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## Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters

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### Abstract

The CONTAM Panel updated the assessment of the risks for human health related to the presence of 3-monochloropropane diol (3-MCPD) and its fatty acid esters in food published in 2016 in view of the scientific divergence identified in the establishment of the tolerable daily intake (TDI) in the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (FAO/WHO) report published in 2017. In this update, dose–response analysis was performed following the recent EFSA Scientific Committee guidance on the use of benchmark dose (BMD) approach in risk assessment, and a review of available data on developmental and reproduction toxicity was included. The outcome of this review indicates that in rats short-term exposure to 3-MCPD above 1 mg/kg body weight (bw) per day can induce reduced sperm motility associated with reduced male fecundity. Decreased sperm count and histopathological changes in the testis and epididymis were observed following longer treatment periods at higher doses. Regarding increased incidence kidney tubular hyperplasia, BMD analysis using model averaging resulted in a BMDL<sub>10</sub> of 0.20 mg/kg bw per day in male rats, which was selected as the new Reference Point (RP) for renal effects. For the effects on male fertility, decreased sperm motility was selected as the most sensitive relevant endpoint and a BMDL<sub>05</sub> of 0.44 mg/kg bw per day was calculated. The RP for renal effects was considered to derive an updated group TDI of 2 µg/kg bw per day for 3-MCPD and its fatty acid esters and was considered protective also for effects on male fertility. The established TDI of 2 µg/kg bw per day is not exceeded in the adult population. A slight exceedance of the TDI was observed in the high consumers of the younger age groups and in particular for the scenarios on infants receiving formula only.

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## Summary

In 2016, the Scientific Opinion of the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) on the risks for human health related to the presence of 3- and 2-monochloropropane diol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food was published. These substances were subsequently assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2016, with some divergence primarily regarding the health-based guidance value derived for 3-MCPD. For 3-MCPD and its fatty acid esters, the CONTAM Panel established a tolerable daily intake (TDI) of 0.8 µg/kg body weight (bw) per day, whereas JECFA established a TDI of 4 µg/kg bw per day. This difference in hazard characterisation was due to methodological differences in the application of the benchmark dose (BMD) approach. In view of the recent EFSA Scientific Committee guidance on the use of BMD approach in risk assessment published in 2017, the CONTAM Panel considered that an update of its 2016 Scientific Opinion is warranted regarding 3-MCPD and its fatty acid esters in food. This update should include a review of the available data on developmental and reproduction toxicity of 3-MCPD and conclusion on the importance of these endpoints on the hazard characterisation of the substance.

A comprehensive search for literature was conducted for peer reviewed publications pertaining to identification and characterisation of reproductive and developmental effects of 3-MCPD and 3-MCPD fatty acid esters, resulting in the selection of 26 papers of which 12 were considered suitable for dose–response analysis.

Most of the animal studies with free 3-MCPD and 3-MCPD esters included in this review were carried out in rats. All studies except one were conducted using racemic 3-MCPD. Various exposure scenarios were tested, with the most sensitive effect being reduced epididymal sperm motility. Continued and higher exposures affected epididymis and testis and a range of related fertility parameters. The sperm motility effects were reversible in a few weeks after cessation of treatment. The available studies in combination lead to an overall no-observed-adverse-effect level (NOAEL) of 1 mg/kg bw per day. 3-MCPD fatty acid esters were tested in two studies and revealed histopathological effects in testis and epididymis at doses corrected for free-3-MCPD content above the NOAEL for free 3-MCPD.

The kidney and testis were found to be the main target organs for 3-MCPD-induced toxicity in animal studies. The toxic effects of 3-MCPD in both target tissues seem to be associated with its oxidative metabolism to β-chlorolactaldehyde and β-chlorolactic acid, which inhibit glycolytic enzymes and causes oxidative stress, leading to the toxicological manifestations observed in kidney and male reproductive system. BMD modelling of renal tubular cell hyperplasia applying the new EFSA Scientific Committee guidance on the use of BMD model averaging, based on the set of models recommended by the guidance and excluding additional parameter constraints, was considered as the preferable approach for the selection of the Reference Point (RP). Tubular hyperplasia was reconfirmed as the key effect in kidneys of rats and a BMDL<sub>10</sub>–BMDU<sub>10</sub> interval for 3-MCPD of 0.20–1.95 mg/kg bw per day was obtained using model averaging. The BMDL<sub>10</sub> using model averaging is about two folds higher than the one derived in the previous EFSA opinion (0.08 mg/kg bw per day). The different outcome of the BMD analyses between JECFA and the European Food Safety Authority (EFSA) using the same data sets on male rat kidney hyperplasia was due to the initial selection of models and parameter constraints applied.

The CONTAM Panel also reviewed available dose–response data on male fertility parameters. A decrease in the percentage of motile sperm was observed in several independent studies in rats (mainly Sprague–Dawley) exposed for 1–2 weeks to 3-MCPD doses ranging from 1 to 30 mg/kg bw per day. A study was selected for BMD modelling, in which a dose-related decrease in the percentage of motile sperm was observed, with concurrent decreases in a series of sperm motility parameters, of which curvilinear velocity (VCL) was considered the most sensitive relevant endpoint. BMD modelling of sperm VCL resulted in a BMDL<sub>05</sub>–BMDU<sub>05</sub> confidence interval of 0.44–3.88 mg/kg bw per day.

3-MCPD seems to have minor effects on sperm count following short-term exposure (2 weeks or less) at doses up to 10–30 mg/kg bw per day. However, exposure of rats for 13 weeks resulted in a dose-dependent decreasing trend of epididymal sperm count, achieving statistical significance at ≥ 4 mg/kg bw per day. Because of high variance of the data (relative standard deviation ranging from 20% to 50%) and fertility being rather insensitive to loss of sperm count in rats, the CONTAM Panel decided to apply one standard deviation of the control group (equivalent to an effect size of 23%) as a benchmark response (BMR). The BMDL<sub>23</sub> of 1.34 mg 3-MCPD/kg bw per day was determined for decrease in rat sperm count.

The loss of sperm in epididymis was generally associated with histopathological changes. BMD analysis of two data sets was carried out applying model averaging and resulted in  $BMDL_{10}$ – $BMDU_{10}$  confidence intervals of 1.55–14.4 mg 3-MCPD/kg bw per day for the chronic increased incidence in seminiferous tubular atrophy, and 1.34–8.50 mg 3-MCPD/kg bw per day for the increased vacuolisation of the epididymal epithelium.

A single study was identified in which potential developmental effects of 3-MCPD were considered. No fetal toxicity, macroscopic malformations, histopathological changes in the testis, or reduced body weights were observed in fetuses from all treatment groups. Although this study has some limitations, it does not suggest a concern towards possible developmental effects of 3-MCPD at doses at which renal and male fertility effects are observed.

Although effects on kidney and male reproductive system start to appear at similar doses, the RP for renal tubule cell hyperplasia was the lowest (0.20 mg 3-MCPD/kg bw per day). It is therefore considered protective also for effects on the male reproductive system and was selected as the critical effect caused by chronic exposure to 3-MCPD and its fatty acid esters. The critical effect is considered to cover any 3-MCPD-related additional cancer risk as well. For the protection of human health from adverse effects of 3-MCPD, the CONTAM Panel applied an overall uncertainty factor of 100 to the selected reference point (0.20 mg/kg bw per day) to account for intraspecies and interspecies differences, and derived an updated group TDI of 2 µg/kg bw per day for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).

Dietary exposure assessment to 3-MCPD and its esters was performed in the previous EFSA opinion of 2016 considering exposure levels to the parent compound, regardless of the original form. The mean and high (P95) chronic dietary exposure estimates were higher for the younger age groups (infants, toddlers and children up to 10 years of age) in comparison to adolescents and the adult age groups. Mean exposure levels (reported as minimum lower bound–maximum upper bound) ranged from 0.5 to 1.5 µg 3-MCPD/kg bw per day across the dietary surveys for the younger age groups, and from 0.2 to 0.7 µg 3-MCPD/kg bw per day in adolescents and adult age groups. High exposure levels ranged from 1.1 to 2.6 µg 3-MCPD/kg bw per day in the younger age groups, and from 0.3 to 1.3 µg 3-MCPD/kg bw per day in adolescents and adults age groups. Specific scenarios were considered for infants receiving formula only, based on mean and P95 occurrence of 3-MCPD in this products. The respective exposure estimates were 2.4 and 3.2 µg 3-MCPD/kg bw per day.

The CONTAM Panel noted that the established TDI of 2 µg/kg bw per day is not exceeded in the adult population (mean and high exposure levels). A slight exceedance of the TDI was observed in the high consumers of the younger age groups and in particular for the scenarios on infants receiving formula only.

Regarding the exposure assessment, the CONTAM Panel confirmed the uncertainties already identified in the 2016 opinion. Additionally, a general lack of data on the developmental and neurodevelopmental effects of 3-MCPD was noted, in particular in infants and juveniles (*ex utero* development). Also, existing chronic studies did not cover adequately male reproductive toxicity and fertility parameters; therefore, there is uncertainty on the long-term effects of 3-MCPD on these endpoints. The exposure assessment most likely underestimated the exposure. Overall, the CONTAM Panel concluded that the impact of the uncertainties on the risk assessment is high.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

In May 2016 the Scientific Opinion of the CONTAM Panel on the risks for human health related to the presence of 3- and 2-monochloropropane diol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food was published (EFSA CONTAM Panel, 2016). These substances were subsequently assessed by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) at their 83rd meeting (8–17 November 2016).

Following the publication of the JECFA meeting summary report JECFA on 23 November 2016 (FAO/WHO, 2016), the CONTAM Panel identified a scientific divergence regarding the risk assessments of 3-MCPD and its fatty acid esters and glycidyl fatty acid esters. While there is a substantial alignment of the two scientific bodies in the identification of the hazards for 3-MCPD and its fatty acid esters and glycidyl fatty acid esters, the divergence consists mainly in the dose-response analyses performed, due to methodological differences in the application of the benchmark dose (BMD) approach. For glycidyl fatty acid esters, the CONTAM Panel, considering the limitations in the design of the pivotal study identified for hazard characterisation, and in view of the high uncertainties associated with the BMD analysis of the aforementioned study dataset, derived a T25 of 10.2 mg/kg bw per day as reference point for the application of the Margin of Exposure (MOE) approach in compliance with the 2005 opinion of the EFSA Scientific Committee (EFSA, 2005). JECFA considered the BMD approach applicable also in the case of glycidyl fatty acid esters. Despite the different approaches used for dose-response analysis, the outcome of the risk assessments on glycidyl fatty acid esters was largely consistent between JECFA and the CONTAM Panel. For 3-MCPD and its fatty acid esters, the CONTAM Panel established a Tolerable Daily Intake (TDI) of 0.8 µg/kg bw per day, whereas JECFA established a TDI of 4 µg/kg bw per day. This difference has an important impact in the outcome of the risk assessment performed by the two bodies. In addition to the differences in the BMD approach, a different interpretation of the weight of evidence of the available information on reproductive and developmental toxicity of 3-MCPD was noted, leading JECFA to apply an extra assessment factor of 2 for the derivation of the TDI to take the inadequacies in the studies of reproductive toxicity into account.

Following a detailed analysis of the divergence, an exchange of views with the EFSA Scientific Committee working group on BMD and consultation with the EFSA Scientific Committee, the CONTAM Panel considers that an update of its Scientific Opinion is warranted in view of the updated guidance of the EFSA Scientific Committee on the use of benchmark dose approach in risk assessment (EFSA Scientific Committee, 2017), in particular to address the divergence identified for the assessment of 3-MCPD and its fatty acid esters.

With regard to the different dose-response approaches used for glycidyl fatty acid esters, it was noted that the large uncertainties due to the limitations in the available experimental data cannot be reduced by application of the new BMD guidance. It was furthermore noted that an adequate refinement of the dose-response analysis can only be achieved with the availability of a carcinogenicity study conducted with an appropriate design.

The following Terms of Reference (TORs) are considered:

- to review the dose-response analysis carried out for 3-MCPD and its fatty acid esters, considering the Scientific Committee guidance on the use of BMD in risk assessment;
- to review the available data on developmental and reproduction toxicity of 3-MCPD and conclude on the importance of these endpoints on the hazard characterisation of the substance;
- if relevant, based on the outcome of the previous tasks, to review the establishment of Health Based Guidance Value, as well as risk characterisations for 3-MCPD and its fatty acid esters; and
- to update the relevant sections of the opinion as adequate depending on the outcome of the previous tasks.



## 2. Data and methodologies

### 2.1. Methodologies

#### 2.1.1. Literature search and appraisal of studies

A comprehensive search for literature was conducted for peer reviewed publications pertaining to identification and characterisation of reproductive and developmental effects of 3-MCPD and 3-MCPD fatty acid esters. A strategy was developed for the literature search and selection of relevant publications. The scope was to focus on studies allowing for the performance of dose–response analysis in a relevant dose range close to that at which renal toxicity was observed. This corresponds to the ‘low-dose’ range for male fertility effects identified in the previous opinion (EFSA CONTAM Panel, 2016).

##### 1) Literature search

- a) Consulted databases: Embase, PubMed and Web of Science
- b) Keywords: Free text search (title and abstract):  
reproduct\*, reprotox\*, development\*, embryo\*, fetus, foetus, fetal, foetal, fertility, infertility, sterility, antifertility, sperm, abnormalit\*, teratogen\*, malformation\*, prenatal exposure, prenatal disorder\*, abortion\*, miscarriage\*, resorption, gestation\*, pregnan\*
- c) MESH/Emtree terms:

|                   | Mesh terms  | Emtree terms  |
|-------------------|---|---|
| <b>Anatomy</b>    | "Urogenital System"[Mesh]   | `genital system'/exp  |
|                   | "Embryonic Structures"[Mesh]: <i>includes embryo, fetus</i>   | `embryonic and placental structures'/exp<br>`animal embryo'/exp   |
| <b>Physiology</b> | "Reproductive Physiological Phenomena"[Mesh]:<br><i>includes fertility, reproduction</i>            | `reproduction'/exp: <i>includes mother fetus relationship</i><br>`genital system function'/exp                              |
|                   | "Developmental Biology"[Mesh]: <i>includes embryology and teratology</i>                            | `developmental biology'/exp<br>`prenatal development'/exp   |
|                   | "Growth and Development"[Mesh]  | `prenatal growth'/exp   |
| <b>Pathology</b>  | "Maternal-Fetal Relations/drugs effects"[Mesh]  |   |
|                   | "Infertility"[Mesh]   | `infertility'/exp   |
|                   | "Pregnancy Complications"[Mesh]: <i>includes: foetal disease; prenatal exposure delayed effects</i> | `pregnancy disorder'/exp<br>`prenatal disorder'/exp<br>`developmental disorder'/exp: <i>includes developmental toxicity</i> |
|                   | "Teratogens"[Mesh]  | `teratogenicity'/exp<br>`embryotoxicity'/exp  |
|                   | "Abnormalities, Drug-Induced"[Mesh]   | `congenital malformation'/exp   |

- d) No filter included for the year of publication

##### 2) Exclusion criteria

The following criteria were used to exclude publication, based only on the reading of titles and abstracts:

- a) *In vivo* studies in non-mammal species
- b) Route of administration: not oral
- c) Papers without English abstract
- d) Papers regarding human male contraception
- e) Conference abstracts, no full text available

The full texts of publications passing the first tier of exclusion were screened and publications not meeting the following criteria were excluded:

- f) At least two doses of 3-MCPD or 3-MCPD esters and a concurrent control group tested
- g) Lowest tested dose < 5 mg/kg body weight (bw) per day

### 3) Study appraisal

Publications passing the inclusion/exclusion phase were appraised by the members of the CONTAM Panel Working Group on 3-MCPD update for their final inclusion in the assessment, based on expert judgement. Any limitations in the information used are documented in this scientific opinion. Selection of scientific papers was based on consideration of the extent to which the study was relevant to the assessment and general consideration on the reliability of the study.

No systematic appraisal was performed for the identified review papers, which were used for the narrative description of the mode of action of 3-MCPD towards the reproductive system.

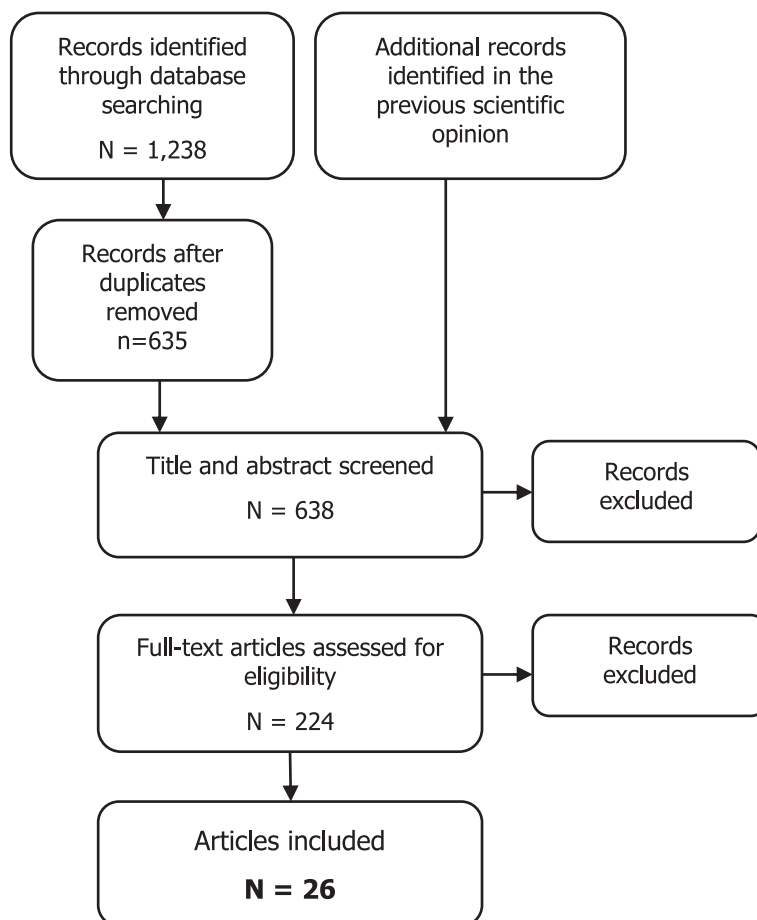
#### 2.1.2. Methodology applied for risk assessment

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO/IPCS (2012), which include hazard identification and characterisation, exposure assessment and risk characterisation. Additionally to the principles described by WHO/IPCS (2012), EFSA guidance pertaining to risk assessment has been applied for the present assessment (see Appendix B).

## 2.2. Data

### 2.2.1. Outcome of literature search

The applied literature search and inclusion/exclusion strategy resulted in the selection of 26 publications. The numbers of initially retrieved hits and of publication excluded in the phases described in Section 2.1.1 is described in Figure 1.



**Figure 1:** Flow chart describing the number of publications retrieved and subsequent selection applying the search and the exclusion criteria detailed in Section 2.1.1



The full texts of the 29 articles meeting the agreed inclusion criteria were appraised for their reliability and relevance to the risk assessment. Twelve articles were considered as suitable for the dose–response analysis. The list of the selected 29 papers is reported in Appendix A.

### 3. Assessment

#### 3.1. Reproductive and developmental toxicity

##### 3.1.1. Free 3-MCPD

Vickery et al. (1974) used a number of study designs in terms of dose levels, timing and duration of exposure and parameters to assess the effects of 3-MCPD on male reproductive organ, sperm and fertility parameters. Groups of five male SD rats (300–350 g) were treated for 21 days to 4 months by daily oral gavage with 0, 0.2, 1.0, 2.5, 5.0, 10 or 25 mg/kg bw, or 1.25, 2.5, 5 or 10 mg 3-MCPD/kg bw. They studied fertility by mating and implantation data, uterine fertilised egg count, uterine sperm counts after mating, and histopathology of male reproductive organs. Following the first dosing regimen, there was an overall no-observed-adverse-effect level (NOAEL) of 1 mg/kg bw per day and a lowest-observed-adverse-effect level (LOAEL) of 2.5 mg/kg bw per day as to fertility determined by mating directly after 21 days exposure. Number of fertilised eggs was zero at the LOAEL of 2.5 mg/kg bw per day directly after a 3-week exposure. Male pituitary weight was reduced after a 3-week exposure to 0.2, 1.0 and 2.5 mg/kg bw per day but not at higher doses. After the second dosing regimen, there was no NOAEL for recovery of uterine fertilised eggs after mating, with a LOAEL of 1.25 mg/kg bw per day. The number of motile sperm flushed from oviduct and uterus after mating was reduced with a LOAEL of 2.5 mg/kg bw per day and a NOAEL at 1.25 mg/kg bw per day. Fertility returned to normal 2 weeks (2.5 mg/kg bw per day) or 4 weeks (10 mg/kg bw per day) after the last dose after 4 months dosing. Pituitary weight was increased at all doses after cessation of 4 month treatment.

Hoyt et al. (1994) treated groups of 10 adult male SD rats (16 weeks of age) with 0, 1, 5 or 25 mg/kg bw 3-MCPD by gavage over 14 days. At day 15, five animals from each group were cohabitated with two virgin female rats (9 weeks of age) each for 14 days and then sacrificed at day 29. The five other male animals were sacrificed at day 15. At a dosage of 25 mg/kg bw, the authors found increased testicular weights at day 15 and changes in testicular cell ploidy at days 15 and 29 combined. A significant decrease in mean sperm concentration was recorded at 25 mg/kg bw per day both at day 15 and 29. Sperm motility was decreased at 5 mg/kg bw, and changes were observed in all other sperm parameters tested at 25 mg/kg bw. Fertility was lost at dose levels of 5 and 25 mg/kg bw. These data indicate a NOAEL of 1 mg 3-MCPD/kg bw per day for effects on male fertility and reproductive functions in rats.

Yamada et al. (1995) treated 7-week-old Crj:CD(SD) rats for 2 weeks by daily oral gavage with 0, 2, 8 mg/kg bw 3-MCPD. One additional 8 mg/kg group was treated for 4 weeks. A number of 19, 7 and 19 rats were used for exposure in each group, respectively, and 14, 7 and 14 rats were selected for subsequent mating. Parameters assessed included motility of epididymal sperm, sperm counts and sperm morphology, and male reproductive organ histopathology. Fertility was assessed by mating and fertility indices and mid-gestation implantation data. Full recovery was observed 2 weeks after last dosing. In both the 2 and 8 mg/kg bw per day groups, sperm motility was decreased immediately after last dosing (with infertility at 8 mg/kg), but both parameters returned to normal 2 weeks later. Other sperm parameters (number, viability, maturation) were unaffected. No effects were observed on prostate and epididymis weights and histopathology. The authors identified an overall LOAEL of 2 mg/kg for sperm motility and fertility immediately after last dosing. No NOAEL could be determined in this study.

Effects of oral exposure to 3-MCPD on fertility in male rats were investigated as part of a consortium effort to determine the optimal period and parameters for detection of male fertility disorders in rats (Takayama et al., 1995). Male Sprague–Dawley rats (N = 7, unknown age and body weight at the start of the test) were dosed with 3-MCPD at 0, 2 or 8 mg/kg bw per day by oral gavage for 2 weeks. At the end of the exposure period, the males were mated with untreated females. Males were then killed for examination. There were no effects on reproductive organ weight and no histopathological changes were observed in the testis and epididymis. Sperm numbers, viability and maturation were unchanged, but sperm activity in the 2 and 8 mg/kg bw per day group, and sperm motility in the 8 mg/kg bw per day group were decreased. Treatment of males with 8 mg/kg bw per

day for 2 weeks resulted in complete infertility without affecting the copulation rate. Full restoration of sperm motility, sperm activity and fertility was observed after 2 weeks of recovery from 3-MCPD treatment.

The relationship between male fertility and loss of sperm motion as a result of oral 3-MCPD exposure was investigated in Sprague–Dawley rats (Crj:CD) (12 weeks of age) (Ban et al., 1999). Four experiments were carried out with 3-MCPD, given to males by gavage daily for 9 days at doses of 0, 1, 3 and 10 mg/kg bw per day (Experiments 1 and 2) or 0, 3 and 10 mg/kg bw per day (Experiments 3 and 4). In the first experiment, 15 males were mated with untreated females. Pregnant dams were killed on day 15 of gestation, and the number of corpora lutea and implants examined. Treatment with 3-MCPD had no effect on copulation. The fecundity was 100% in the control and 1 mg/kg per day groups, but fell significantly to 60% and 0% in the 3 and 10 mg/kg bw per day groups, respectively. In experiment 2, 3-MCPD-treated males (N = 12–13) were killed the day following the final dose and cauda epididymis removed and prepared for histology and sperm count. Sperm were retrieved from vas deferens and analysed for sperm motions using an automated analyser. Exposure to 3-MCPD had no effect on weight of cauda epididymis, testes or whole body. There were also no effects of 3-MCPD on epididymal sperm numbers or histopathology. In contrast, the percentage of motile sperms was reduced in the 10 mg/kg bw per day group, and curvilinear velocity and amplitude of head displacement were decreased at  $\geq 3$  mg/kg bw per day. Experiment 3 was carried out to investigate if the reduced sperm motion caused by 3-MCPD exposure influenced the ability of sperm to reach the oviduct. Male rats (N = 8–12 per group) were mated with untreated females after the final dose. There was a dose-dependent reduction in the number of sperms collected in the oviducts, to less than 1% of controls in females mated with males exposed to 10 mg/kg bw per day. A fourth experiment was carried out to study the fertilisation in the oviducts of females mated with males (N = 11–12 per group) exposed to 3-MCPD. The percentage of fertilised eggs decreased significantly from 95% in females mated with control males, to 29 and 4% in males mated with males exposed to 3-MCPD at 3 and 10 mg/kg bw per day, respectively. There was no histopathological effect in testes or epididymis up to the highest dose. These data indicate a NOAEL of 1 mg 3-MCPD/kg bw per day, based on dose-dependent decrease in sperm motility and consequential decrease in fecundity observed at 3 and 10 mg 3-MCPD/kg bw per day.

Mineshima et al. (2000) reports on a ring study involving 11 laboratories, comparing five different rat sperm motility test methods. Using 3-MCPD as a test compound affecting epididymis sperm motility, 10–15-week-old male rats, in most labs of the Crj:CD strain, were orally treated for 1 week (in one lab for 2 weeks) with dose levels of 2.5, 5 and 10 mg/kg bw per day. Four to ten animals per dose group were treated. Sperm parameters assessed included motility of epididymal sperm, sperm counts and sperm morphology. Male fertility was assessed by 1:1 mating with females, copulatory plug observation and midterm observation of fertility index and litter data. There was an overall LOAEL of 2.5 mg/kg for motility (1 week exposure) and fertility, with fertility reduced to 10% only after 2 weeks exposure. At 5 mg/kg bw per day after 1 week exposure, motility was affected and fertility reduced to 0–30%; at 10 mg/kg bw per day after one week exposure, motility was affected and fertility reduced to 0%. No NOAEL could be determined from this study.

The effects of oral exposure to 3-MCPD on sperm motility and fecundity was studied in Sprague–Dawley rats (Crj:CD) as a part of a larger investigation into the performance of an automated sperm motion analyser (Ban et al., 2001). The study included experiments previously published by Ban et al. (1999) and Kaneto et al. (1999). Importantly, this study also looked at the recovery of male sperm motility and other fertility variables up to 4 weeks following the last dose. Oral gavage of male rats to 0, 3 or 10 mg 3-MCPD/kg bw per day for 9 days resulted in the reproductive effects described by Ban et al. (1999). After a 2-week recovery period of the high-dose group, mobility of sperm collected from vas deferens and fecundity metrics started to recover and all variables measured were back to control levels after 4 weeks of recovery. In a separate experiment, male Sprague–Dawley rats (Crj:CD) were dosed with 0 or 16 mg 3-MCPD/kg bw per day for 2 weeks and sperm were collected from vas deferens after 1 day, 3 days, 3 weeks and 4 weeks following the last dose. One day after the last dose, percentage motile sperm and almost all parameters used to describe sperm mobility were decreased. After recovery for 3 and 4 weeks, all sperm mobility values were completely restored. In addition to confirming previous published results by Ban et al. (1999) and Kaneto et al. (1999), this study importantly shows complete reversibility within 4 weeks of the effects of 3-MCPD on sperm motility caused by short-term (2 weeks) exposure up to 16 mg/kg body weight per day (the highest dose tested).

Li et al. (2003) treated male SD rats (22 animals per group, unknown age and body weight at the start of the test) with 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 or 16.0 mg 3-MCPD/kg bw per day by gavage over 90 days. A decrease in epididymis sperm count was found at the end of the study in the 4.0 mg/kg bw group and at higher dose levels. Sperm viability was reduced at 8.0 mg 3-MCPD/kg bw per day and above. The authors reported histopathological evidence for decreases in the number of mature sperm in the epididymal duct in rats exposed to 8 and 16 mg/kg bw per day. Testicular lactate dehydrogenase-X activity was decreased at 8.0 mg/kg bw per day and above. In comparison, adverse renal effects (increase in relative kidney weight, increase in urinary *N*-acetyl- $\beta$ -glucosidase activity) were found at 4.0 mg 3-MCPD/kg bw per day and above. From these data, a NOAEL of 2.0 mg/kg bw per day could be derived for adverse effects of 3-MCPD on both rat testis and kidney after 90 days.

A subchronic study in B6C3F1 mice was performed by Cho et al. (2008a). Ten mice/sex per group were exposed to 0, 5, 25, 100, 200 or 400 mg 3-MCPD/kg bw per day in drinking water over a 13-week period. Average doses of 0, 0.94, 4.59, 18.05, 36.97 or 76.79 mg/kg bw per day and 0, 0.79, 3.94, 15.02, 32.23 or 61.34 mg/kg bw per day were calculated by the study authors for males and females, respectively. Animals were checked daily for clinical signs of discomfort, morbidity and mortality and body weight. Food and water consumption were recorded on a weekly basis during the exposure period. Haematology and blood chemistry analyses were performed at the end of the exposure period. Sample of vaginal fluid and cells were collected for 12 consecutive days before the sacrifice and cellular populations were studied to determine the oestrus cycle stage. Testis and epididymis were weighed and the cauda epididymis was isolated and analysed for sperm motility. A complete necropsy of the sacrificed animals was carried out and histopathology analyses were performed at the end of the exposure period. No changes in testis and ovary weights were observed in mice treated with 3-MCPD compared to the control groups. Decreased sperm motility was observed only at 76.8 mg 3-MCPD/kg bw per day. An increase in mean length of the oestrus cycle was observed in all treatment groups without a clear dose–response trend, achieving statistical significance at 61.3 mg 3-MCPD/kg bw per day. An increase in incidence and severity of germinal epithelium degeneration was observed in the testes of animals exposed to  $\geq 4.59$  mg 3-MCPD/kg bw per day, achieving statistical significance at  $\geq 37.0$  mg 3-MCPD/kg bw per day. No significant changes were observed in ovaries in any treatment group. Overall, a NOAEL of 0.94 mg/kg bw per day for reproductive effects can be identified from this study.

Groups of SD rats (50 rats/sex per dose group, 4 weeks old at the beginning of the study) were exposed over a 2-year period to 0, 25, 100 or 400 mg 3-MCPD/L in drinking water by Cho et al. (2008b). Average doses of 0, 1.97, 8.27 or 29.50 mg/kg bw per day and 0, 2.68, 10.34 or 37.03 mg/kg bw per day were calculated by the study authors for males and females, respectively. Animals were checked daily for clinical signs of discomfort, morbidity and mortality, and body weight, food and water consumption were recorded on a weekly basis during the exposure period. Histopathology analyses were performed at the end of the exposure period. The results of the study are reported in detail in EFSA CONTAM Panel (2016). Focussing on the effects relevant to reproduction, the following histopathological changes were observed in the male reproductive system: statistically significant increases in the incidence of seminiferous tubular atrophy and arteritis/periarteritis were reported at 1.97 mg/kg bw per day and above; incidence of epididymal atrophy was observed only at the highest tested dose; finally increased incidence of Leydig cell tumours was observed at 8.27 mg/kg bw per day, achieving statistical significance only at 29.5 mg/kg bw per day. Overall, a LOAEL of 1.97 mg/kg bw per day for reproductive effects can be identified from this study.

Zhang et al. (2012) treated groups of five adult male SD rats (initial bw of  $435 \pm 35$  g) with 0, 2.5, 5.0 or 10 mg (*S*)-3-MCPD/kg bw by daily gavage over 52 days. At the end of the study, sperm motility and sperm glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, adenosine triphosphate (ATP) and cyclic adenosine monophosphate (cAMP) levels were reduced at all dose levels including the lowest dose level, i.e. at 2.5 mg/kg bw. No NOAEL could be identified from this study, and a LOAEL of 2.5 mg/kg bw per day could be derived for adverse effects on sperm function in rats.

A dose–response study of 3-MCPD administered orally for 7 days to adult male Sprague–Dawley rats (10 weeks of age) was published by Kim et al. (2012). Three treatment groups were chosen 3, 10 or 30 mg 3-MCPD/kg bw per day, based upon previous *in vivo* mechanistic studies of male antifertility. (Jelks et al., 2001; Kawaguchi et al., 2004), and the rats killed on the last day of treatment. The study was focussed upon the epididymal toxicity of 3-MCPD and this tissue was slightly increased in weight at the end of treatment whereas no effects were seen upon the weights of the testis, seminal vesicle or prostate. There was no effect on the sperm content of the testis, but there were no data on epididymal sperm content, making a conclusion difficult regarding a potential effect in sperm numbers

in this study. Dose-dependent increases in histopathological effects in the epididymal epithelium were observed starting at the lowest dose.

### 3.1.2. 3-MCPD fatty acid esters

In a study commissioned by EFSA, the effects of 3-MCPD and 3-MCPD dipalmitate administered daily by gavage for 90 days to adult Wistar rats of both sexes were studied by Barocelli et al. (2011). The treatments were equimolar with respect to 3-MCPD (0.267, 0.067 or 0.017 mmol/kg bw per day) and the primary treatments were: (a) MCPD at 29.5, 7.37 or 1.84 mg/kg bw per day or (b) 3-MCPD dipalmitate at 156.75, 39.19 or 9.87 mg/kg bw per day. The highest 3-MCPD dose was selected from the carcinogenicity study of Cho et al. (2008b). The study provided a general clinical toxicological profile which included some male and female reproductive tissue toxicology but no developmental parameters. Twenty four hours after the last dose of either 3-MCPD or its dipalmitate ester there were no significant dose effects on the weights of either the gonads from either sex or male secondary sex organs. No other reproductive effects were reported in female rats. However, in male rats treated with the high dose of 3-MCPD dipalmitate (156.75 mg/kg bw per day), some rats showed moderate or total degeneration of the seminiferous tubules whilst others showed atrophy or necrosis of spermatogenic cells and Sertoli cells. In rats treated with the intermediate and low dose of 3-MCPD dipalmitate (39.19 and 9.78 mg/kg bw per day), mild degenerative changes of the spermatogenic epithelium were observed. Similar extensive pathological testis changes, including lymphomononuclear infiltrates in the epididymis, were seen in rats treated with high doses of 3-MCPD (29.5 mg/kg bw per day). Rats receiving the intermediate dose, 7.37 mg/kg bw per day of 3-MCPD, showed a mild decrease of spermatids and increased atrophy of spermatogenic and Sertoli cells in the seminiferous tubules. Low doses of 3-MCPD (1.84 mg/kg bw per day) caused some degenerative phenomena, with decreased spermatids density, slight atrophy of supporting cells and spermatogenic cells. Qualitative data were combined by the authors to give a total testis pathological score, which showed statistically significant changes only at high doses and effects were three times more severe after administration of 3-MCPD (29.5 mg/kg bw per day) than after administration of equimolar doses of 3-MCPD dipalmitate. The NOAEL and LOAELs could not be determined with any confidence using the changes in testicular pathology scores across the dose range used. The approach of producing total pathological scores in a complex tissue such as the testis is controversial, especially if the pathological markers do not reflect the same toxicological lesion, for example combining scores for Leydig cell hyperplasia and degenerative spermatogenic epithelium (Creasy et al., 2012). Additionally, it fails to take into account the incidence of a particular lesion within a treatment group, which ranged from being absent to severe. The CONTAM Panel agreed that the incidence data of a particular pathological change were more reliable when considering the dose effects of 3-MCPD or its ester. Some difficulties in interpretation of the scores below the qualitative value of 1 was encountered and resolved with the authors who provided a more detailed description.<sup>1</sup> Using, in particular, the incidence of a histological fall in testis spermatid/spermatozoa numbers a LOAEL for 3-MCPD could be identified at 1.84 mg/Kg bw per day, and for 3-MCPD dipalmitate at 9.78 mg/kg bw per day.

Onami et al. (2014) conducted a 13-week repeated dose study of three 3-MCPD esters administered by intragastric intubation to 6-weeks old male (weighing from 98 to 125 g) and female (from 82 to 104 g) F344 rats. 3-MCPD was used for reference and the esters chosen were 3-MCPD palmitate diester (CDP), 3-MCPD palmitate monoester (CMP) and 3-MCPD oleate diester (CDO). The doses used were; 3-MCPD (40 mg/kg bw per day) and the doses for 3-MCPD fatty acid esters were 14, 55 and 220 mg/kg bw per day for CDP, 8, 32 and 130 mg/kg bw per day for CMP and 15, 60 and 240 mg/kg bw per day for CDO. The doses being equimolar MCPD doses of 2.5, 10 and 40 mg/kg bw, respectively, and chosen with reference to the carcinogenicity of MCPD reported by Cho et al. (2008b). Treatment was 5 daily doses per week for 13 consecutive weeks. The authors conducted a general toxicological profile including blood, kidney, testis, liver, and included histopathology and clinical biochemistry. Testicular focal granuloma, unilateral or bilateral seminiferous tubular atrophy and aspermatogenesis were observed without clear dose response. In the initial segment of the epididymis, the sperm maturation and storage tissue, dose-dependent apoptotic cell death was noted, limited to the columnar epithelium of the initial segment. In rats treated with 3-MCPD and high-dose 3-MCPD fatty acid esters apoptosis was significantly increased as compared to the vehicle control group. The

<sup>1</sup> The following scoring system was indicated by the study authors: 0 within normal limits, 0.5 minimal, 1 slight, 2 moderate, 3 severe (A. Mutti and A. Corradi, personal communication, 2017).



figures showing histology demonstrated the presence of epididymal sperm. Based on apoptosis in the epididymal epithelium the NOAELs were estimated to be 55 mg/kg bw per day for CDP, 32 mg/kg bw per day for CMP and 60 mg/kg bw per day for CDO, equimolar to 10 mg 3-MCPD/kg bw per day. For comparison, based on changes in absolute and relative kidney weights, NOAELs were 14 mg/kg bw per day for CDP, 8 mg/kg bw per day for CMP and 15 mg/kg bw per day for CDO, equimolar to 2.5 mg 3-MCPD/kg bw per day in both males and females. The severity of toxicological effects in rats treated with either CDP, CMP or CDO for 13 weeks were almost equivalent to those with equimolar 3-MCPD.

### 3.1.3. Conclusions

Most of the animal studies with free 3-MCPD and 3-MCPD esters included in this review were carried out in rats. With the exclusion of one study using the (*S*)-3-MCPD (Zhang et al., 2012), all studies were conducted using racemic 3-MCPD. Various exposure scenarios were tested, with the most sensitive effect being reduced epididymal sperm motility. Continued and higher exposures affected epididymis and testis and a range of related fertility parameters. The sperm motility effects were reversible in a few weeks after cessation of treatment. The available studies in combination lead to an overall NOAEL of 1 mg/kg bw per day. Vickery et al. (1974), Hoyt et al. (1994) and Ban et al. (1999) showed a NOAEL of 1 mg/kg, whereas Li et al. (2003) reported a NOAEL of 2 mg/kg bw per day based on decreased sperm count. Other studies did not provide a NOAEL, the lowest doses tested representing LOAELs above 1 mg/kg bw per day (Yamada et al., 1995; Mineshima et al., 2000; Kim et al., 2012; Zhang et al., 2012). 3-MCPD fatty acid esters were tested in Barocelli et al. (2011) and Onami et al. (2014) and revealed histopathological effects in testis and epididymis at doses corrected for free-3-MCPD content above the NOAEL for free 3-MCPD.

## 3.2. Mode of action

The kidney and testis were found to be the main target organs for 3-MCPD-induced toxicity in animal studies. The molecular mechanisms of the toxic potential of 3-MCPD are not yet fully understood. Generally, the toxic effects of 3-MCPD in both target tissues were associated with its oxidative metabolism to  $\beta$ -chlorolactaldehyde and  $\beta$ -chlorolactic acid (Lynch et al., 1998).

### Renal toxicity

The inhibition of glycolysis by metabolites associated with the  $\beta$ -chlorolactate pathway was suggested as possible nephrotoxic mechanism of 3-MCPD.  $\beta$ -Chlorolactaldehyde has been shown to inhibit the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase (Jones and Porter, 1995). The impairment of the glycolytic pathway and energy production was supposed to contribute to the kidney damage. Additionally, the accumulation of oxalic acid (the degradation product of  $\beta$ -chlorolactic acid) in the kidney was also thought to contribute to the kidney toxicity of 3-MCPD (Jones et al., 1981).

In a metabolomics study in rats exposed to 3-MCPD (30 mg/kg bw per day for 40 days), urine galactosylglycerol was identified as a possible early biomarker for the effects of 3-MCPD exposure. The authors suggested that 3-MCPD disrupts the homeostasis of the lysosomal enzyme  $\beta$ -galactosidase in kidney, leading to decreased hydrolysis of galactosylglycerol to galactose and glycerol and elevation of galactosylglycerol in the urine (Li et al., 2010).

To further get insight into the molecular mechanisms of 3-MCPD toxicity, a 28-day oral toxicity study was conducted with rats. Animals were treated with either 3-MCPD (10 mg/kg bw) or an equimolar dose of or its dipalmitate ester. Alterations induced by 3-MCPD and its dipalmitate ester were characterised by two-dimensional gel-based proteomics analysis of liver, kidney and testis of the animals. This revealed an increased expression of the alcohol dehydrogenase (the enzyme postulated to be involved in the oxidative metabolism of 3-MCPD) as well as a downregulation of several glycolytic enzymes (e.g. triosephosphate isomerase, which has previously been shown to be inhibited by  $\beta$ -chlorolactaldehyde) in 3-MCPD and 3-MCPD dipalmitate treated animals. Furthermore, the cellular antioxidant protein DJ-1 was one of the most deregulated proteins in kidney, as well as in liver and testis of rats treated with 3-MCPD or its dipalmitate ester (Braeuning et al., 2015; Sawada et al., 2015, 2016). In a recent mechanistic study investigating the molecular effects of 3-MCPD on DJ-1, it was shown that the observed substance-induced deregulation of DJ-1 was due to a post-translational modification, a 3-MCPD-mediated oxidation of a conserved, redox-sensitive cysteine residue of the DJ-1 protein (Buhrke et al., 2017).

## Male reproductive toxicity

3-MCPD is considered to cause male reproductive toxicity at least partly through the inhibition of glycolysis enzymes by its metabolites with energy depletion as result (Ford and Waites, 1982; Jones, 1983; Kwack et al., 2004). The activity of all glycolytic enzymes in the epididymal and testicular tissue of rats was reduced following daily subcutaneous injections of 6.5 mg/kg bw 3-MCPD for 9 days (Kaur and Guraya, 1981). The inhibition of the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase by the 3-MCPD metabolite  $\beta$ -chlorolactaldehyde, was suggested as possible mechanism (Jones and Porter, 1995; Lynch et al., 1998).

Significantly decreased levels of total RNA and protein contents were observed in the testis and epididymis of rats that received 6.5 mg/kg bw 3-MCPD per day for 9 days. These changes were paralleled by increases in the concentrations of proteinase and ribonuclease, whereas the DNA content was unchanged (Kaur and Guraya, 1981). The spermatotoxic effect of 3-MCPD were also suggested to be mediated by reduction of  $H^+$ -ATPase expression and subsequent alteration of the pH level in the cauda epididymis, leading to a disruption of sperm maturation and acquisition of motility (Kwack et al., 2004). 3-MCPD inhibited progesterone production in R2C rat Leydig cells in a time- and dose-dependent inhibitory manner. Disruption of progesterone production induced by 3-MCPD was considered to be potentially related to the inhibition of the cAMP signal transduction cascade. Authors supposed that reduced cAMP levels interfere with the male steroidogenic capacity (Sun et al., 2013).

A comparative proteomic analysis, based on a 28-day oral toxicity study in male Wistar rats treated with 3-MCPD or equimolar 3-MCPD dipalmitate dose (see above) revealed a deregulation of several proteins associated to lipid metabolism, reproductive system disease and cancer in testes of treatment groups. Similarly to the kidney, the cellular antioxidant protein DJ-1 was identified to be one of the most deregulated proteins in testes of all treated animals (Sawada et al., 2015). At the molecular level, reactive oxygen species (ROS) formation with subsequent oxidation of the redox-sensitive cysteine residue of the DJ-1 protein was suggested to be involved in the development of 3-MCPD-mediated toxicity (Buhrke et al., 2017).

In summary, a general picture emerges in which 3-MCPD, after metabolism, inhibits glycolytic enzymes and causes oxidative stress, leading to the toxicological manifestations observed in kidney and male reproductive system.

### 3.3. Identification of critical effects and dose–response assessment

#### 3.3.1. Renal effects

##### 3.3.1.1. Updated BMD analysis

The CONTAM Panel confirmed the kidney to be one of the main target organs of 3-MCPD. The dose–response analysis of different renal effects observed in long-term toxicity studies on oral exposure to 3-MCPD in rats indicated the increased incidence of kidney tubular cell hyperplasia as the most relevant effect for the performance of dose–response analysis (EFSA CONTAM Panel, 2016 and FAO/WHO, 2017).

The CONTAM Panel reviewed the BMD modelling of tubular cell hyperplasia applying the new guidance on the use of BMD in risk assessment (EFSA Scientific Committee, 2017), in which the use of additional parameter constraints is not recommended. This resulted in the BMD confidence intervals reported in Table 1. The full details of the newly performed BMD modelling are reported in Appendices A–D.

**Table 1:** Benchmark dose (BMD) modelling of incidence of renal tubular cell hyperplasia in rats using the new EFSA guidance on BMD (EFSA Scientific Committee, 2017)

| Study                  | Sex | Overall BMDL <sub>10</sub> –BMDU <sub>10</sub> <sup>(a)</sup><br>(mg/kg bw per day) | BMDL <sub>10</sub> –BMDU <sub>10</sub> (BMD <sub>10</sub> ) applying<br>model averaging <sup>(b)</sup> (mg/kg bw per day) |
|------------------------|-----|---|---|
| Sunahara et al. (1993) | M   | 0.22–4.23   | 0.54–4.91 (1.47)  |
|                        | F   | 0.29–3.26   | 0.55–3.70 (1.15)  |
| Cho et al. (2008b)     | M   | 0.08–1.96   | 0.20–1.95 (0.68)  |
|                        | F   | 17.0–37.3   | 21–36 (28)  |

M: males; F: females; bw: body weight.

(a): Lowest BMDL<sub>10</sub>–highest BMDU<sub>10</sub> across acceptable models using the Akaike information criterion (AIC) method.

(b): Model averaging calculated using the Wheeler and Bailer (2008) software.



The CONTAM Panel noted that the modelling of tubular cell hyperplasia for male SD rats of the Cho et al. (2008b) study and male and female F344 rats from the Sunahara et al. (1993) study give consistent results and could in principle be used in a combined modelling, as already shown by Rietjens et al. (2012). However, female SD rats appear to be far less sensitive to the renal effects of 3-MCPD. As the possible biochemical reasons for this difference are unknown, the combined modelling of the data sets from the two studies was not considered as justified (data not shown).

Overall, the CONTAM Panel concluded that the model average BMDL<sub>10</sub>–BMDU<sub>10</sub> interval of 0.20–1.95 mg/kg bw per day calculated for the incidence of tubular cell hyperplasia in male SD rats from the Cho et al. (2008b) study provides an adequate RP of 0.20 mg/kg bw per day for the renal effects of 3-MCPD and molar equivalents of 3-MCPD fatty acid esters.

### 3.3.1.2. Assessment of the identified EFSA-JECFA divergence on BMD analysis

Methodological differences in the performance of the BMD modelling of data sets on tubular cell hyperplasia from the Sunahara et al. (1993) and Cho et al. (2008b) studies led to the selection of different reference points by the CONTAM Panel (EFSA CONTAM Panel, 2016) and JECFA (FAO/WHO, 2017). The CONTAM Panel applied models allowing for additional parameter constraints both with and without the default restrictions as available in the US Environmental Protection Agency BMDS software and selected the lowest acceptable BMDL<sub>10</sub> using the criteria outlined in the previous opinion of the EFSA Scientific Committee on the use of BMD (EFSA, 2009). JECFA applied default restrictions to models allowing for additional parameter constraints and selected the lowest BMDL<sub>10</sub> from acceptable models (i.e. non-statistically different from the full model using the likelihood ratio ( $p > 0.1$ )). In addition, JECFA compared the selected BMDL<sub>10</sub> with the model averaging estimates using the software of Wheeler and Bailer (2008) applied to 'all models, except the quantal-quadratic' (FAO/WHO, 2017). The outcome of the different modelling approaches are summarised in Table 2.

**Table 2:** Comparison of the results obtained from the BMD modelling of the incidence of renal tubular cell hyperplasia in rats by EFSA (EFSA CONTAM Panel, 2016) and JECFA (FAO/WHO, 2017)

| Study                  | Sex | EFSA selected BMDL <sub>10</sub> –BMD <sub>10</sub> (mg/kg bw per day) <sup>(a)</sup> | JECFA selected BMDL <sub>10</sub> –BMD <sub>10</sub> (mg/kg bw per day) <sup>(d)</sup> | JECFA BMDL <sub>10</sub> –BMD <sub>10</sub> applying model averaging (mg/kg bw per day) <sup>(d)</sup> |
|------------------------|-----|---|--|--|
| Sunahara et al. (1993) | M   | 0.22–1.20 <sup>(b)</sup>  | 1.08–1.64 <sup>(e)</sup>   | 1.74–2.47  |
|                        | F   | 0.29–0.83 <sup>(b)</sup>  | 1.30–1.89 <sup>(e)</sup>   | 1.60–1.96  |
| Cho et al. (2008b)     | M   | 0.077–0.54 <sup>(b)</sup>   | 0.87–1.21 <sup>(e)</sup>   | 0.89–1.29  |
|                        | F   | 14–27 <sup>(c)</sup>  | 14.4–23.5 <sup>(e)</sup>   | 20.4–28.0  |

M: males; F: females; bw: body weight.

(a): EFSA selected the lowest BMDL<sub>10</sub> using the statistical criteria outlined in EFSA (2009), using models both with and without additional parameter constraints.

(b): Gamma (unrestricted).

(c): Quantal-linear.

(d): JECFA applied additional parameter constraints to models allowing for them, and selected the lowest acceptable BMDL<sub>10</sub>.

In addition JECFA performed BMD analysis using the Wheeler and Bailer (2008) software for model averaging, only for comparison purposes.

(e): Log-logistic (restricted).

In the updated BMD analysis described in Section 3.3.1.1, the CONTAM Panel applied the Scientific Committee updated guidance on the use of BMD in risk assessment (EFSA Scientific Committee, 2017), and derived BMDL<sub>10</sub> using model averaging, based on a set of models recommended by the guidance and excluding additional parameter constraints (see Table 1). Also, this analysis resulted in somewhat different values from those obtained by JECFA, as can be observed by comparing the BMDL<sub>10</sub> and BMD<sub>10</sub> derived using model averaging in Tables 1 and 2. The different outcome of the BMD analyses was mainly due to the different views of the two organisations regarding the use of additional parameter constraints, and probably to some extent also to the different sets of BMD models applied. The BMDLs calculated by JECFA are within the BMDL–BMDU intervals calculated by EFSA using model averaging, suggesting a minor divergence from a scientific point of view.

### 3.3.2. Reproductive effects

#### Effects on male fertility

The CONTAM Panel reviewed available dose–response data on male fertility parameters. As noted previously, 3-MCPD can induce a range of effects in the male reproductive system. Most of the selected studies indicated the decreased sperm motility as the main effect caused by repeated exposure to 3-MCPD. Sperm motility was assessed using different methods, e.g. manual motility scoring, computer-assisted sperm analysis (CASA) or automated Sperm Quality Analyzer (SQA). Differences were noted also in the experimental protocols applied in different studies, including exposure duration and sperm sampling and incubation procedures. Mineshima et al. (2000) showed that different test methods and incubation protocols may influence the outcome of the experiment. Decreased sperm count was also identified as a possible endpoint for the dose–response assessment of the effects of 3-MCPD on male fertility. However, an inconsistent trend was observed in the results on sperm count reported in different studies. 3-MCPD seems to have minor effects on sperm count following short-term exposure (2 weeks or less) at doses up to 10–30 mg/kg bw per day. However, exposure of rats for 13 weeks resulted in a dose-dependent decreasing trend of epididymal sperm count, reaching statistical significance at  $\geq 4$  mg/kg bw per day (Li et al., 2003). Finally, histopathological analysis revealed changes in the male reproductive system, in particular in epididymis and seminiferous tubules, in some cases associated to the observed decrease in epididymal sperm count (see e.g. Li et al., 2003; Barocelli et al., 2011; Kim et al., 2012).

#### Sperm motility

A decrease in the percentage of motile sperm and sperm motility parameters was observed in several independent studies in rats (mainly Sprague–Dawley) exposed for 1–2 weeks to 3-MCPD doses ranging from 1 to 30 mg/kg bw per day. The application of different experimental procedures and techniques to measure the fraction of motile sperm resulted in a considerable variability in the outcome of the studies. The CONTAM Panel decided to limit the dose–response analysis to studies in which automated sperm analysis (CASA) was deployed, because a standardised technique was considered less subject to experimental bias. Out of the three studies using CASA identified in the literature search (Hoyt et al., 1994; Ban et al., 1999, 2001), the one from Ban et al. (1999) was selected for BMD analysis due to the higher statistical power of the study. In this study, a dose-related decline in percentage of motile sperm was observed, with concurrent decreases in a series of sperm motility parameters, including curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and radius of circular cells. VCL and ALH were the most sensitive parameters associated with decreased sperm motility. The Panel selected VCL for BMD modelling, since it is a key parameter to predict human male subfertility (e.g. Larsen et al., 2000; Youn et al., 2011).

BMD modelling resulted in a  $\text{BMDL}_{05}$ – $\text{BMDU}_{05}$  confidence interval of 0.44–3.88 mg/kg bw per day.

The details of the BMD analysis are reported in Appendix F.

#### Epididymal sperm count

A statistically significant decrease in epididymal sperm count was reported in a 13-week study (Li et al., 2003). The CONTAM Panel noted a substantial variability in the data (standard deviations ranging from 20% to more than 50% of the mean levels through the control and treatment groups), which is not unexpected for this type of data (see e.g. Zenick and Clegg, 1986). Furthermore, it is known that male rat fertility is not affected by small changes in sperm count and successful fertilisation occurs even in the presence of 90% depletion of sperm reserves (see e.g. Mangelsdorf et al., 2003; Perobelli et al., 2012). Overall, the CONTAM Panel did not consider the default benchmark response (BMR) of 5%, recommended by the EFSA guidance, as a relevant effect size for this parameter in rats and a higher BMR was justified from a biological point of view. The CONTAM Panel decided to apply one standard deviation of the control group (equivalent to an effect size of 23%) as recommended by US EPA (2012). The BMD modelling of epididymal sperm counts resulted in a  $\text{BMDL}_{23}$ – $\text{BMDU}_{23}$  interval of 1.34–4.25 mg 3-MCPD/kg bw per day (details of the BMD analysis reported in Appendix I). The  $\text{BMDL}_{23}$  of 1.34 mg 3-MCPD/kg bw per day was selected as reference point for the decrease in rat sperm count.

The details of the BMD analysis for this endpoint are reported in Appendix G.

## Histopathological changes

Dose-dependent decrease in number of spermatids and increased atrophy of spermatogenic cells and Sertoli cells were observed in rats exposed to 1.84 mg 3-MCPD/kg bw per day and above in a subchronic study, and similar effects were observed in rats exposed to 3-MCPD dipalmitate, starting at higher 3-MCPD equivalent doses (Barocelli et al., 2011). In view of the uncertainties in the reported data discussed in Section 3.1.2, no quantitative dose–response analysis could be performed on this data set. Increased incidence of seminiferous tubular atrophy was also reported by Cho et al. (2008b) in rats exposed to  $\geq 2$  mg/kg bw per day for two consecutive years. Increased incidence of vacuolisation of the epididymal epithelium was observed at 3 mg 3-MCPD/kg bw per day and above by Kim et al. (2012). BMD analysis of the data sets from Cho et al. (2008b) and Kim et al. (2012) was carried out applying model averaging and resulted in BMDL<sub>10</sub>–BMDU<sub>10</sub> confidence intervals of 1.55–14.4 mg 3-MCPD/kg bw per day for the chronic increased incidence in seminiferous tubular atrophy, and 1.34–8.50 mg 3-MCPD/kg bw per day for the increased vacuolisation of the epididymal epithelium.

The details of the BMD analyses for the histopathological changes are reported in Appendices H and I.

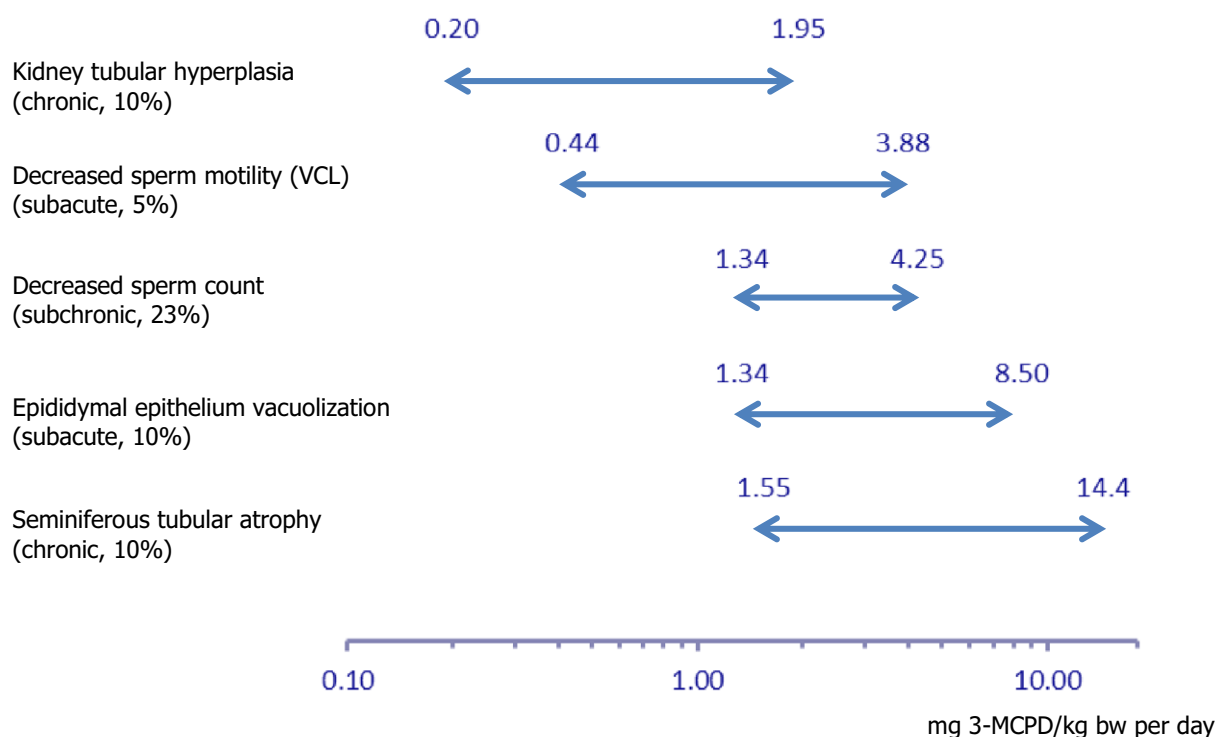
## 3.4. Derivation of health-based guidance values

The selected BMDL–BMDU intervals (in mg/kg bw per day) for the identified renal and fertility effects of 3-MCPD are shown in Figure 2. In accordance with the previous opinions by the CONTAM Panel (EFSA CONTAM Panel, 2016) and JECFA (FAO/WHO, 2017), tubular hyperplasia in kidneys of rats was reconfirmed as the critical effect. A BMDL<sub>10</sub>–BMDU<sub>10</sub> interval of 0.20–1.95 mg 3-MCPD/kg bw per day was obtained using model averaging of data from male SD rats from the study by Cho et al. (2008b).

Several parameters relating to male fertility were affected at 3-MCPD doses around 1 mg/kg bw per day. Sperm motility was the most sensitive effects on the male reproductive system in rats. BMD analysis of the VCL from the data set by Ban et al. (1999) in SD rats, resulted in a BMDL<sub>05</sub>–BMDU<sub>05</sub> interval of 0.44–3.88 mg/kg bw per day. Because of the large variance in epididymal sperm count data, a BMR of 23%, equalling one standard deviation of the control group, was used for this parameter. Applying this approach to BMD modelling of data from SD rats from a 90-day study by Li et al. (2003) resulted in a BMDL<sub>23</sub>–BMDU<sub>23</sub> interval of 1.34–4.25 mg 3-MCPD/kg bw per day. As discussed in Section 3.3.2, such a decrease in sperm count is not expected to have any effect on rat fertility). The effect on sperm count was supported by increased incidence of histopathological changes in epididymis and seminiferous tubules occurring at similar doses.

The terms of reference stipulated assessment of data on developmental effects of 3-MCPD and its fatty acid esters. No data on developmental effects were identified in the literature search performed in this opinion. In the previous opinion (EFSA CONTAM Panel, 2016), a single study was identified in which pregnant SD rats were exposed to 0, 5, 10 or 25 mg 3-MCPD/kg bw per day from gestational days 11.5 to 18.5 (El Ramy et al., 2006). No fetal toxicity, macroscopic malformations, histopathological changes in the testis, or reduced bw were observed in fetuses from any of the treatment groups. Although the study has some limitations (the exposure period did not cover the whole organogenesis and histopathology was confined to the testis), it does not suggest a concern towards possible developmental effects of 3-MCPD at doses at which renal and male fertility effects are observed.

Although effects on kidney and male reproductive system start to appear at similar doses, the RP for renal tubule cell hyperplasia was the lowest (0.20 mg/kg bw per day). This RP would correspond to less than 10% in reduction of epididymal sperm count and less than 5% in reduction of sperm motility. It is therefore considered to be protective also for effects on the male reproductive system and was selected as the critical effect caused by chronic exposure to 3-MCPD and its fatty acid esters. As discussed in the previous EFSA opinion on 3-MCPD and its esters, sustained hyperplasia might lead to cancer by a non-genotoxic action (EFSA CONTAM Panel, 2016). Therefore, taking into account the key event, i.e. renal cell hyperplasia, is considered to cover any 3-MCPD-related additional cancer risk as well. For the protection of human health from adverse effects of 3-MCPD, the CONTAM Panel applied an overall uncertainty factor of 100 to the selected reference point (0.20 mg/kg bw per day) to account for intraspecies and interspecies differences and derived an updated group TDI of 2 µg/kg bw per day for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).



Exposure duration and selected BMR are reported in brackets.

**Figure 2:** Comparison of selected BMDL–BMDU intervals (in mg/kg bw per day) for the identified key renal and fertility effects of 3-MCPD

### 3.5. Risk characterisation

In the previous opinion of the EFSA CONTAM Panel (2016), dietary exposure assessment to 3-MCPD and its esters was performed considering exposure levels to the parent compound, regardless of the original form. The mean and high (P95) chronic dietary exposure estimates were higher for the younger age groups (infants, toddlers and children up to 10 years of age) in comparison to adolescents and to the adult age groups. Mean exposure levels (reported as minimum lower bound–maximum upper bound) ranged from 0.5 to 1.5  $\mu\text{g}$  3-MCPD/kg bw per day across the dietary surveys for the younger age groups, and from 0.2 to 0.7  $\mu\text{g}$  3-MCPD/kg bw per day in the adolescents and adult age groups. High exposure levels ranged from 1.1 to 2.6  $\mu\text{g}$  3-MCPD/kg bw per day in the younger age groups, and from 0.3 to 1.3  $\mu\text{g}$  3-MCPD/kg bw per day in adolescents and adults age groups. Specific scenarios were considered for infants receiving formula only, based on mean and P95 occurrence of 3-MCPD in these products. The respective exposure estimates were 2.4 and 3.2  $\mu\text{g}$  3-MCPD/kg bw per day.

The CONTAM Panel noted that the TDI of 2  $\mu\text{g}$ /kg bw per day is not exceeded in the adult population (mean and high exposure levels). A slight exceedance of the TDI was observed in the high consumers of the younger age groups and in particular for the scenarios on infants receiving formula only.

### 3.6. Uncertainty analysis

Regarding the exposure assessment, the CONTAM Panel confirmed the uncertainties already identified in its 2016 opinion (EFSA CONTAM Panel, 2016). Additional uncertainties were identified in the hazard characterisation of 3-MCPD in particular related to its effect on male reproductive system. A lack of data on the developmental and neurodevelopmental effects of 3-MCPD was noted, in particular in infants and juveniles (*ex utero* development). In addition, existing chronic studies did not cover adequately male reproductive toxicity and fertility parameters; therefore there is uncertainty on the long-term effects of 3-MCPD on these endpoints.

In Table 3, a summary of the uncertainty evaluation for 3-MCPD, considering both the main uncertainties identified in the previous and present opinions, is presented. It highlights the main sources of uncertainty and indicates an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

The exposure assessment most likely underestimated the exposure. Overall, the CONTAM Panel concluded that the impact of the uncertainties on the risk assessment is high.

**Table 3:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to 3-MCPD identified in the present opinion and in the EFSA CONTAM Panel (2016) opinion

| Sources of uncertainty   | Direction <sup>(a)</sup> |
|--|--------------------------|
| Data from few sources and from a limited number of samples   | +/-                      |
| For some food groups not represented in the analytical data set occurrence data were imputed using a model | +/-                      |
| Missing occurrence data on some food groups  | -                        |
| Data on only one form (free or ester-bound) of 3-MCPD in samples that may contain both forms               | -                        |
| Use of occurrence data from a specific food group for a broader food group                                 | +/-                      |
| Consumption data from few dietary surveys  | +/-                      |
| Extent of hydrolysis of esterified forms of 3-MCPD   | +                        |
| Extrapolation from animal data for human risk assessment   | +/-                      |
| Conversion of glycidol to 3-MCPD may occur   | +/-                      |
| Lack of data on toxicity in infants and juveniles especially male <i>ex utero</i> development              | -                        |
| Lack of appropriate studies on long-term effects of 3-MCPD on male fertility                               | -                        |

(a): +: uncertainty with potential to cause overestimation of exposure/risk; -: uncertainty with potential to cause underestimation of exposure/risk.

## 4. Conclusions

- 3-MCPD induced a dose-dependent reduction in sperm motility, which was associated with a reduced ability to fertilise oocytes. This was reflected in a fall in fecundity measured by reduced litter size and eventually complete infertility. There was no effect on mating behaviour, and in general the weight of reproductive tissues was not changed.
- Effects on sperm motility could be seen in rats shortly after the start of daily treatment above 1 mg 3-MCPD/kg bw per day, and were completely reversed 2–4 weeks after treatment cessation.
- In the short term, there was no effect on spermatogenesis or sperm number at doses lower than 10 mg 3-MCPD/kg bw per day, but more prolonged treatment in rats below this dose was occasionally associated with reduction in sperm count and pathological changes in the testis and epididymis.
- Equimolar doses of fatty acid esters of 3-MCPD and free 3-MCPD produce similar effects.
- The updated guidance of the EFSA Scientific Committee on the use of BMD in risk assessment was used for the dose–response analyses for renal and male fertility effects. The resulting BMDLs indicated that oral exposure to 3-MCPD affects kidney and the male reproductive system at a similar dose range.
- Sperm motility (VCL) was reduced following 1–2 weeks of oral exposure to 3-MCPD with a BMDL<sub>05</sub>–BMDU<sub>05</sub> interval of 0.44–3.88 mg/kg bw per day.
- Because of large variance in epididymal sperm counts, a benchmark dose response of 23%, equal to one standard deviation of the control group, was selected for this parameter. The BMDL<sub>23</sub>–BMDU<sub>23</sub> interval for decrease in epididymal sperm count was 1.34–4.25 mg 3-MCPD/kg bw per day.
- Reduction in sperm count was supported by histological effects on epididymis and testes occurring at similar doses as those affecting sperm numbers.
- Renal tubular cell hyperplasia was reconfirmed as the critical effect in rats during chronic oral exposure to 3-MCPD with a BMDL<sub>10</sub>–BMDU<sub>10</sub> interval of 0.20–1.95 mg/kg bw per day using model averaging. The BMDL<sub>10</sub> derived using model averaging was about twofolds higher than the one derived in the previous EFSA opinion (0.08 mg/kg bw per day).



- The different outcome of the BMD analyses between JECFA and EFSA using the same data sets on male rat kidney hyperplasia was due to the initial selection of models and parameter constraints applied.
- The CONTAM Panel applied an overall uncertainty factor of 100 to the selected reference point (renal tubular cell hyperplasia; 0.20 mg/kg bw per day) to account for intraspecies and interspecies differences and derived an updated group TDI of 2 µg/kg bw per day for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).
- Mean chronic dietary exposure levels (reported as minimum lower bound–maximum upper bound) estimated for 3-MCPD and its fatty acid esters in the previous opinion of the EFSA CONTAM Panel of 2016 ranged from 0.5 to 1.5 µg 3-MCPD/kg bw per day across the dietary surveys for the younger age groups, and from 0.2 to 0.7 µg 3-MCPD/kg bw per day in the adolescents and adult age groups. High (P95) exposure levels ranged from 1.1 to 2.6 µg 3-MCPD/kg bw per day in the younger age groups, and from 0.3 to 1.3 µg 3-MCPD/kg bw per day in adolescents and adults age groups. Exposure levels of 2.4 and 3.2 µg 3-MCPD/kg bw per day were estimated in specific scenarios for infants receiving formula only, based on mean and P95 occurrence of 3-MCPD in this products, respectively.
- The CONTAM Panel noted that the established TDI of 2 µg/kg bw per day is not exceeded in the adult population (mean and high exposure levels). A slight exceedance of the TDI was observed in the high consumers of the younger age groups and in particular for the scenarios on infants receiving formula only.

## 5. Recommendations

- *In vitro* studies should be carried out on the comparison of adverse effects of 3-MCPD on human and rat sperm.
- Human pharmacokinetics studies should be performed for 3-MCPD and its esters, in particular multiple doses across a substantial time frame.
- Human data to confirm the possible effects of 3-MCPD should be generated, by developing reliable biomarkers of exposure and effects, and conducting epidemiological studies both in the general population and in susceptible population subgroups (e.g. to look for association between low male fertility and high intake of 3-MCPD).
- More data should be generated on fertility effects following long-term exposure to 3-MCPD, and on developmental, neurodevelopmental and juvenile toxicity.

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## Abbreviations

|        |   |
|--------|---|
| 3-MCPD | 3-monochloropropane-1,2-diol                            |
| AIC    | Akaike information criterion                            |
| ALH    | amplitude of lateral head displacement                  |
| ATP    | adenosine triphosphate                                  |
| BMD    | benchmark dose  |
| BMDL   | benchmark dose lower confidence limit                   |
| BMR    | benchmark response                                      |
| BMDU   | benchmark dose upper confidence limit                   |
| bw     | body weight   |
| cAMP   | cyclic adenosine monophosphate                          |
| CDO    | 3-MCPD oleate diester                                   |
| CDP    | 3-MCPD palmitate diester                                |
| CMP    | 3-MCPD palmitate monoester                              |
| CASA   | computer-assisted sperm analysis                        |
| CONTAM | EFSA Panel on Contaminants in the Food Chain            |
| EPA    | Environmental Protection Agency                         |
| F      | Female  |
| FAO    | Food and Agriculture Organization of the United Nations |
| GAPDH  | glyceraldehyde-3-phosphate dehydrogenase                |
| JECFA  | Joint FAO/WHO Expert Committee on Food Additives        |
| LDH    | lactate dehydrogenase                                   |
| LOAEL  | lowest-observed-adverse-effect level                    |
| M      | male  |
| MOE    | Margin of Exposure                                      |
| NOAEL  | no-observed-adverse-effect level                        |
| ROS    | reactive oxygen species                                 |
| RP     | Reference Point   |
| SD     | standard deviation                                      |
| SE     | standard error  |
| SQA    | Sperm Quality Analyzer                                  |
| TDI    | tolerable daily intake                                  |
| TORs   | Terms of Reference                                      |
| VCL    | curvilinear velocity                                    |

## Appendix A – Studies selected for full text appraisal

| Reference                                   | Species, strain            | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)                        | Sex  | Doses tested   | Developmental and reproductive endpoints  | NOAEL  | LOAEL  | Comments  |
|---|----------------------------|--|------|--|---|--|--|---|
| <b>Studies selected for risk assessment</b> |                            |  |      |  |   |  |  |   |
| Ban et al. (1999)                           | Rat, Sprague–Dawley Crj:CD | 3-MCPD, 9 days, gavage, 14–15 animals/dose group, 1/day  | M    | 0, 1, 3 or 10 mg/kg bw   | Reproductive organs weight; Epididymis sperm motility parameters and count; Fertility Pre/post-implantation loss; Fetal viability | 1 mg/kg bw per day (sperm motility, fertility)                     | 3 mg/kg bw per day (sperm motility, fertility)   | Data also included in Ban et al. (2001)   |
| Ban et al. (2001)                           | Rat, Sprague–Dawley Crj:CD | 3-MCPD, 9–28 days exposure (w/or w/o 3–28 days recovery), gavage, 14–15 animals/dose group, 1/day or single dose | M    | Range: 1–16 mg/kg bw   | Reproductive organs weight; Sperm count and motility; Fertility   | 1 mg/kg bw per day (sperm motility, fertility)                     | 3 mg/kg bw per day (sperm motility, fertility)   | Collaborative study, include data from Ban et al. (1999) and Kaneto et al. (1999)   |
| Barocelli et al. (2011)                     | Rat, Wistar                | 3-MCPD or 3-MCPD dipalmitate, 90 days, gavage, 10–20 animals/sex per dose group, 1/day                           | M, F | 3-MCPD: 1.84, 7.37 or 29.5 mg/kg bw<br><br>3-MCPD dipalmitate: 9.87, 39.19 or 156.75 mg/kg bw (equimolar to 1.84, 7.37 and 29.5 mg/kg bw of free 3-MCPD, respectively) | Reproductive organs histopathology  | 3-MCPD: –<br><br>3-MCPD dipalmitate: 39.19 (testis histopathology) | 3-MCPD: 1.84 (testis histopathology)<br><br>3-MCPD dipalmitate: 156.75 (testis histopathology) | Minor limitations of the study included a low concentration of 3-MCPD in the rat food and corn oil administration vehicle (over 1,000-fold less than the lowest dose of 3-MCPD); timeframes of administration of the different compounds and doses were staggered, although each phase had its own concurrent control group |

| Reference          | Species, strain     | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing) | Sex   | Doses tested   | Developmental and reproductive endpoints  | NOAEL   | LOAEL   | Comments |
|--------------------|---------------------|---|---|--|---|---|---|----------|
| Cho et al. (2008a) | Mouse, B6C3F1       | 3-MCPD, 13 weeks, diet (drinking water), 10 animals/sex per dose group, <i>ad libitum</i> | M, F  | M: 0.94, 4.59, 18.05, 36.97 or 76.79 mg/kg bw per day<br>F: 0.79, 3.94, 15.02, 30.23 or 61.34 mg/kg bw per day | Reproductive organs weight and histopathology; Cauda epididymis sperm motility; Vaginal cytology  | 36.97 mg/kg bw (sperm motility)<br><br>4.59 mg/kg bw (germinal epithelium degeneration) | 76.79 mg/kg bw (sperm motility)<br><br>18.05 mg/kg bw (germinal epithelium degeneration)                            |          |
| Cho et al. (2008b) | Rat, Sprague-Dawley | 3-MCPD, 2 years, diet (drinking water), 50 animals/sex/per dose group, <i>ad libitum</i>  | M, F  | M: 1.97, 8.27 or 29.50 mg/kg bw per day<br><br>F: 2.68, 10.34 or 37.03 mg/kg bw per day                        | Reproductive organs histopathology  | <br><br>8.27 mg/kg bw (epididymal atrophy)  | 1.97 mg/kg bw (seminiferous tubular atrophy and arteritis/periarteritis)<br><br>29.50 mg/kg bw (epididymal atrophy) |          |
| Hoyt et al. (1994) | Rat, Sprague-Dawley | 3-MCPD, 2 weeks (w/o 2 weeks recovery), gavage, 10 animals/dose group, 1/day              | M Fertility was studies mating treated with untreated F | 0, 1, 5 or 25 mg/kg bw   | Reproductive organs weight and histopathology; Cauda epididymis sperm count morphology and motility; Fertility                              | 1 mg/kg bw (sperm motility, decreased fertility)  | 5 mg/kg bw (sperm motility, decreased fertility)  |          |
| Kim et al. (2012)  | Rat, Sprague-Dawley | 3-MCPD, 7 days, gavage, 6 animals/dose group, 1/day                                       | M   | 0, 3, 10 or 30 mg/kg bw  | Reproductive organs weight and histopathology; testis sperm count; Cauda epididymis sperm motility and morphology; Epididymis enzyme levels | –   | 3 mg/kg bw per day (vacuolisation of epididymal epithelium)   |          |

| Reference               | Species, strain                     | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)   | Sex  | Doses tested  | Developmental and reproductive endpoints  | NOAEL   | LOAEL  | Comments   |
|-------------------------|-------------------------------------|---|------|---|---|---|--|--|
| Li et al. (2003)        | Rat, Sprague-Dawley                 | 3-MCPD, 90 days, gavage, 22 animals/dose group, 1/day   | M    | 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 or 16.0 mg/kg bw  | Reproductive organs weight and histopathology; Epididymis sperm count, viability and morphology; Testicular enzymes (LDH, LDH-X) levels | 2.0 mg/kg bw per day (sperm count)<br>4.0 mg/kg bw per day (sperm viability)  | 4.0 mg/kg bw per day (sperm count)<br>8.0 mg/kg bw per day (sperm viability)   |  |
| Mineshima et al. (2000) | Rat, Sprague-Dawley Crj:CD (mostly) | 3-MCPD, 1–2 weeks, gavage, 4–10 animals/dose group, 1/day   | M    | 2.5, 5.0 or 10.0 mg/kg bw   | Cauda epididymis/testis sperm motility, count and morphology; Fertility   | –   | 2.5 mg/kg bw (sperm motility and fertility)  | Study designed as a multilaboratory comparison of different methods for sperm motility assessment. Data presentation other than for motility is very limited |
| Onami et al. (2014)     | Rat, F344                           | 3-MCPD, 3-MCPD palmitate diester (CDP), 3-MCPD palmitate monoester (CMP) or 3-MCPD oleate diester (CDO), 13 weeks, gavage, 10 animals/sex per dose group, 1/day | F, M | 3-MCPD: 40 mg/kg bw<br>CDP: 14, 55 or 220 mg/kg bw*<br>CMP: 8, 32 or 130 mg/kg bw*<br>CDO: 15, 60 or 240 mg/kg bw*<br>(common control group included)<br>*Doses equimolar to 2.5, 10.0 and 40.0 mg/kg bw of free 3-MCPD, respectively | Reproductive organs weight and histopathology   | CDP: 55 mg/kg bw per day<br>CMP: 32 mg/kg bw per day<br>CDO: 60 mg/kg bw per day (apoptosis in epididymal epithelium) | CDP: 220 mg/kg bw per day<br>CMP: 130 mg/kg bw per day<br>CDO: 240 mg/kg bw per day (apoptosis in epididymal epithelium) |  |



| Reference              | Species, strain            | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)   | Sex | Doses tested   | Developmental and reproductive endpoints   | NOAEL   | LOAEL  | Comments  |
|------------------------|----------------------------|---|-----|--|--|---|--|---|
| Takayama et al. (1995) | Rat, Sprague–Dawley        | 3-MCPD, 2 weeks, gavage, 7 animals/dose group, 1/day  | M   | 0, 2 or 8 mg/kg bw   | Reproductive organs weight and histopathology; Sperm motility, activity and viability; Fertility   | 2.0 mg/kg bw per day (fertility)                        | 2.0 mg/kg bw per day (sperm motility)<br>8.0 mg/kg bw per day (fertility)  | Lack of details in the study report (e.g. age and bw of animals at the beginning of the study, SD or SE not reported)                                       |
| Vickery et al. (1974)  | Rat, Sprague–Dawley        | Exp.1 = 3-MCPD, 3 weeks, gavage, 5 animals/dose group, 1/day<br>Exp.2 = 3-MCPD, 9 days, gavage, 5 animals/dose group, 1/day<br>Exp.3 = 3-MCPD, 4 months + 20 days recovery, gavage, 5 animals/dose group, 1/day | M   | Exp.1/3 = 0.2, 1.0, 2.5, 5.0, 10 or 25 mg/kg bw<br>Exp.2 = 1.25, 2.5, 5 or 10 mg/kg bw | Reproductive organs histopathology; Sperm count (uterine, after mating); Fertility   | Exp. 1: 1.0 mg/kg bw per day (decreased pregnancy rate) | Exp. 1: 2.5 mg/kg bw per day (decreased pregnancy rate)<br>Exp. 2: 1.25 mg/kg bw per day (recovery of uterine fertilised eggs) | Fertility was studied in unexposed females mated with exposed males. Sperm was only collected from uterus and oviduct of unexposed females following mating |
| Yamada et al. (1995)   | Rat, Sprague–Dawley Crj:CD | 3-MCPD, 4 weeks (w/or w/o 2 weeks recovery), gavage, 7–12 animals/dose group, 1/day   | M   | 0, 2 or 8 mg/kg bw   | Reproductive organs weight and histopathology; Cauda epididymis sperm motility, count and morphology; Fertility; Post-implantation loss; Foetal viability and weight |   | 2.0 mg/kg bw per day (sperm motility and decreased fertility)  |   |

| Reference                 | Species, strain            | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)  | Sex | Doses tested  | Developmental and reproductive endpoints  | NOAEL                            | LOAEL                                 | Comments   |
|---------------------------|----------------------------|--|-----|---|---|----------------------------------|---------------------------------------|--|
| Zhang et al. (2012)       | Rat, Sprague–Dawley        | (S)-3-MCPD, 52 days, gavage, 5 animals/dose group, 1/day   | M   | 0, 2.5, 5.0 or 10.0 mg/kg bw  | Cauda epididymis sperm motility and hyperactivity; Sperm enzymes (ATP, cAMP, GAPDS) levels                            |                                  | 2.5 mg/kg bw per day (sperm motility) | Only sperm parameters measured   |
| <b>Supporting studies</b> |                            |  |     |   |   |                                  |                                       |  |
| Banik et al. (1972)       | Rat, Sprague–Dawley Crj:CD | 3-MCPD, 13–50 days, gavage, 5–13 animals/dose group, 1/day<br>Animals treated for 50 days (low-dose group), 17 (or 34) days (mid-dose group), or 20 days (high-dose group) | M   | 0, 0.5, 2.5 or 25 mg/rat per day (corresponding to ~ 1.3, 2.7 and 27 mg/kg bw, respectively, based on mean rat weights) | Fertility   | 1.3 mg/kg bw per day (fertility) | 2.7 mg/kg bw per day (fertility)      | Different dose groups were treated for periods varying between 17 and 50 days (treatment periods decreasing with increasing dosage). Data are not suitable for hazard characterisation |
| Coppola (1969)            | Rat, Wistar                | 3-MCPD, 14 days, gavage, 6–24 animals/dose group, 1/day  | M   | 0, 0.1, 0.5, 1, 5, 10, 25, 50 mg/kg bw per day (main study)<br>0 and 8 mg/kg bw per day (satellite study)               | Reproductive Main study: prostate and testes weights, fertility; Satellite study: Sperm count and motility; Fertility | 1.0 mg/kg bw per day (fertility) | 5 mg/kg bw per day (fertility)        | No dose–response analysis possible for sperm quality parameters (only one dose tested)   |
| Johnson and Pursel (1972) | Pigs                       | 3-MCPD, 5 days, diet, 1–2 animals/dose group, 1/day  | M   | Exp. 1 = 1, 5, 25 or 50 mg/kg bw  | Reproductive organs histopathology; Sperm count, motility and morphology; Fertility                                   |                                  | 1 mg/kg bw per day (fertility)        | No independent control group, very low number of animals per group   |

| Reference                 | Species, strain             | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)  | Sex | Doses tested   | Developmental and reproductive endpoints  | NOAEL  | LOAEL  | Comments  |
|---------------------------|-----------------------------|--|-----|--|---|--|--|---|
| Kwack et al. (2004)       | Rat, Sprague–Dawley         | 3-MCPD, 4 weeks, gavage, 15 animals/dose group,  | M   | 0, 0.01, 0.05, 0.25, 1 or 5 mg/kg bw   | Reproductive organs weight and histopathology; Sperm count and motility; Fertility; Number of corpora lutea, implants and resorptions; Fetal viability, weight and morphology; Placental weight | 0.05 mg/kg bw per day (decreased epididymal sperm count) | 0.25 mg/kg bw per day (decreased epididymal sperm count) | Lack of methodological details and inconsistency in the reporting results on fertility and reproduction<br>Decrease in epididymal sperm count was difficult to explain in the absence of histopathological findings |
| Rooney and Jackson (1980) | Rat, Wistar American strain | 3-MCPD, 3-MCPD nitrobenzoate or 3-MCPD acetamidobenzoate, single administration or 1–25 weeks, gavage, 5 animals/dose group, 1/day | M   | 3-MCPD: 10 or 20 mg/kg bw;<br>3-MCPD nitrobenzoate: 25, 75, 600 or 3,000 mg/kg bw (equimolar to 0.06, 0.18, 1.47 and 7.35 nmol/kg bw of free 3-MCPD, respectively);<br>3-MCPD acetamidobenzoate: 25 or 400 mg/kg bw (equimolar to 0.09 and 1.54 nmol/kg bw of free 3-MCPD, respectively) | Fertility   | –  | –  | The study is limited in both detail and scope. The esters used would not be likely to occur in food although the data does support that esterification of 3-MCPD decreases the potency in comparison to free 3-MCPD |

| Reference   | Species, strain                                       | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)  | Sex | Doses tested  | Developmental and reproductive endpoints                                       | NOAEL | LOAEL | Comments  |
|---|---|--|-----|---|--|-------|-------|---|
| Sawada et al. (2015)                                      | Rat, Wistar Crl:WI                                    | 3-MCPD or 3-MCPD dipalmitate, 28 days, gavage, 2–6 animals dose group, 1/day   | M   | 3-MCPD = 10 mg/kg bw<br>3-MCPD dipalmitate = 13.3 or 53.0 mg/kg bw (corresponding to 2.5 and 10 mg/kg bw, respectively) | Testis weight, histopathology and proteomics analysis                          | –     | –     | Little value for risk assessment with exception that it suggests esters effects due to metabolism to free MCPD. Proteomic analysis was on whole testis and not on the epididymis or sperm |
| <b>Cited but not used as not meeting quality criteria</b> |   |  |     |   |  |       |       |   |
| Ericsson and Baker (1970)                                 | Rat, Sprague–Dawley (Spartan and Upjohn); Guinea-pigs | Exp.1 (rats) = 3-MCPD, 8 days, s.c. injection, 3 animals/dose group, 1/day;<br>Exp.2 (rats) = 3-MCPD, 1–8 days, gavage, 3–63 animals/dose group, 1/day<br>Exp.3 (guinea pigs) = 3-MCPD, 45 days, s.c. injection, 4 animals/dose group, 1/day | M   | Exp.1 = 0.5, 1.5, 3.0 mg/day;<br>Exp.2 = 1.0, 1.5, 2.0, 3.0, 5.0, 10, 15 or 30 mg/day;<br>Exp.3: 50 mg/day              | Reproductive organs weight and histopathology;<br>Sperm motility;<br>Fertility | –     | –     | No control group included, dose–response analysis not possible  |
| Ericsson and Ericsson (1996)                              | Rat, Sprague–Dawley (Spartan)                         | Exp.1 = 3-MCPD, single administration, gavage, 9–10 animals/dose group<br>Exp.2 = 3-MCPD, 7 days, 3–5 animals/dose group, 1/day<br>Exp.3 = 3-MCPD, 78 days, 7–9 animals/dose group, 2/day  | M   | Exp.1 = 30, 40, 50 or 60 mg/kg bw;<br>Exp.2 = 20, 40, 60 or 80 mg/kg bw;<br>Exp.3 = 15, 30 or 45 mg/kg bw               | Epididymis histopathology  | –     | –     | No control group included, dose–response analysis not possible  |

| Reference                | Species, strain                                 | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)  | Sex | Doses tested   | Developmental and reproductive endpoints           | NOAEL | LOAEL | Comments   |
|--------------------------|---|--|-----|--|--|-------|-------|--|
| Ford and Harrison (1983) | Rat, CD; Mice, CD-1; guinea pig; golden hamster | <p>Exp.1 (rats) = 3-MCPD, 10 days, gavage, 4 animals/dose group, 1/day;</p> <p>Exp.2 (rats) = 3-MCPD, 1–4 days, gavage, 4 animals/dose group, 1/day;</p> <p>Exp.3 (rats) = 3-MCPD, 3–10 days, gavage (after ligation of epididymis), 5 animals/dose group, 1/day;</p> <p>Exp.4 (hamsters) = 3-MCPD, 10 days, gavage, 4 animals/dose group, 1/day;</p> <p>Exp.5 (guinea pigs): 3-MCPD, 10 days, s.c. injection, 4 animals/dose group, 1/day;</p> <p>Exp.6 (mice) = 3-MCPD, 10 days, gavage, 8 animals/dose group, 1/day</p> | M   | <p>Exp. 1 = 4, 8 or 25 mg/kg bw</p> <p>Exp.2/3 = 8 mg/kg bw</p> <p>Exp.4 = 50 or 100 mg/kg bw</p> <p>Exp.5 = 66 mg/kg bw</p> <p>Exp. 6 = 44 mg/kg bw</p> | Epididymis sperm count and enzyme activity (GAPDH) | –     | –     | Experiments mainly focussing on the identification of the mode of action of fertility effects. Data not suitable for hazard characterisation |
| Helal (1982)             | Rat, Nile field and Albino                      | 3-MCPD, single administration, gavage, 5 animals/dose group  | M   | 0.5, 1, 2, 4, 6, 10, 20, 40, 60 or 80 mg/kg bw   | Reproductive organs histopathology; Fertility      | –     | –     | Limitations in the reporting of the study design and performance. Single administration study  |



| Reference             | Species, strain            | Compound(s) and exposure protocol (duration, recovery, regime, number of animals, timing)   | Sex | Doses tested   | Developmental and reproductive endpoints  | NOAEL  | LOAEL | Comments  |
|-----------------------|----------------------------|---|-----|--|---|--|-------|---|
| Jackson et al. (1977) | Rat (strain not specified) | Exp.1 = (S)- or (R)-3-MCPD, single administration, gavage, 5 animals/dose group<br>Exp.2 = (S)-3-MCPD, 13 days, gavage, 5 animals/dose group, 1/day | M   | Exp.1 = 0, 12.5 (racemic mixture) or 25 (S) mg/kg bw<br>Exp.2 = 0, 2.5 or 5 mg/kg bw | Fertility   | –  | –     | No statistics, no threshold dose calculation possible |
| Zhang et al. (2008)   | Mouse, CD-1 (ICR)          | 3-MCPD, 2 or 8 months, gavage, 4–16 animals/dose group, 1/day (presumed, not explicitly stated)   | M   | 0, 2.9, 5.9 or 11.7 mg/kg bw   | Reproductive organs weight and histopathology; Epididymis sperm count; Fertility and mating | 11.7 mg/kg bw per day (mating and fertility after 2 months; epididymis sperm count, testis and epididymis histopathology after 8 months) | –     | Unclear dose regime and randomisation                 |

bw: body weight; CDO: 3-MCPD oleate diester; CDP: 3-MCPD palmitate diester; CMP: 3-MCPD palmitate monoester; F: female; LOAEL: lowest-observed-adverse-effect level; M: male; MCPD: 3- and 2-monochloropropane diol; SD: standard deviation; SE: standard error; LDH: lactate dehydrogenase; ATP: glyceraldehyde-3-phosphate dehydrogenase; cAMP: cyclic adenosine monophosphate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

## Appendix B – Benchmark dose modelling of incidence of kidney tubular cell hyperplasia in male Fischer rats (Sunahara et al., 1993)

### A. Data description

As discussed in detail in the previous EFSA opinion (EFSA CONTAM Panel 2016), F344 rats (50/sex per dose group) were exposed to nominal concentrations of 0, 20, 100 or 500 mg/L 3-MCPD via drinking water in a 2-year study. Analytical measures revealed an average background concentration of 2.47 mg/L 3-MCPD in drinking water and average concentrations of 26.5, 105.9 and 502.8 mg/L 3-MCPD for the three treatment groups, respectively. For these, calculated mean daily intakes were given in the report as 1.1, 5.2 or 28.3 mg/kg bw per day for males. The CONTAM Panel calculated a 3-MCPD daily intake of 0.11 mg/kg bw per day for male controls. Incidence of kidney tubular cell hyperplasia was as reported below:

| Dose (mg/kg bw per day) | Incidence of tubular cell hyperplasia | Number of animals |
|-------------------------|---------------------------------------|-------------------|
| 0.11                    | 3                                     | 50                |
| 1.10                    | 6                                     | 50                |
| 5.20                    | 15                                    | 50                |
| 28.30                   | 34                                    | 50                |

bw: body weight.

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017).
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017).

### D. Results

Table with summary of the fitted models

| Model           | Number of parameters | Log-likelihood | AIC    | BMD  | BMDL | BMDU | Converged | Accepted AIC |
|-----------------|----------------------|----------------|--------|------|------|------|-----------|--------------|
| Null            | 1                    | -120.43        | 242.86 | NA   | NA   | NA   | Yes       |              |
| Full            | 4                    | -91.58         | 191.16 | NA   | NA   | NA   | Yes       |              |
| Logistic        | 2                    | -94.54         | 193.08 | 6.43 | NA   | NA   | Yes       | No           |
| Probit          | 2                    | -94.32         | 192.64 | 6.00 | NA   | NA   | Yes       | No           |
| Log-logistic    | 3                    | -91.58         | 189.16 | 1.64 | 0.42 | 4.11 | Yes       | Yes          |
| Log-probit      | 3                    | -91.60         | 189.20 | 1.80 | 0.58 | 4.23 | Yes       | Yes          |
| Weibull         | 3                    | -91.60         | 189.20 | 1.32 | 0.28 | 3.96 | Yes       | Yes          |
| Gamma           | 3                    | -91.62         | 189.24 | 1.22 | 0.22 | 4.03 | Yes       | Yes          |
| Two-stage       | 3                    | -92.11         | 190.22 | 2.59 | 1.95 | 3.60 | Yes       | Yes          |
| LVM: Expon. m3- | 3                    | -91.63         | 189.26 | 0.34 | 0.89 | 3.62 | Yes       | Yes          |
| LVM: Hill m3-   | 3                    | -91.61         | 189.22 | 0.31 | 1.10 | 3.74 | Yes       | Yes          |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

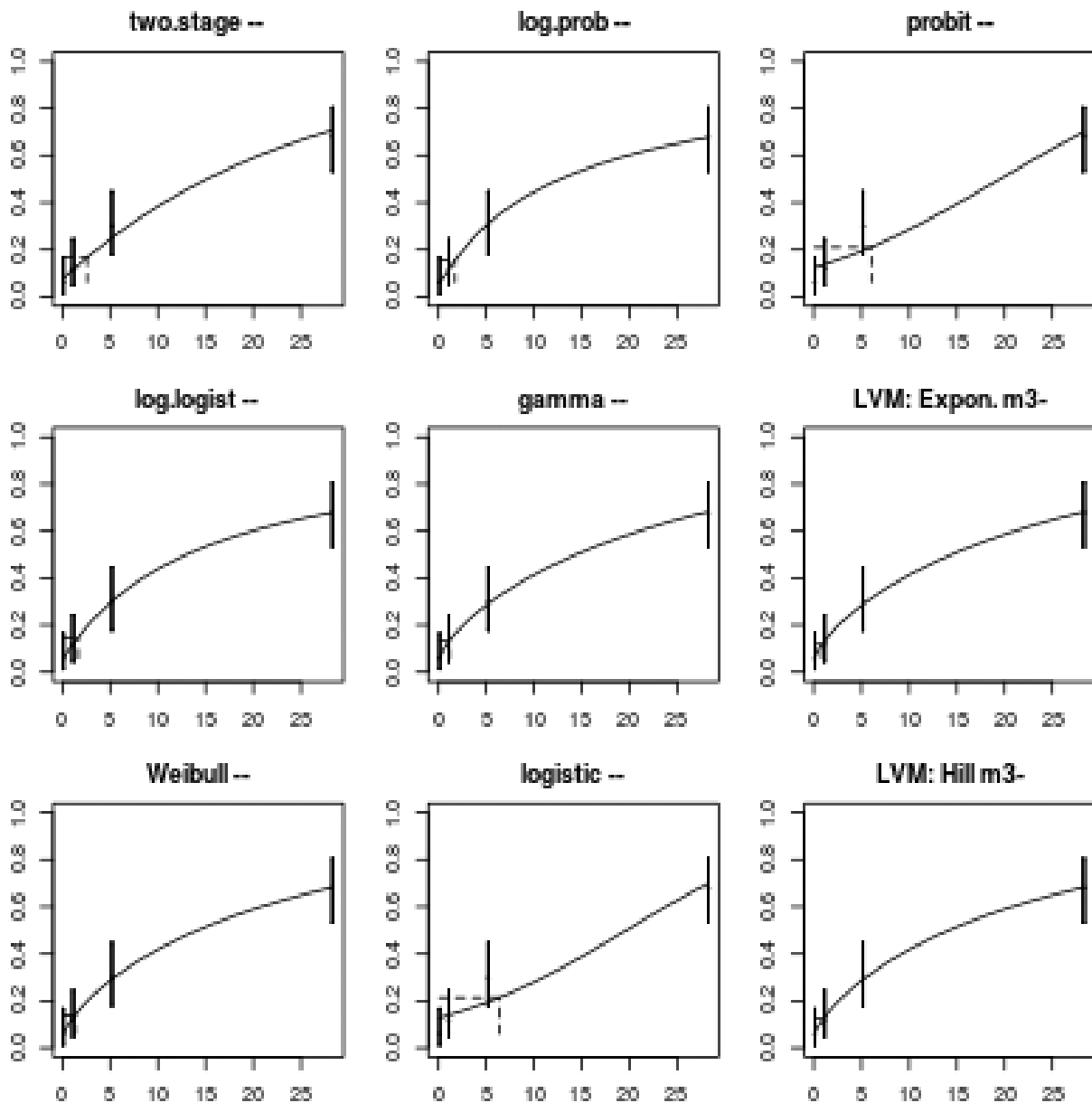
## Weights for Model Averaging

| two.stage | log.logist | Weibull | log.prob | Gamma | Logistic | Probit | EXP  | HILL |
|-----------|------------|---------|----------|-------|----------|--------|------|------|
| 0.09      | 0.15       | 0.15    | 0.15     | 0.14  | 0.02     | 0.03   | 0.14 | 0.14 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

| BMD  | BMDL | BMDU |
|------|------|------|
| 1.47 | 0.54 | 4.91 |



## Appendix C – Benchmark dose modelling of incidence of kidney tubular cell hyperplasia in female Fischer rats (Sunahara et al., 1993)

### A. Data description

As discussed in detail in the previous EFSA opinion (EFSA CONTAM Panel, 2016), F344 rats (50/sex per dose group) were exposed to nominal concentrations of 0, 20, 100 or 500 mg/L 3-MCPD via drinking water in a 2-year study. Analytical measures revealed an average background concentration of 2.47 mg/L 3-MCPD in drinking water and average concentrations of 26.5, 105.9 and 502.8 mg/L 3-MCPD for the three treatment groups, respectively. For these, calculated mean daily intakes were given in the report as 1.4, 7.0 or 35.3 mg/kg bw per day for females. The CONTAM Panel calculated a 3-MCPD daily intake of 0.14 mg/kg bw per day female controls. Incidence of kidney tubular cell hyperplasia was as reported below:

| Dose (mg/kg bw per day) | Incidence of tubular cell hyperplasia | Number of animals |
|-------------------------|---------------------------------------|-------------------|
| 0.14                    | 2                                     | 50                |
| 1.4                     | 4                                     | 50                |
| 7.0                     | 20                                    | 50                |
| 35.3                    | 31                                    | 50                |

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017)
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

### D. Results

Table with summary of the fitted models

| Model           | Number of parameters | Log-likelihood | AIC    | BMD  | BMDL | BMDU | Converged | Accepted AIC |
|-----------------|----------------------|----------------|--------|------|------|------|-----------|--------------|
| Null            | 1                    | -119.52        | 241.04 | NA   | NA   | NA   | Yes       |              |
| Full            | 4                    | -89.19         | 186.38 | NA   | NA   | NA   | Yes       |              |
| Logistic        | 2                    | -97.97         | 199.94 | 8.49 | NA   | NA   | Yes       | No           |
| Probit          | 2                    | -97.61         | 199.22 | 7.94 | NA   | NA   | Yes       | No           |
| Log-logistic    | 3                    | -90.34         | 186.68 | 1.23 | 0.41 | 2.98 | Yes       | Yes          |
| Log-probit      | 3                    | -90.19         | 186.38 | 1.55 | 0.47 | 3.26 | Yes       | Yes          |
| Weibull         | 3                    | -90.71         | 187.42 | 0.84 | 0.33 | 1.68 | Yes       | Yes          |
| Gamma           | 3                    | -90.92         | 187.84 | 0.83 | 0.29 | 1.72 | Yes       | Yes          |
| Two-stage       | 3                    | -93.01         | 192.02 | 3.24 | NA   | NA   | Yes       | No           |
| LVM: Expon. m5- | 4                    | -89.19         | 186.38 | 1.00 | 0.48 | 2.08 | Yes       | Yes          |
| LVM: Hill m5-   | 4                    | -89.19         | 186.38 | 0.95 | 0.42 | 2.04 | Yes       | Yes          |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

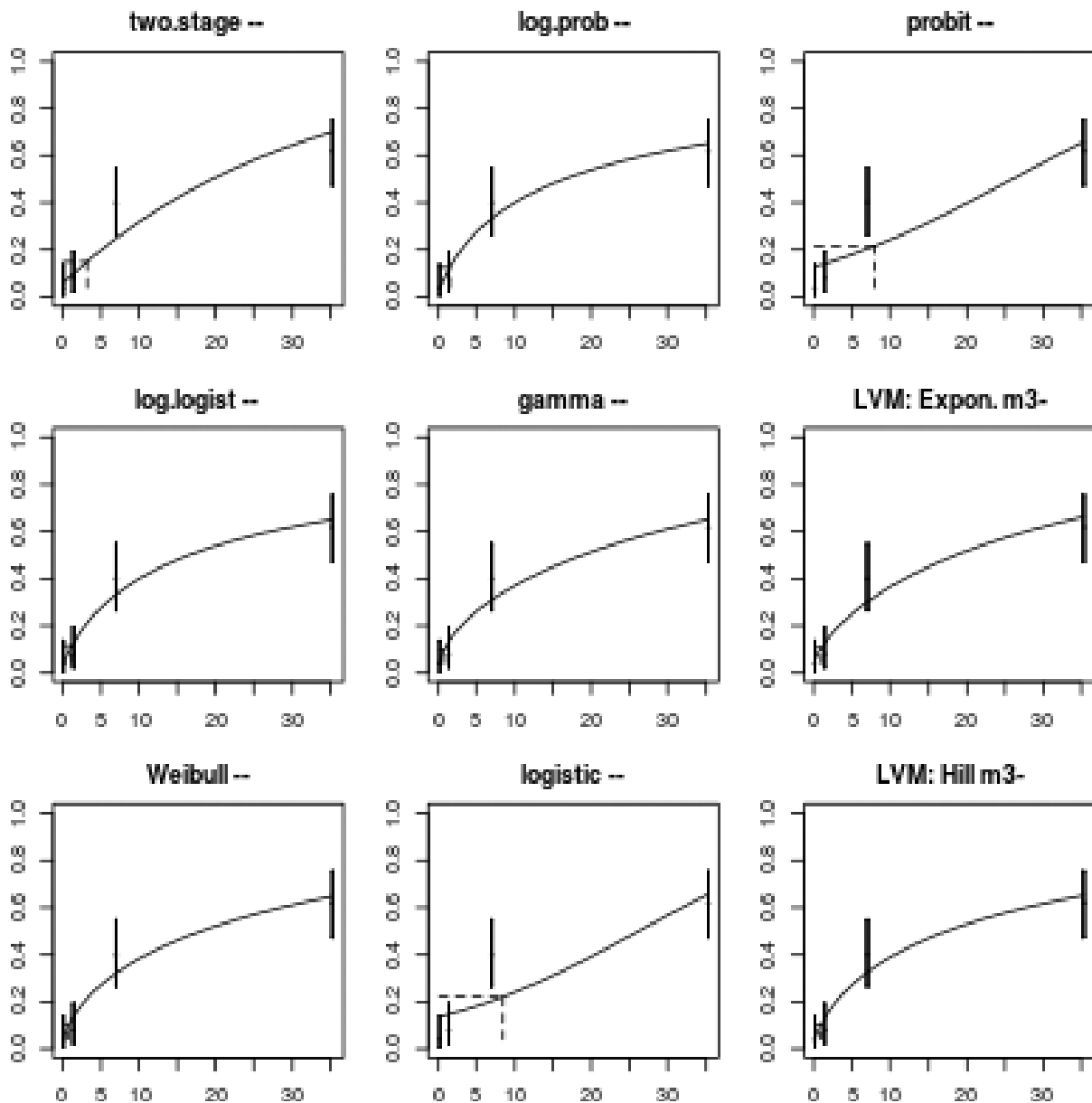
## Weights for Model Averaging

| two.stage | log.logist | Weibull | log_prob | Gamma | Logistic | Probit | EXP | HILL |
|-----------|------------|---------|----------|-------|----------|--------|-----|------|
| 0.01      | 0.21       | 0.14    | 0.24     | 0.12  | 0        | 0      | 0.1 | 0.17 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

| BMD  | BMDL | BMDU |
|------|------|------|
| 1.15 | 0.55 | 3.7  |





## Appendix D – Benchmark dose modelling of incidence of kidney tubular cell hyperplasia in male Sprague–Dawley rats (Cho et al., 2008b)

### A. Data description

As discussed in detail in the previous EFSA opinion (EFSA CONTAM Panel, 2016), SD rats (50/sex per dose group) were exposed to 0, 25, 100 or 400 mg/L 3-MCPD via drinking water in a 2-year study. Doses were converted to average daily intake of 0, 2.0, 8.3 or 29.5 mg/kg bw for males. Incidence of kidney tubular cell hyperplasia was as reported below:

| Dose (mg/kg bw per day) | Incidence of tubular cell hyperplasia | Number of animals |
|-------------------------|---------------------------------------|-------------------|
| 0.0                     | 1                                     | 50                |
| 2.0                     | 11                                    | 50                |
| 8.3                     | 21                                    | 50                |
| 29.5                    | 36                                    | 50                |

bw: body weight.

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017).
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017).

### D. Results

Table with summary of the fitted models

| Model           | Number of parameters | Log-likelihood | AIC    | BMD  | BMDL | BMDU | Converged | Accepted AIC |
|-----------------|----------------------|----------------|--------|------|------|------|-----------|--------------|
| Null            | 1                    | -128.86        | 259.72 | NA   | NA   | NA   | Yes       |              |
| Full            | 4                    | -94.91         | 197.82 | NA   | NA   | NA   | Yes       |              |
| Logistic        | 2                    | -102.47        | 208.94 | 5.63 | NA   | NA   | Yes       | No           |
| Probit          | 2                    | -102.18        | 208.36 | 5.36 | NA   | NA   | Yes       | No           |
| Log-logistic    | 3                    | -95.07         | 196.14 | 0.85 | 0.23 | 1.91 | Yes       | Yes          |
| Log-probit      | 3                    | -95.10         | 196.20 | 0.93 | 0.28 | 1.96 | Yes       | Yes          |
| Weibull         | 3                    | -94.94         | 195.88 | 0.64 | 0.14 | 1.66 | Yes       | Yes          |
| Gamma           | 3                    | -94.91         | 195.82 | 0.54 | 0.08 | 1.61 | Yes       | Yes          |
| Two-stage       | 3                    | -97.44         | 200.88 | 2.14 | NA   | NA   | Yes       | No           |
| LVM: Expon. m3- | 3                    | -94.92         | 195.84 | 0.49 | 0.17 | 1.42 | Yes       | Yes          |
| LVM: Hill m3-   | 3                    | -94.93         | 195.86 | 0.59 | 0.13 | 1.55 | Yes       | Yes          |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

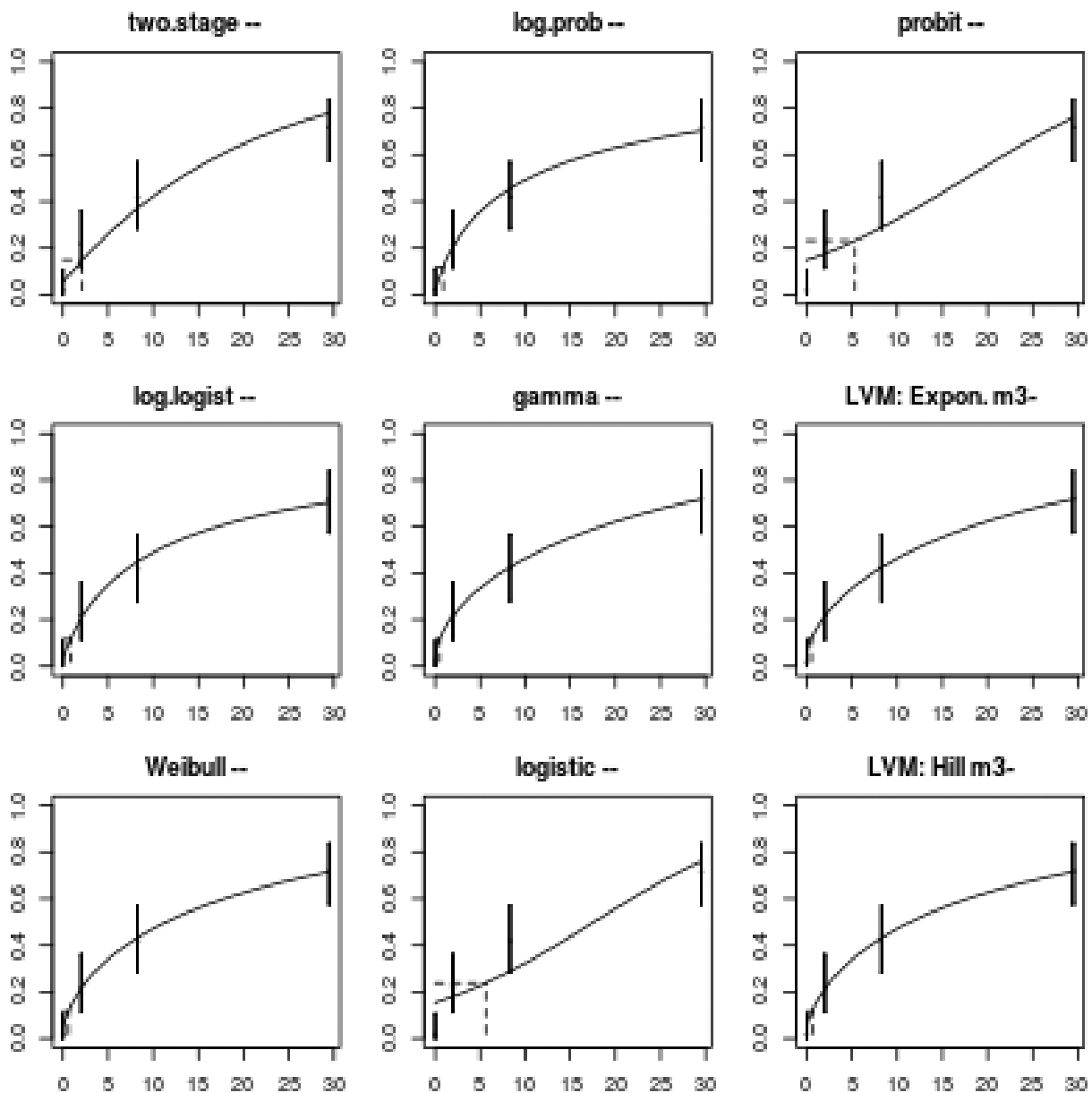
## Weights for model averaging

| two.stage | log.logist | Weibull | log.prob | Gamma | Logistic | Probit | EXP  | HILL |
|-----------|------------|---------|----------|-------|----------|--------|------|------|
| 0.01      | 0.15       | 0.17    | 0.14     | 0.18  | 0        | 0      | 0.17 | 0.17 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

| BMD  | BMDL | BMDU |
|------|------|------|
| 0.68 | 0.20 | 1.95 |



## Appendix E – Benchmark dose modelling of incidence of kidney tubular cell hyperplasia in female Sprague–Dawley rats (Cho et al., 2008b)

### A. Data description

As discussed in detail in the previous EFSA opinion (EFSA CONTAM Panel, 2016), SD rats (50/sex per dose group) were exposed to 0, 25, 100 or 400 mg/L 3-MCPD via drinking water in a 2-year study. Doses were converted to average daily intake of 0, 2.7, 10.3 or 37.0 mg/kg bw for females. Incidence of kidney tubular cell hyperplasia was as reported below:

| Dose (mg/kg bw per day) | Incidence of tubular cell hyperplasia | Number of animals |
|-------------------------|---------------------------------------|-------------------|
| 0.0                     | 1                                     | 50                |
| 2.7                     | 0                                     | 50                |
| 10.3                    | 1                                     | 50                |
| 37.0                    | 10                                    | 50                |

bw: body weight.

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017)
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

### D. Results

Table with summary of the fitted models

| Model           | Number of parameters | Log-likelihood | AIC   | BMD | BMDL | BMDU | Converged | Accepted AIC |
|-----------------|----------------------|----------------|-------|-----|------|------|-----------|--------------|
| Null            | 1                    | -45.39         | 92.78 | NA  | NA   | NA   | Yes       |              |
| Full            | 4                    | -34.82         | 77.64 | NA  | NA   | NA   | Yes       |              |
| Logistic        | 2                    | -35.66         | 75.32 | 29  | 24.1 | 35.8 | Yes       | Yes          |
| Probit          | 2                    | -35.71         | 75.42 | 28  | 22.4 | 35.7 | Yes       | Yes          |
| Log-logistic    | 3                    | -35.54         | 77.08 | 28  | 18.2 | 36.8 | Yes       | Yes          |
| Log-probit      | 3                    | -35.53         | 77.06 | 26  | 17.0 | 37.2 | Yes       | Yes          |
| Weibull         | 3                    | -35.54         | 77.08 | 28  | 18.6 | 37.2 | Yes       | Yes          |
| Gamma           | 3                    | -35.53         | 77.06 | 27  | 18.4 | 37.0 | Yes       | Yes          |
| Two-stage       | 3                    | -35.61         | 77.22 | 26  | 20.6 | 35.8 | No        | Yes          |
| LVM: Expon. m3- | 3                    | -35.57         | 77.14 | 31  | 20.2 | 36.7 | Yes       | Yes          |
| LVM: Hill m3-   | 3                    | -35.56         | 77.12 | 30  | 19.7 | 36.6 | Yes       | Yes          |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

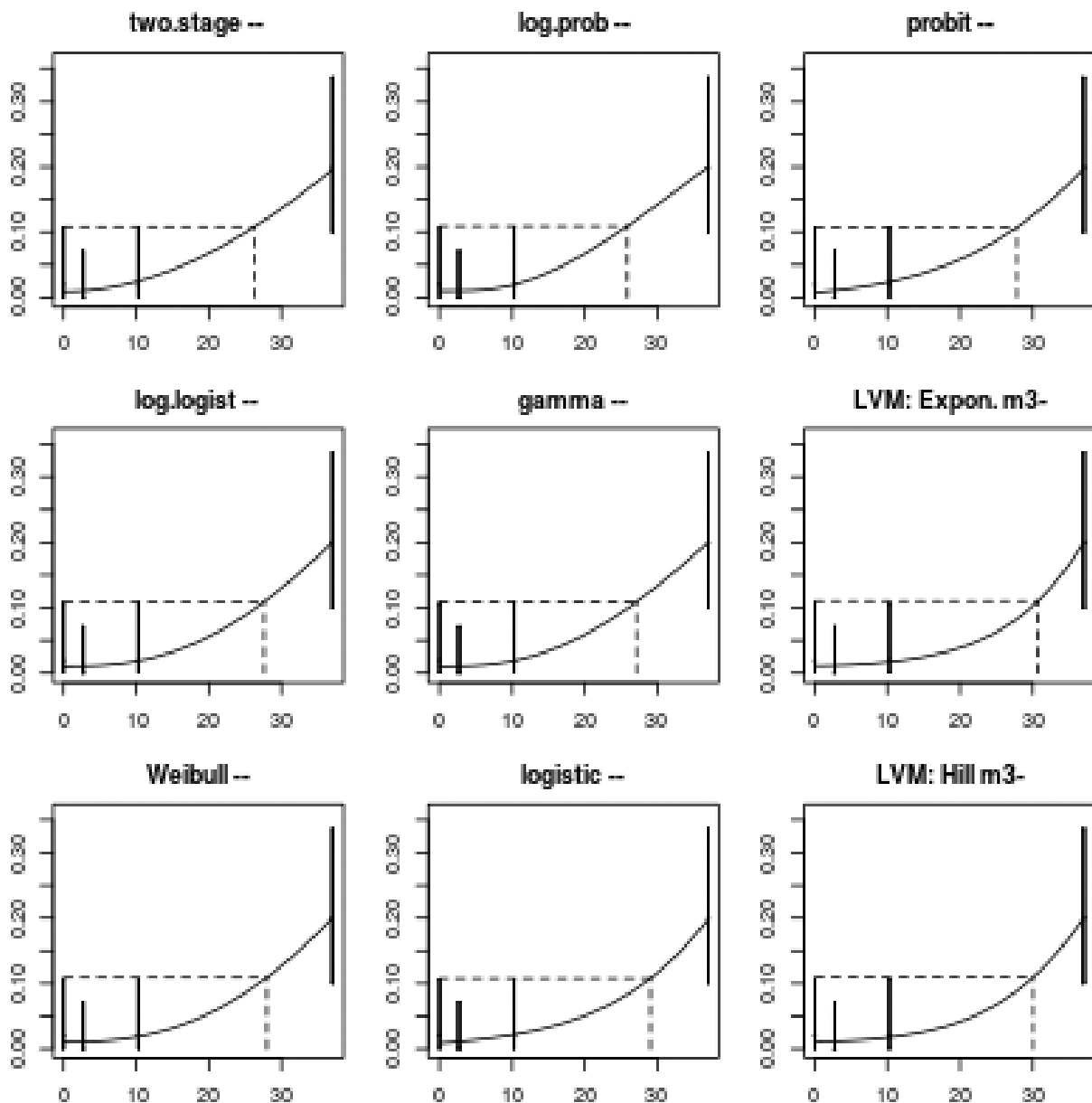
## Weights for Model Averaging

| two.stage | log.logist | Weibull | log.prob | Gamma | Logistic | Probit | EXP  | HILL |
|-----------|------------|---------|----------|-------|----------|--------|------|------|
| 0.08      | 0.09       | 0.09    | 0.09     | 0.09  | 0.21     | 0.2    | 0.08 | 0.08 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

| BMD | BMDL | BMDU |
|-----|------|------|
| 28  | 20   | 36   |



## Appendix F – Benchmark dose modelling of decreased sperm curvilinear velocity in Sprague–Dawley rats (Ban et al., 1999)

### A. Data description

Sprague–Dawley male rats (N = 12–13/group) were exposed by gavage to 0, 1, 3 or 10 mg 3-MCPD/kg bw per day for nine days. The curvilinear velocity (VCL) was measured and mean levels  $\pm$  standard deviations were reported:

| Dose (mg/kg bw per day) | VCL ( $\mu\text{m/s}$ ) | SD ( $\mu\text{m/s}$ ) | N  |
|-------------------------|-------------------------|------------------------|----|
| 0                       | 449.9                   | 27.8                   | 12 |
| 1                       | 441.3                   | 39.8                   | 12 |
| 3                       | 402.0                   | 20.4                   | 13 |
| 10                      | 309.2                   | 73.2                   | 13 |

bw: body weight; VCL: curvilinear velocity; SD: standard deviation.

### B. Selection of the BMR

A BMR of 5% and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017). BMR of 5% was considered appropriate both from a statistical and biological standpoint.

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017)
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

### D. Results

| Model      | Converged | loglik | npar | AIC    |
|------------|-----------|--------|------|--------|
| Full       | Yes       | 31.93  | 5    | –53.86 |
| m1- (null) | Yes       | 8.17   | 2    | –12.34 |
| Expon. m3- | Yes       | 31.89  | 4    | –55.78 |
| Expon. m5- | Yes       | 31.93  | 5    | –53.86 |
| Hill m3-   | Yes       | 31.90  | 4    | –55.80 |
| Hill m5-   | Yes       | 31.93  | 5    | –53.86 |

AIC: Akaike information criterion.

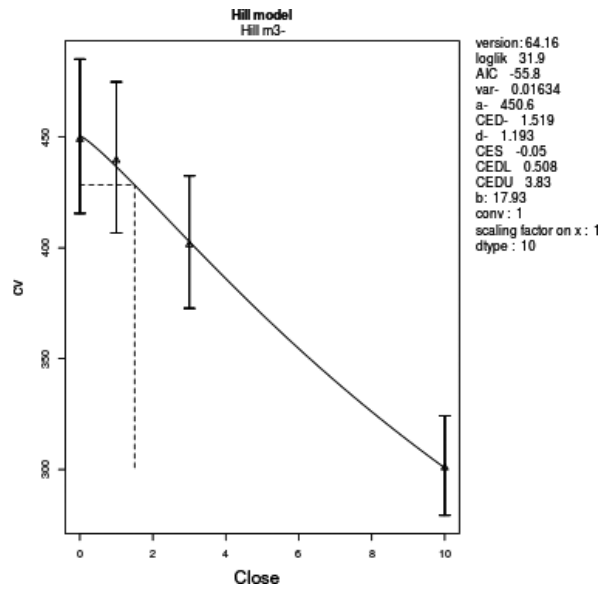
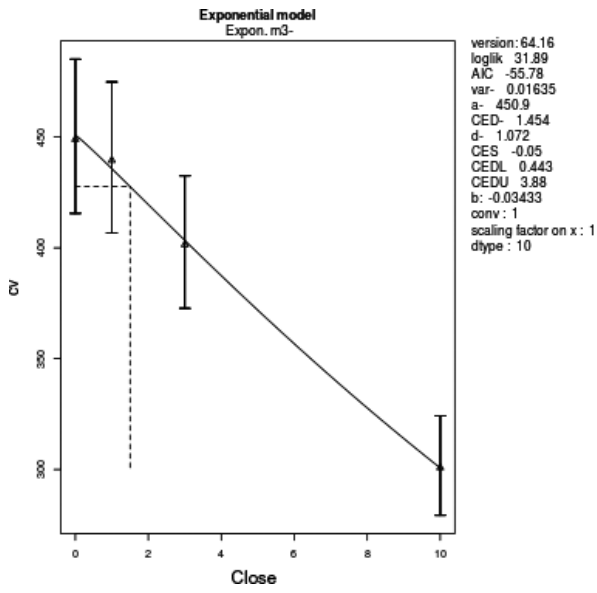
### Final BMD Values

| Model      | BMDL | BMDU | BMD |
|------------|------|------|-----|
| Expon. m3- | 0.44 | 3.88 | 1.5 |
| Hill m3-   | 0.51 | 3.83 | 1.5 |

BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.



## Plots



## Appendix G – Benchmark dose modelling of decreased sperm count in Sprague–Dawley rats (Li et al., 2003)

### A. Data description

Sprague–Dawley male rats (N = 22/group) were exposed by gavage to 0, 0.25, 0.5, 1, 2, 4, 8 or 16 mg 3-MCPD/kg bw per day for 90 days. The epididymal sperm count was measured as number of sperm/g epididymis and mean levels  $\pm$  standard deviations were reported:

| Dose  | Sperm count ( $1 \times 10^6$ /g) | SD ( $1 \times 10^6$ /g) | N  |
|-------|-----------------------------------|--------------------------|----|
| 0.00  | 202.0                             | 46.4                     | 22 |
| 0.25  | 197.0                             | 41.7                     | 22 |
| 0.50  | 185.7                             | 97.2                     | 22 |
| 1.00  | 178.4                             | 57.0                     | 22 |
| 2.00  | 170.0                             | 71.7                     | 22 |
| 4.00  | 151.4                             | 68.6                     | 22 |
| 8.00  | 91.8                              | 43.9                     | 21 |
| 16.00 | 52.9                              | 29.7                     | 20 |

### B. Selection of the BMR

As discussed in Section 3.3.2, the CONTAM Panel did not consider the selection of the default BMR of 5% for continuous data as appropriate for changes in epididymal sperm count. Instead, the Panel considered to use one standard deviation calculated for the control group as a BMR, as recommended by US EPA (2012), resulting in a BMR of 23%.

A 90% interval around the BMD was selected as recommended.

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017).
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017).

### D. Results

#### Fitted Models

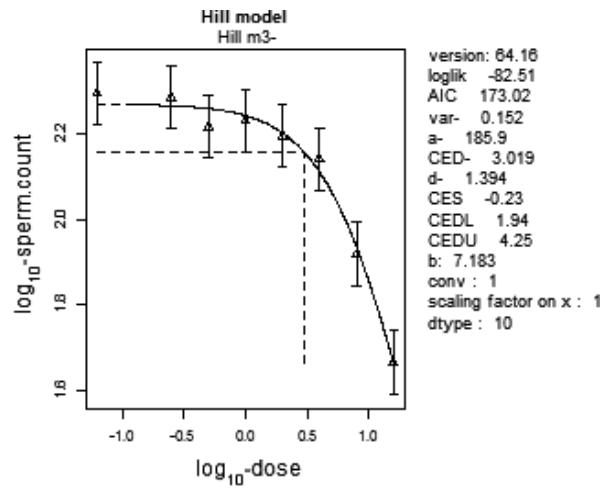
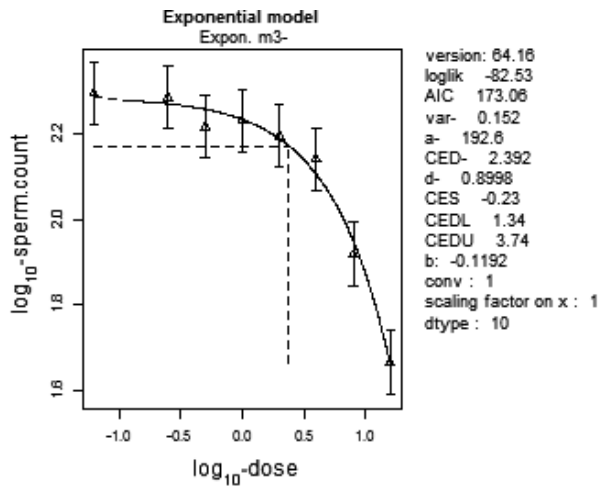
| Model      | Converged | loglik  | npar | AIC    |
|------------|-----------|---------|------|--------|
| Full       | Yes       | -80.91  | 9    | 179.82 |
| m1-        | Yes       | -157.13 | 2    | 318.26 |
| Expon. m3- | Yes       | -82.53  | 4    | 173.06 |
| Expon. m5- | Yes       | -82.34  | 5    | 174.68 |
| Hill m3-   | Yes       | -82.51  | 4    | 173.02 |
| Hill m5-   | Yes       | -82.51  | 5    | 175.02 |

AIC: Akaike information criterion.

#### Final BMD Values

| Model      | BMDL | BMDU | BMD  |
|------------|------|------|------|
| Expon. m3- | 1.34 | 3.74 | 2.39 |
| Hill m3-   | 1.94 | 4.25 | 3.02 |

BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.



## Appendix H – Benchmark dose modelling of incidence of seminiferous tubule atrophy in Sprague–Dawley rats (Cho et al., 2008b)

### A. Data description

As discussed in detail in the previous EFSA opinion (EFSA CONTAM Panel, 2016), SD rats (50/sex per dose group) were exposed to 0, 25, 100 or 400 mg/L 3-MCPD via drinking water in a 2-year study. Doses were converted to average daily intake of 0, 2.0, 8.3 or 29.5 mg/kg bw for males. Incidence of testis atrophy was as reported below:

| Dose (mg/kg bw per day) | Testis atrophy | N  |
|-------------------------|----------------|----|
| 0.0                     | 6              | 50 |
| 1.97                    | 16             | 50 |
| 8.3                     | 13             | 50 |
| 29.5                    | 34             | 50 |

bw: body weight.

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017).
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017).

### D. Results

#### Table with summary of the fitted models

| Model           | No. of parameters | loglik  | AIC    | Accepted | BMDL  | BMDU  | BMD | conv |
|-----------------|-------------------|---------|--------|----------|-------|-------|-----|------|
| Null            | 1                 | -128.86 | 259.72 |          | NA    | NA    | NA  | NA   |
| Full            | 4                 | -109.69 | 227.38 |          | NA    | NA    | NA  | NA   |
| Two.stage       | 3                 | -112.35 | 230.70 | No       | NA    | NA    | 5.8 | Yes  |
| Log.logist      | 3                 | -112.64 | 231.28 | No       | NA    | NA    | 9.7 | Yes  |
| Weibull         | 3                 | -112.57 | 231.14 | No       | NA    | NA    | 7.2 | Yes  |
| Log.prob        | 3                 | -113.40 | 232.80 | No       | NA    | NA    | 2.2 | Yes  |
| Gamma           | 3                 | -112.53 | 231.06 | No       | NA    | NA    | 2.0 | Yes  |
| Logistic        | 2                 | -112.32 | 228.64 | Yes      | 5.030 | 7.93  | 6.2 | Yes  |
| Probit          | 2                 | -112.32 | 228.64 | Yes      | 4.860 | 7.63  | 6.0 | Yes  |
| LVM: Expon. m3- | 3                 | -112.17 | 230.34 | No       | 0.195 | 24.10 | 2.7 | Yes  |
| LVM: Hill m3-   | 3                 | -112.31 | 230.62 | No       | 0.218 | 17.80 | 3.0 | Yes  |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

#### Weights for Model Averaging

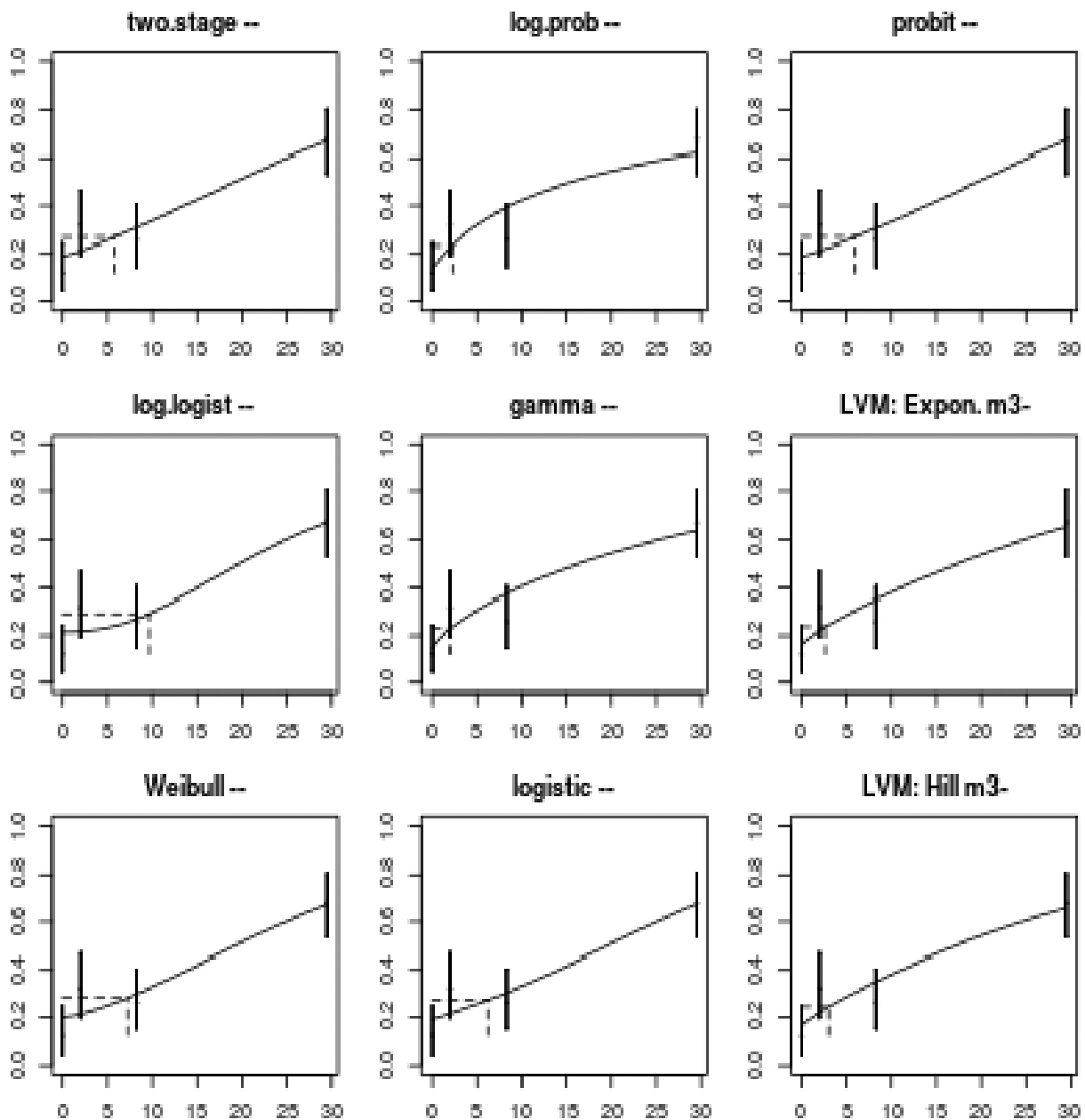
| two.stage | log.logist | Weibull | log.prob | Gamma | Logistic | Probit | EXP | HILL |
|-----------|------------|---------|----------|-------|----------|--------|-----|------|
| 0.09      | 0.06       | 0.07    | 0.03     | 0.07  | 0.24     | 0.24   | 0.1 | 0.09 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

### Final BMD Values

| BMD  | BMDL | BMDU |
|------|------|------|
| 4.86 | 1.55 | 14.4 |





## Appendix I – Benchmark dose modelling of incidence of vacuolisation of epididymal epithelium in Sprague–Dawley rats (Kim et al., 2012)

### A. Data description

Sprague–Dawley male rats (N = 6/group) were exposed to 0, 3, 10 or 30 mg 3-MCPD/kg bw per day for 7 days. Histopathology analyses of epididymis showed an increase in vacuolisation in epithelial cells with the following dose response:

| Dose | Incidence | N |
|------|-----------|---|
| 0    | 0         | 6 |
| 3    | 1         | 6 |
| 10   | 4         | 6 |
| 30   | 6         | 6 |

### B. Selection of the BMR

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017).

### C. Software used and specifications

Results are obtained using the R-package 'bmdModeling'.

- Fitting benchmark dose models is based on the R-package [proast64.9](#).
- Model averaging results from multiple fitted benchmark dose models is based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017).
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017).

### D. Results

Table with summary of the fitted models

| Model           | No. of parameters | loglik | AIC   | Accepted | BMDL  | BMDU  | BMD | conv |
|-----------------|-------------------|--------|-------|----------|-------|-------|-----|------|
| Null            | 1                 | –16.55 | 35.10 |          | NA    | NA    | NA  | NA   |
| Full            | 4                 | –6.52  | 21.04 |          | NA    | NA    | NA  | NA   |
| Two.stage       | 3                 | –6.53  | 19.06 | Yes      | 0.592 | 4.74  | 2.0 | Yes  |
| Log.logist      | 3                 | –6.77  | 19.54 | No       | NA    | NA    | 2.5 | Yes  |
| Weibull         | 3                 | –6.54  | 19.08 | Yes      | 0.269 | 9.93  | 2.2 | Yes  |
| Log.prob        | 3                 | –6.69  | 19.38 | Yes      | 0.581 | 10.70 | 2.6 | Yes  |
| Gamma           | 3                 | –6.57  | 19.14 | Yes      | 0.154 | 9.53  | 2.3 | Yes  |
| Logistic        | 2                 | –6.83  | 17.66 | Yes      | 1.740 | 6.12  | 3.4 | Yes  |
| Probit          | 2                 | –6.76  | 17.52 | Yes      | 1.670 | 5.60  | 3.2 | Yes  |
| LVM: Expon. m3- | 3                 | –6.55  | 19.10 | Yes      | 0.224 | 7.93  | 2.2 | Yes  |
| LVM: Hill m3-   | 3                 | –6.59  | 19.18 | Yes      | 0.309 | 7.54  | 2.4 | Yes  |

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.

### Weights for Model Averaging

| two.stage | log.logist | Weibull | log.prob | Gamma | Logistic | Probit | EXP  | HILL |
|-----------|------------|---------|----------|-------|----------|--------|------|------|
| 0.09      | 0.07       | 0.09    | 0.08     | 0.09  | 0.19     | 0.2    | 0.09 | 0.09 |

Given 1,000 generated data sets, the BMDL is the 5th percentile of all parametric bootstrap BMD values and the BMDU is the 95th percentile.

Estimated BMD (in mg/kg bw per day) based on the averaged response model which is a weighted average of the accepted models' response values.

### Final BMD values from model averaging

| BMD  | BMDL | BMDU |
|------|------|------|
| 2.59 | 1.34 | 8.50 |

