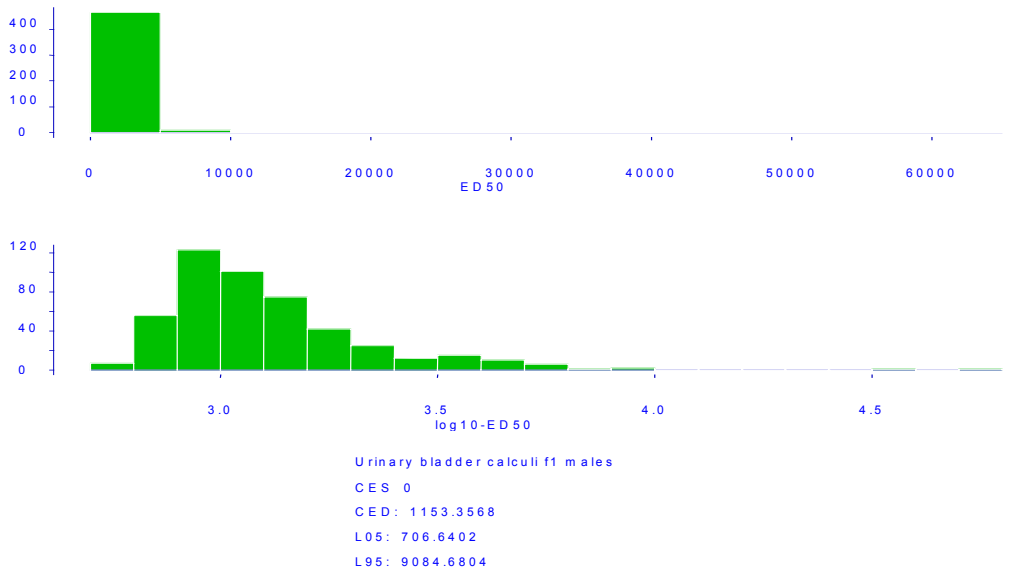

Figure 4C.16 Uncertainty distribution around CED (ED50) for urinary bladder calculi in F1 males after 500 bootstrap runs

Appendix 5A. White spirits: selection of dose-response models

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

White spirits		sex-dependent parameter								male	female	male + female
		none	a	b	var	a, b	a, var	b, var	a, b, var			
Relative liver weights												
y=a	(1)	236.10										
y=a·exp(bx)	(2)	283.06	284.76	**286.75**						143.74	143.11	286.85
y=a·exp(b·x^c)	(3)	283.18										
y=a·[c-(c-1)exp(bx)]	(4)	283.27										
y=a·[c-(c-1)exp(bx^d)]	(5)	283.62										
Relative kidney weight												
y=a	(1)	238.09										
y=a·exp(bx)	(2)	262.44	263.92	262.60	262.62	**268.30**				130.80	137.68	268.48
y=a·exp(b·x^c)	(3)	263.81										
y=a·[c-(c-1)exp(bx)]	(4)	263.99										
y=a·[c-(c-1)exp(bx^d)]	(5)	264.06										
Relative spleen weight												
y=a	(1)	159.82										
y=a·exp(bx)	(2)	165.28	186.85	172.97	180.80	187.98	**200.91**			77.19	124.85	202.04
y=a·exp(b·x^c)	(3)	165.74										
y=a·[c-(c-1)exp(bx)]	(4)	166.03										
y=a·[c-(c-1)exp(bx^d)]	(5)	167.07										

Appendix 5A. (White spirits, continued)

Log-likelihoods associated with various model fits. The result given in bold indicates the model selected.

Endpoint		sex-dependent parameter								male	female	male + female
		none	a	b	var	a, b	a, var	b, var	a, b, var			
Red blood cells												
y=a	(1)		295.28									
y=a·exp(bx)	(2)		300.29				309.74					
y=a·exp(b·x^c)	(3)		302.78									
y=a·[c-(c-1)exp(bx)]	(4)		302.83			304.26	**311.83**			143.67	170.18	313.85
y=a·[c-(c-1)exp(bx^d)]	(5)											

Appendix 5B. White Spirits: dose-response data and fitted models

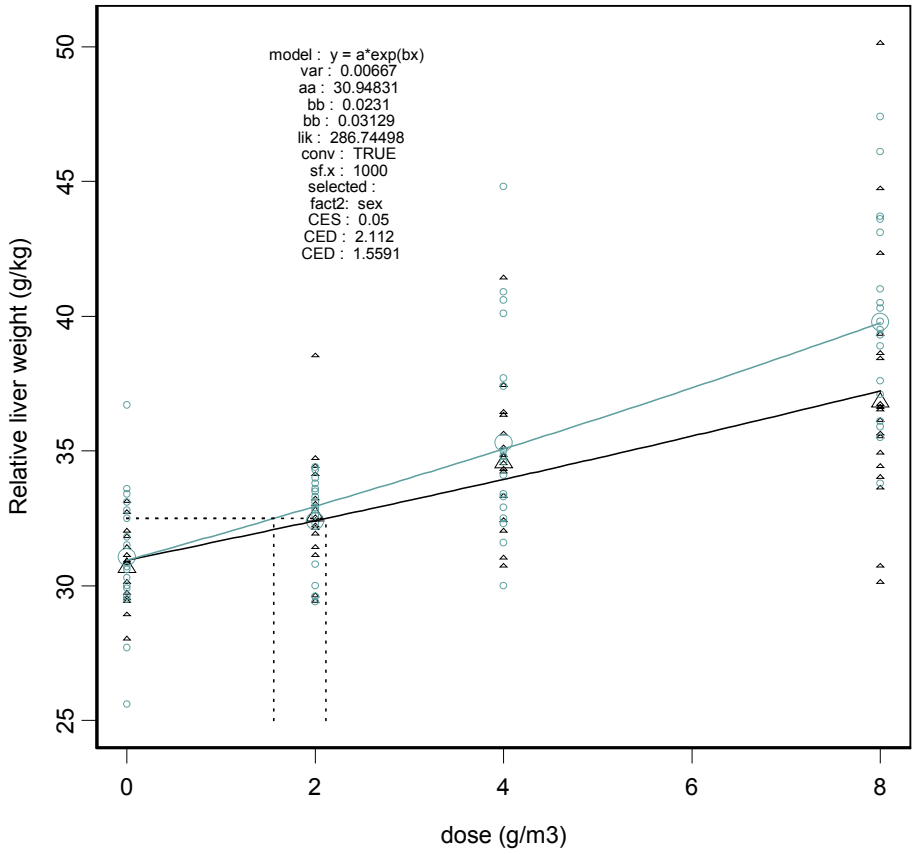

Figure 5B.1 Relative liver weights as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 5B. (White Spirits, continued)

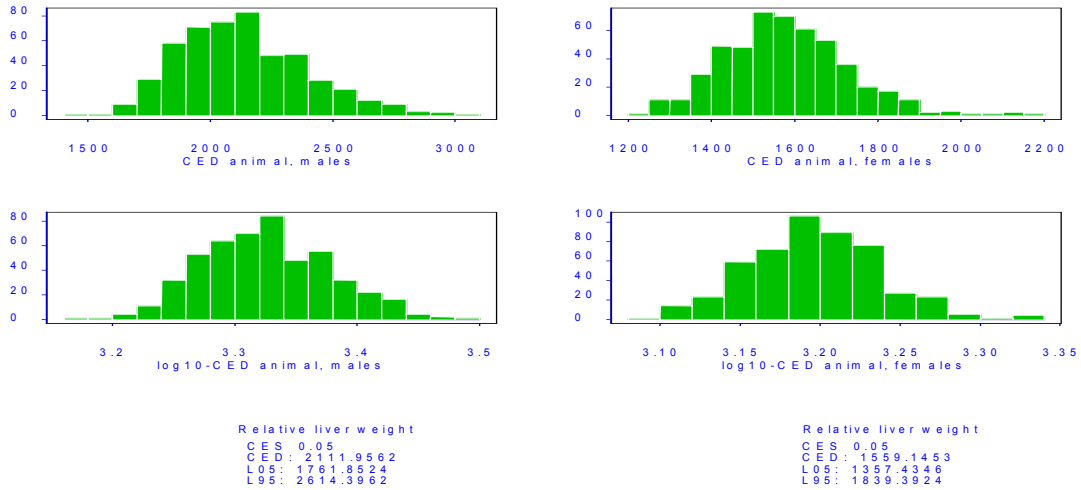

Figure 5B.2 Uncertainty distribution around the CED05 for increased relative liver weights obtained by 500 bootstrap runs

Appendix 5B. (White Spirits, continued)

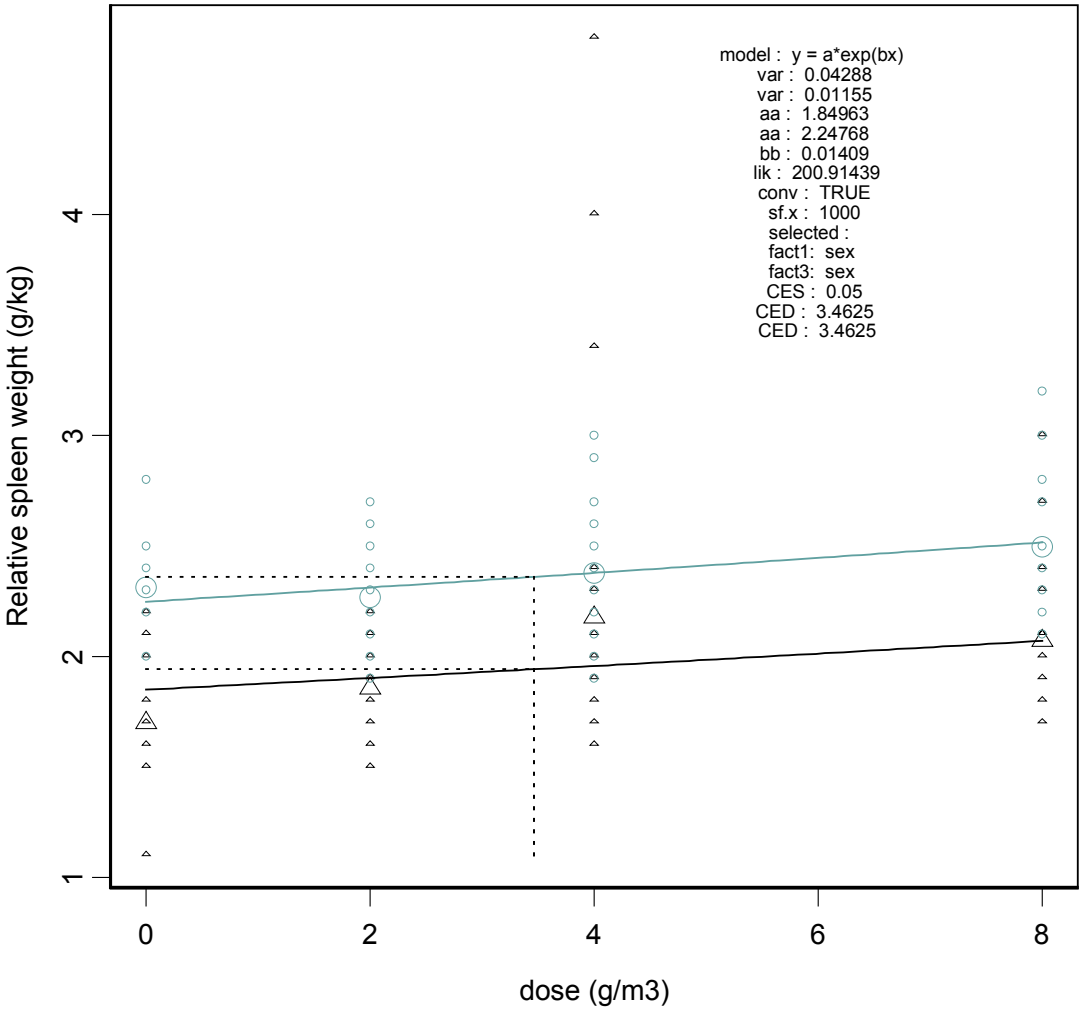

Figure 5B.3 Relative spleen weights as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 5B. (White Spirits, continued)

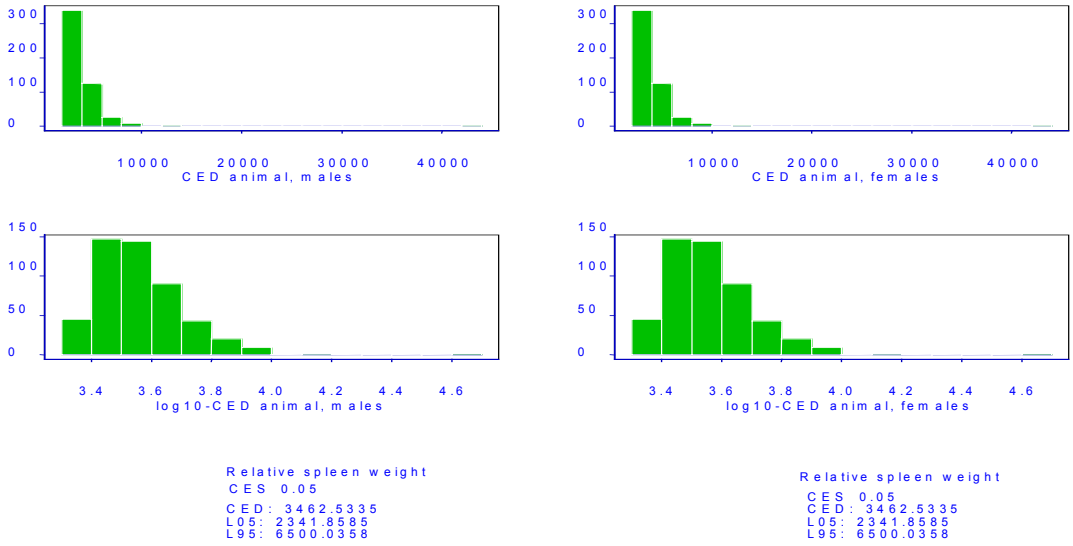

Figure 5B.4 Uncertainty distribution around the CED05 for increased relative spleen weights obtained by 500 bootstrap runs

Appendix 5B. (White Spirits, continued)

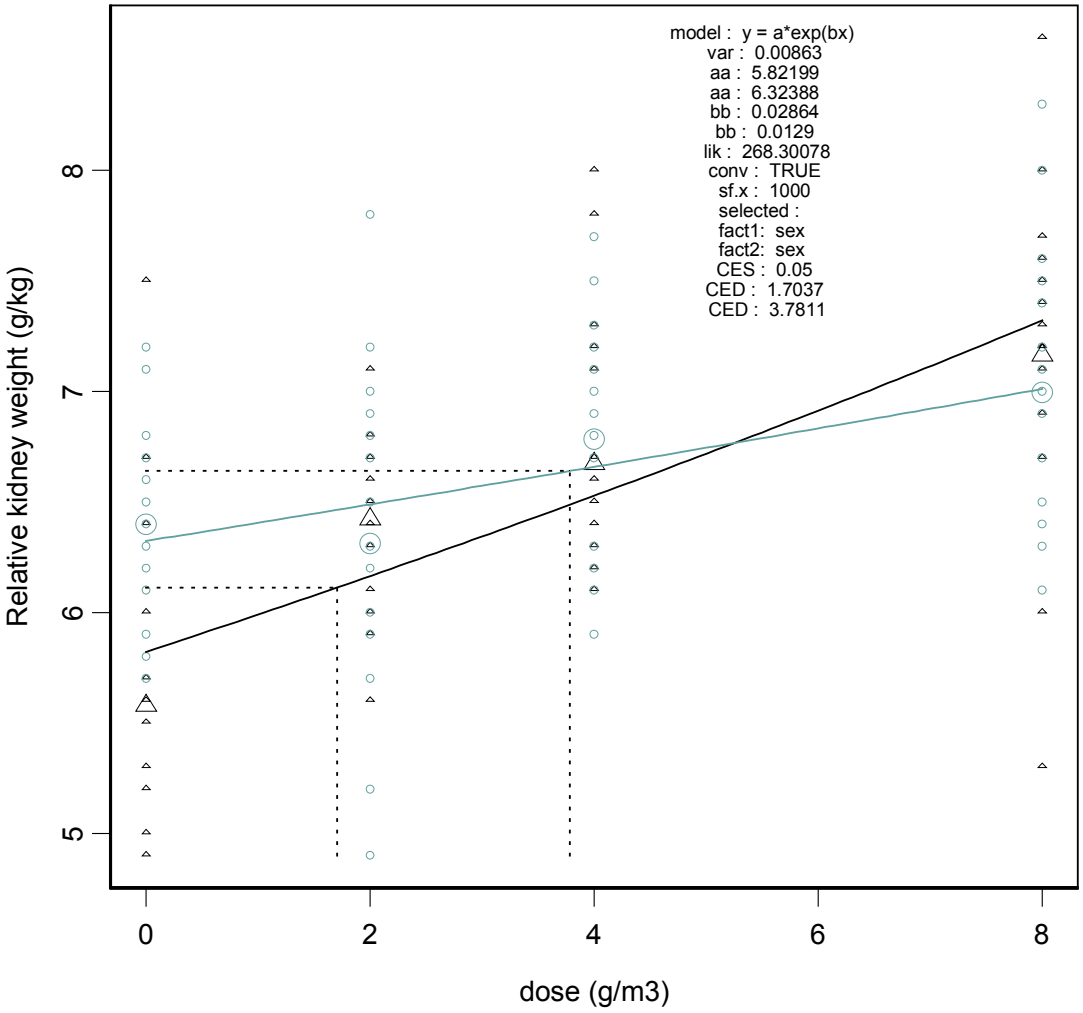

Figure 5B.5 Relative kidney weights as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

Appendix 5B. (White Spirits, continued)

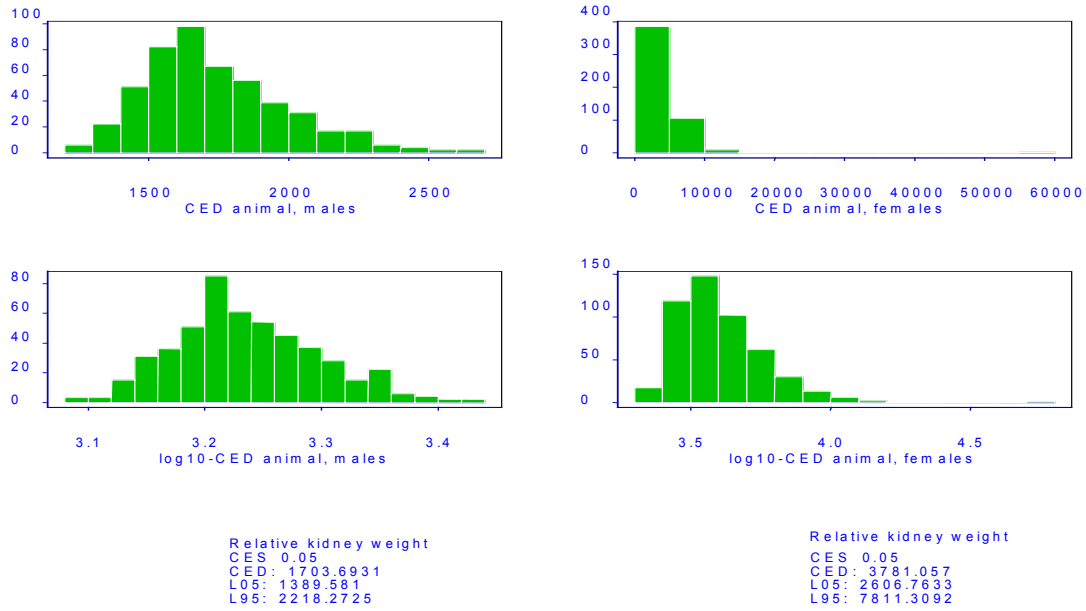

Figure 5B.6 Uncertainty distribution around the CED05 for increased relative kidney weights obtained by 500 bootstrap runs

Appendix 5B. (White Spirits, continued)

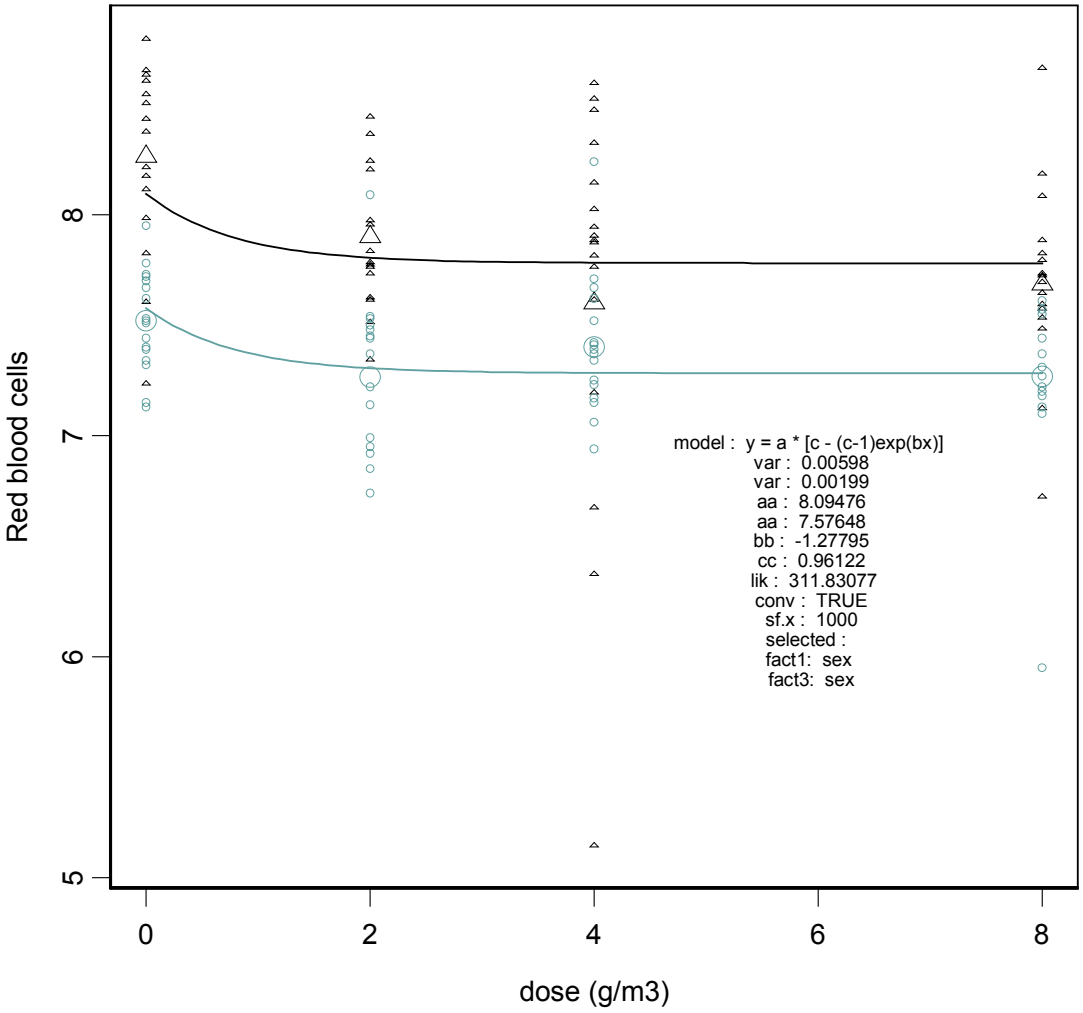

Figure 5B.7 Red blood cell counts as a function of dose for males (triangles) and females (circles). Larger marks denote the geometric group means.

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