Scientific opinion on the risks for human health related to the presence of 3-and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food

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Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food

EFSA Panel on Contaminants in the Food Chain (CONTAM)

Abstract

EFSA was asked to deliver a scientific opinion on free and esterified 3- and 2-monochloropropane-1, 2-diol (MCPD) and glycidyl esters in food. Esters of 3- and 2-MCPD and glycidol are contaminants of processed vegetable oils; free MCPDs are formed in some processed foods. The Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated 7,175 occurrence data. Esters of 3- and 2-MCPD and glycidyl esters were found at the highest levels in palm oil/fat, but most vegetable oil/fats contain substantial quantities. Mean middle bound (MB) dietary exposure values to total 3-MCPD, 2-MCPD and glycidol, respectively, across surveys and age groups in μg/kg body weight (bw) per day were 0.2–1.5, 0.1–0.7 and 0.1–0.9; high exposure (P95) values were 0.3–2.6, 0.2–1.2 and 0.2–2.1. Animal studies show extensive hydrolysis of esterified 3-MCPD and glycidol following oral administration; esterified and free forms were assumed to contribute equally to internal exposures. Nephrotoxicity was consistently observed in rats treated with 3-MCPD. Data on 2-MCPD toxicity were insufficient for dose–response assessments. Chronic treatment with glycidol increased the incidence of tumours in several tissues of rats and mice, likely via a genotoxic mode of action. The Panel selected a $BMDL_{10}$ value for 3-MCPD of 0.077 mg/kg bw per day for induction of renal tubular hyperplasia in rats and derived a tolerable daily intake (TDI) of 0.8 μg/kg bw per day. The mean exposure to 3-MCPD was above the TDI for 'Infants', 'Toddlers' and 'Other children'. For glycidol, the Panel selected a T25 value of 10.2 mg/kg bw per day for neoplastic effects in rats. The margins of exposure (MoEs) were 11,300–102,000 and 4,900–51,000 across surveys and age groups at mean and P95 exposures, respectively. An exposure scenario for infants receiving formula only resulted in MoEs of 5,500 (mean) and 2,100 (P95). MoEs of 25,000 or higher were considered of low health concern.

Keywords: MCPD, glycidol, glycidyl fatty acid esters, process contaminant, refined oil fat

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Summary

Following a request from the European Commission, the Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on the risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters (GE) in food.

3-Monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) are chlorinated derivatives of glycerol (1,2,3-propanetriol). 3- and 2-MCPD and their fatty acid esters are among non-volatile chloropropanols, identified in the late 1970s in the composition of hydrolysed vegetable protein (HVP) which is used as a savoury flavour-enhancing food ingredient. 3- and 2-MCPD fatty acid esters are produced in vegetable oils on refining, and they contain the fatty acids common to the parent oils and fats in a similar ratio.

Glycidol is associated with the formation and decomposition of 3- and 2-MCPD. It forms monoesters with fatty acids (GE) during the refining of vegetable oils.

Chloropropanols are formed in HVP during the hydrochloric acid-mediated hydrolysis step of the manufacturing process. In food production, chloropropanols form from the reaction of endogenous or added chloride with glycerol or acylglycerols, although the precise mechanism has not yet been elucidated. The major proposed routes of formation of 3- and 2-MCPD fatty acid esters include a direct nucleophilic attack by chloride ions on the acylglycerol carbon atom attached to an ester or hydroxyl group; the formation via chlorination of a GE; and formation via a cyclic acyloxonium ion or a cyclic acyloxonium free radical.

The only identified source of GE in food is refined vegetable oil where they appear to be formed during the heating of diacylglycerol (DAG) or monoacylglycerol under the high temperature conditions of deodorisation.

GE formation is believed to be independent from MCPD fatty acid ester formation, although they may also be formed by elimination of hydrochloric acid from MCPD monoesters that have a vicinal chlorohydrin structure. These monoesters are 3-MCPD that is esterified in the 1- or 2-position and 2-MCPD monoesters.

Processing conditions used in the manufacture of HVP produce free 3- and 2-MCPD. Steps are now taken routinely to both reduce their formation and to lower their levels, but the problem is not eliminated. Glycidyl esters are formed from DAG on heating vegetable oils to temperatures in excess of 200°C, for example during the deodorisation stages of refining, and are therefore a particular problem in palm oil, which can have a high (4–12%) DAG content.

Analytical methods for free 3- and 2-MCPD in foods are well characterised, validated for a suitable range of foods and fit for purpose. There are no suitable methods for unstable free glycidol. Indirect methods for ester-bound 3- and 2-MCPD and glycidol in foods are well characterised for the important range of foods. Three validated American Oil Chemists' Society (AOCS) methods exist and provide directly comparable results. The methods for free MCPD do not include MCPD released from esters, and the methods for ester-bound 3- and 2-MCPD and glycidol do not provide data for the free compounds. Two methods were therefore applied – one for free MCPD and one for MCPD and glycidol released from bound forms.

In its exposure assessment, the CONTAM Panel considered a total of 7,175 occurrence data on 3-MCPD, 2-MCPD and glycidol (collated as measures were implemented to reduce the levels of these compounds in edible fats/oils). Data on glycidol were only available from the ester-bound form. Three categories of analytical data were considered – one on 3-MCPD (in free form) in soy sauce, HVP and related products (702 data points); another on 3- and 2-MCPD from esters and glycidol from esters in oils/fats (4,754 data points); and a third one on 3- and 2-MCPD (free and from esters) and glycidol (from esters) in food groups other than those mentioned above (1,719 data points). In the third category, in most cases, the contribution to the total 3- and 2-MCPD from the free form was included, while the results on glycidol were only from esters. In fats and oils, only the ester-bound forms were analysed because the contribution of the free forms was considered negligible. More than half of the data referred to fats and oils, but other food groups where the presence of these substances is expected were also represented in the data set. Where possible, for food groups not represented in the data set, the occurrence of 3-, 2-MCPD and glycidol was calculated using a model based on the available data on fats and oils, taking into account the market share of the different oils in Europe. This model was also used to estimate the contribution of 3- and 2-MCPD from ester-bound forms in food groups for which data were available only for the free forms.

The highest occurrence values were found in the food group 'Fats and oils', with 'Palm oil/fat' showing a mean middle bound (MB) level of 2,912 μg/kg for 3-MCPD (from esters), 1,565 μg/kg for 2-MCPD (from esters) and 3,955 μg/kg for glycidol (from esters). Lower mean MB levels were calculated for other oils, ranging between 48 and 608 μg/kg for 3-MCPD (from esters), between 86 and 270 μg/kg for 2-MCPD (from esters) and between 15 and 650 μg/kg for glycidol (from esters). 'Margarine, normal fat' had mean MB levels of 668 μg/kg for 3-MCPD (from esters), 236 μg/kg for 2-MCPD (from esters) and 582 μg/kg for glycidol (from esters). Among food groups other than fats and oils, the highest levels were observed in 'Potato crisps' (mean MB levels of 216 μg/kg for total 3-MCPD, 135 μg/kg for total 2-MCPD and 110 μg/kg for glycidol from esters), 'Hot surface cooked pastries' (mean MB levels of 247 μg/kg for total 3-MCPD, 123 μg/kg for total 2-MCPD and 137 μg/kg for glycidol from esters), 'Cookies' (mean MB levels of 200 μg/kg for total 3-MCPD, 103 μg/kg for total 2-MCPD and 134 μg/kg for glycidol from esters) and 'Short crusts' (mean MB levels of 154 μg/kg for total 3-MCPD, 79 μg/kg for total 2-MCPD and 149 μg/kg for glycidol from esters).

The exposure assessment for 3- and 2-MCPD was based upon the level of exposure to the parent compounds, regardless of their original form (i.e. as free or as ester of fatty acids), and referred to as 3-MCPD and 2-MCPD. Likewise, exposure to glycidol referred to the parent compound, although in this case, the original forms were exclusively as fatty acid esters.

Chronic dietary exposure to 3- and 2-MCPD and glycidol was assessed as mean and high (95th percentile, P95) exposure across dietary surveys. The exposure levels showed relatively little difference between lower bound (LB) and upper bound (UB) estimates, and the risk characterisation was therefore based on MB estimates of exposure. In all scenarios, the youngest population groups ('Infants', 'Toddlers' and 'Other children') showed the highest dietary exposure estimates.

The mean exposure to 3-MCPD was 0.5–1.5 μg/kg body weight (bw) per day (MB) across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to 3-MCPD was 1.1–2.6 μg/kg bw per day (MB) across dietary surveys in these age groups. In adolescents and adult population groups (adults, elderly, very elderly), the mean exposure to 3-MCPD ranged from 0.2 to 0.7 μg/kg bw per day (MB) and the high exposure (P95) ranged from 0.3 to 1.3 μg/kg bw per day (MB).

The mean 2-MCPD exposure (MB) across dietary surveys ranged from 0.2 to 0.7 μg/kg bw per day, for 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to 2-MCPD was 0.5–1.2 μg/kg bw per day (MB) across dietary surveys in these age groups. In adolescents and adult population groups (adults, elderly, very elderly), the mean exposure to 2-MCPD ranged from 0.1 to 0.3 μg/kg bw per day (MB) and the high exposure (P95) ranged from 0.2 to 0.6 μg/kg bw per day (MB).

The mean exposure to glycidol was 0.3–0.9 μg/kg bw per day (MB) across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to glycidol was 0.8–2.1 μg/kg bw per day (MB) across dietary surveys in these age groups. In adolescents and adult population groups (adults, elderly, very elderly), the mean exposure to glycidol ranged from 0.1 to 0.5 μg/kg bw per day (MB). The high exposure (P95) in 'Adolescents' ranged from 0.4 to 1.1 μg/kg bw per day (MB) and in adults and older population groups ranged from 0.2 to 0.7 μg/kg bw per day (MB).

Exposure scenarios of infants receiving formula only, based on mean consumption and mean occurrence in the formula, resulted in daily intake of 2.4 μg/kg bw for 3-MCPD, 1.0 μg/kg bw for 2-MCPD and 1.9 μg/kg bw for glycidol. Using P95 occurrence data resulted in daily intake of 3.2 μg/kg bw for 3-MCPD, 1.6 μg/kg bw for 2-MCPD and 4.9 μg/kg bw for glycidol.

For 'Infants', the food groups 'Infant and follow-on formulae', 'Vegetable fats and oils' and 'Cookies' were the major contributors to 3- and 2-MCPD and glycidol exposure. For 'Toddlers', the food groups 'Vegetable fats and oils', 'Cookies' and 'Pastries and cakes' were the major contributors to 3- and 2-MCPD and glycidol exposure. 'Infant formula' and follow-on formula' were also important contributors to 3- and 2-MCPD exposure. For 'Other children', the food groups with highest contribution to exposure to 3- and 2-MCPD and glycidol were 'Pastries and cakes', 'Margarine and similar' and 'Cookies'. For glycidol, 'Fried or roast meat' was an additional relevant contributor. 'Vegetable fats and oils' also contributed to 3- and 2-MCPD, and glycidol exposure. For 'Adolescents', 'Adults', 'Elderly' and 'Very elderly', the major sources of 3- and 2-MCPD and glycidol were 'Margarine and similar' and 'Pastries and cakes'. Additionally, 'Fried or baked potato products' were important contributors to 3- and 2-MCPD exposure while 'Fried or roast meat' and in some cases 'Chocolate spreads and similar' were important contributors to glycidol exposure.

3-MCPD and its dipalmitate fatty acid esters appear to be rapidly and efficiently absorbed following ingestion with extensive presystemic de-esterification occurring in the gastrointestinal tract of rats.

Elimination of 3-MCPD from serum was also rapid following dosing with either the parent compound or its dipalmitate ester. 3-MCPD is extensively metabolised by routes including conjugation to glutathione and oxidation to b-chlorolactate and oxalic acid, with less than 5% appearing in the urine and faeces as parent compound. The majority of 3-MCPD is eliminated from serum within a few hours of dosing with either the parent compound or its dipalmitate ester.

No toxicokinetic data for 2-MCPD were identified. However, the difference in the structural localisation of the chorine within the molecule makes it unlikely that 2-MCPD exhibits the same metabolic pattern as 3-MCPD.

Glycidol and its fatty acid esters are efficiently absorbed following ingestion. Significant presystemic hydrolysis of GE occurs, although the de-esterification process appears to be more extensive in rats than in monkeys. Metabolism of the glycidol moiety proceeds rapidly by several enzymatic pathways, including glutathione conjugation and mercapturate formation. The glycidol moiety is predominantly excreted in urine as poorly described metabolites.

In short-term studies in rat, 3-MCPD produces severe renal toxicity at single intraperitoneal (i.p.) doses between 100 and 120 mg/kg bw, which persists for several weeks. Repeated oral doses also result in renal toxicity, and progressive nephropathy and renal tubule dilation can be seen after a daily dose as low as 5.2 mg/kg bw. The renal toxicity of 3-MCPD appears to reside with the R isomer.

3-MCPD administered to rats at 30 mg/kg bw per day impaired red blood cell function by decreasing haemoglobin content and inducing volume fraction changes consistent with normocytic and normochromic anaemia.

Neurotoxic effects such as hind limb paralysis were reported only at doses over 50 mg/kg bw per day following short-term exposure in mice.

In long-term studies, doses as low as 2 mg/kg bw per day 3-MCPD caused progressive nephrotoxicity (characterised by tubular hyperplasia), testicular toxicity (atrophy and arteritis) and mammary glandular hyperplasia in male rats and nephrotoxicity in female rats. Related to these effects, benign tumours of the testes (Leydig cell tumours), mammary gland (fibroadenoma) and kidney (tubular adenoma) developed.

Doses between 5 and 10 mg/kg bw per day 3-MCPD administered to rats can completely impair male fertility without changing sperm production. This effect has been demonstrated in several species including primates and is reversible. The no observed adverse effect level (NOAEL) of 3-MCPD on male fertility is not clear. Single and multiple doses of 3-MCPD administered to pregnant rats decreased the number of implantations and increased fetal loss but were not teratogenic. The NOAEL for multiple doses was 10 mg/kg bw per day for maternal toxicity and 30 mg/kg bw per day for fetal toxicity.

Despite some positive genotoxicity tests *in vitro*, there is no evidence that 3-MCPD is genotoxic *in vivo* in any organ tested, including the kidney and testis.

From the available information on 3-MCPD fatty acid esters, it can be concluded that the range of toxic effects for esterified 3-MCPD is the same as that seen for the free 3-MCPD, supporting the view that the esters are cleaved and toxicity is primarily exerted by 3-MPCD.

After equimolar multiple doses of 3-MCPD and 3-MCPD dipalmitate, the biochemical changes associated with renal toxicity are similar in pattern and magnitude. Both compounds produce an array of renal histopathology including glomerular lesions and tubular epithelial hyperplasia.

There is limited evidence that some esters of 3-MCPD have male antifertility effects at a similar molar dose to 3-MCPD, and degenerative changes in the spermatogenic tubules have been recorded after treatment with 3-MCPD fatty acid esters.

No studies on the *in vitro* genotoxicity of 3-MCPD fatty acid esters were identified. From the limited evidence (one study with different endpoints) available, there is no indication that 3-MCPD fatty acid esters are genotoxic *in vivo*.

The CONTAM Panel concluded that the kidney and testis appeared to be the main target organs for 3-MCPD-induced toxicity, the toxic effects being associated with oxidative metabolism of 3-MCPD to β-chlorolactaldehyde and β-chlorolactic acid. The inhibition of glycolysis by metabolites associated with the β-chlorolactate pathway was suggested as the possible nephron- and spermo-toxic mechanism of 3-MCPD.

The CONTAM Panel concluded that the Leydig cell and mammary gland tumours observed following long-term exposure to 3-MCPD were probably not relevant to humans.

The CONTAM Panel selected two long-term exposure studies where rats received 3-MCPD via drinking water to perform dose–response analysis for effects in the kidney and testis. The results of both studies were analysed and those showing a monotonic dose–response trend were selected for benchmark dose (BMD) analysis.

The CONTAM Panel established a tolerable daily intake (TDI) of 0.8 μg/kg bw per day for 3-MCPD. This was based on a chronic study in rats in which the lowest $BMDL_{10}$ of 0.077 mg/kg bw per day for renal tubular hyperplasia in males was derived and application of an overall uncertainty factor of 100.

Noting the lack of specific data on 3-MCPD fatty acid esters and their hydrolysis, the CONTAM Panel confirmed that the toxicity of 3-MCPD fatty acid esters should be considered equivalent (on a molar basis) to that of the parent compound. Therefore, the CONTAM Panel concluded that the TDI of 0.8 μg/kg bw per day constitutes a group TDI for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).

The mean exposure to 3-MCPD was below the established group TDI of 0.8 μg/kg bw per day in 'Adolescents', 'Adults' and older age classes in all dietary surveys. In 'Infants', 'Toddlers' and 'Other children', half of the dietary surveys had mean exposure at or above the group TDI, up to a maximum of about 1.5 μg/kg bw per day in 'Toddlers' and 'Other children'. The high exposure (P95) to 3-MCPD for 'Infants', 'Toddlers' and 'Other children' was above the group TDI in all dietary surveys, ranging between a minimum of 1.1 μg/kg bw per day in 'Other children' or roughly 1.5 μg/kg bw per day in 'Infants' and 'Toddlers' up to about 2.5 μg/kg bw per day in all the three age classes. The estimated exposure to 3-MCPD of infants receiving formula only was 2.4 μg/kg bw per day using mean occurrence and 3.2 μg/kg bw per day using P95 of occurrence; both values are above the group TDI, which is exceeded up to fourfold. The high exposure (P95) to 3-MCPD for adolescents was at or above the group TDI in half of the dietary surveys, with exposure estimates up to 1.4 μg/kg bw per day. For 'Adults' and the older age classes, only the maximum P95 of dietary exposure to 3-MCPD was around the group TDI.

There are limited data on the short-term toxicity of 2-MCPD. Acute median lethal dose (LD_{50}) was estimated to be between 50 and 60 mg/kg bw in rats. A single i.p. dose of 200 mg/kg bw, although generally toxic, did not cause signs of renal toxicity. In a 28-day study in rats, daily doses of 16 or 30 mg/kg bw caused severe myopathy and nephrotoxicity. From 8 days of treatment, severe lesions leading to cell death developed in striated muscle, particularly in cardiac myocytes that resulted in heart failure and the death of some animals. These effects were not observed at 2 mg/kg bw per day. No data on long-term studies for 2-MCPD or 2-MCPD fatty acid esters were identified.

In vitro genotoxicity data on 2-MCPD were too limited to make any conclusion. No mammalian *in vivo* genotoxicity studies have been identified for 2-MCPD and 2-MCPD fatty acid esters.

2-MCPD did not induce kidney toxicity at doses at which 3-MCPD produced renal failure, enlarged kidneys and long-lasting diuresis. These differences were explained by the fact that metabolism of 2-MCPD to β-chlorolactaldehyde and β-chlorolactate cannot occur, which is believed to play an important role in nephrotoxicity of 3-MCPD. The underlying mechanisms for renal toxicity and the destruction of striated muscles, including the heart, are unknown.

Although the exposure data were available, it was not possible to undertake risk characterisation for 2-MCPD due to the lack of information.

For glycidol, neurotoxicity was observed after 28 days of treatment of rats with 200 mg/kg bw per day. Glycidol caused renal toxicity in repeated dose studies in rats and mice at doses in the range 150–400 mg/kg bw per day.

Two-year carcinogenicity studies in mice (25 and 50 mg/kg bw per day) and rats (37.5 and 75 mg/kg bw per day) showed induction of tumours in multiple organs from both sexes. Supporting evidence for carcinogenicity of glycidol was provided by a short-term study in a transgenic mouse strain.

Male anti-fertility effects have been noted in rats and mice. The lowest observed adverse effect level (LOAEL) was 25 mg/kg bw per day in the rat, resulting in a 36% reduction in epididymal sperm count. This may be attributed to conversion of glycidol to 3-MCPD in the stomach. Glycidol was maternally toxic in mice without producing any major external abnormalities in the fetus. Neurotoxicity was observed in male pups of rats exposed to a maternal dose of 49 mg glycidol/kg bw per day during pregnancy and weaning.

Glycidol and its esters, from which the free compound can be derived, possess a reactive epoxide moiety. There is strong evidence from *in vitro* data and some evidence from *in vivo* studies that glycidol is a genotoxic compound.

The CONTAM Panel only considered toxicity studies in animals with glycidol as no *in vivo* data were identified for glycidyl esters. Dose–response considerations were made for glycidol assuming a complete hydrolysis of the esters to free glycidol following ingestion. However, the dose–response data were considered inadequate for BMD modelling. Based on the EFSA Guidance on substances that are genotoxic and carcinogenic, T25 values were calculated for the incidence of tumours observed in rats

and mice following long-term exposure to glycidol. A T25 of 10.2 mg/kg bw per day for peritoneal mesothelioma in male rats was used as the reference point.

In view of the genotoxic and carcinogenic potential of glycidol, a margin of exposure (MoE) approach was applied. MoE estimates were calculated by dividing the reference point of 10.2 mg/kg bw per day by the exposure levels. A MoE of 25,000 or higher was considered of low health concern.

For 'Infants', 'Toddlers' and 'Other children', the MoE estimates for the mean exposure ranged from 34,000 to 11,300; the MoE for high (P95) exposure ranged from 12,800 to 4,900. For 'Adolescents' and 'Adults', 'Elderly' and 'Very elderly' age groups, the MoE for the mean exposure ranged from 102,000 to 20,400, whereas at high (P95) exposure the range was from 51,000 to 9,300.

The MoE estimates corresponding to the P95 of exposure for 'Infants' were particularly low due to the contribution of glycidyl esters from infant formulae. The scenarios calculated for 'Infants' receiving only formula diet resulted in a MoE of about 5,400 for the mean occurrence and 2,100 for the P95 of occurrence.

In conclusion, estimated exposure substantially exceeding the group TDI for 3-MCPD is of concern; this is particularly seen in the younger age groups. Although there is a high uncertainty in the reference point used as a basis for the calculation of the MoEs for glycidol, the MoEs lower than 25,000 indicate a health concern.

The CONTAM Panel recommended to include all food groups potentially contaminated and foods where mitigation measures have been enforced in the future monitoring activities for 3-, 2-MCPD and glycidol. The enantiomeric composition of 3-MCPD and its fatty acid esters present in food should be studied. Further studies on the rates and degree of de-esterification and the metabolic fate for 3- and 2–MCPD fatty acid esters and GE were recommended. For 2-MCPD, the CONTAM Panel recommended generation of additional data to elucidate the long-term toxicity and the mode and mechanism of action of the substance. More extensive testing of the dose–response for carcinogenesis from chronic lifetime oral administration of glycidol and its esters in rats would reduce uncertainty in the risk assessment.

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1. Introduction

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

3-Monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant for which a tolerable daily intake (TDI) of 2 μg/kg bw has been established. A maximum level of 20 μg/kg for hydrolysed vegetable protein (HVP) and soy sauce has been established for liquid products containing 40% dry matter, corresponding to a maximum of 50 μg/kg in the dry matter by Commission Regulation (EC) 1881/2006[1].

Esters of 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) and glycidyl esters are important contaminants of processed edible oils used as foods or food ingredients. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) agreed with the estimate of 100% release of 3-MCPD from its esters in humans.[2]

Glycidyl fatty acid esters (GE) are process contaminants generated during the deodorisation step of edible oil refining. The toxicological relevance of GE has not yet been fully elucidated. Glycidol itself is categorised as probably carcinogenic to humans. Latest scientific studies indicate an (almost) entire release of glycidol from fatty acid esters within the human digestive tract.

EFSA has published on 20 September 2013 a scientific report on the analysis of occurrence of 3-MCPD in food in Europe for the years 2009–2011 and preliminary exposure assessment.[3]

The European Commission would like to request from EFSA a scientific opinion on the risk for public health as the consequence of the presence of 3- and 2-MCPD and glycidyl esters in food, with a view to taking permanent risk management measures.

The opinion should address the possible acute and chronic health effects, including risks for specific vulnerable population groups, and address the question whether an acute reference dose (ARfD) is needed. It should also address the question whether the use of a variability factor would be appropriate.

In order to enable EFSA to carry out such risk assessment, Member States with the active involvement of food business operators were requested to monitor the presence of 3- and 2-MCPD and glycidyl esters in food and to submit those data to EFSA and the Commission before 1 October 2014. Monitoring guidelines, defining the data to be submitted and their format, have been circulated among Member States and food business operators.

Terms of Reference as provided by the European Commission

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risks for human health related to the presence of 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane1,3-diol (2-MCPD) (3- and 2-MCPD) and 3- and 2-MCPD fatty acid esters and GE in food.

The scientific opinion should, *inter alia,* comprise evaluation of the toxicity of 3- and 2-MCPD, 3- and 2-MCPD fatty acid esters and GE for humans, considering all relevant toxicological endpoints;

the exposure of the EU population to 3- and 2-MCPD fatty acid esters and GE in addition to the exposure to 3- and 2-MCPD, including the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc.).

1.2. Additional information

1.2.1. Definitions

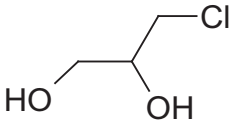
This Opinion refers to 3-MCPD and 2-MCPD and their fatty acid esters, and also to fatty acid esters of glycidol.

[1] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5.

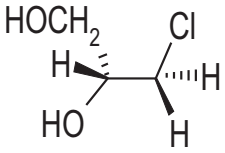
[2] Statement of the Scientific Panel on Contaminants in the Food chain (CONTAM) on a request from the European Commission related to 3-MCPD esters. Available online: http://www.efsa.europa.eu/en/efsajournal/doc/1048.pdf

[3] European Food Safety Authority, 2013 Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009–2011 and preliminary exposure assessment. EFSA Journal 2013;11(9):3381, 45 pp. doi:10.2903/j.efsa.2013.3381. Available online: www.efsa.europa.eu/efsajournal

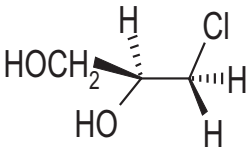
3-MCPD refers to 3-monochloropropane-1,2-diol [CAS Number 96-24-2].



Its IUPAC name is 3-chloropropane-1,2-diol (3-CPD).
It has also been called α-chlorohydrin and glycerol α-monochlorohydrin.
Its empirical formula is $C_3H_7ClO_2$ and molecular weight is 110.5.
3-MCPD exists as enantiomers (R)-(-)-3-chloropropane-1,2-diol [CAS Number 57090-45-6] and (S)-(+)-3-chloropropane-1,2-diol [CAS Number 60827-45-4].

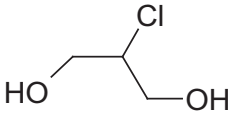


(*R*)-3-MCPD



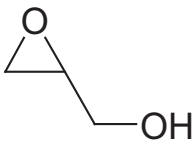
(*S*)-3-MCPD

2-MCPD refers to 2-monochloropropane-1,3-diol [CAS Number 497-04-1].



Its IUPAC name is 2-chloropropane-1,3-diol (2-CPD).
It has also been called beta-chlorohydrin and glycerol β-monochlorohydrin.
Its empirical formula is $C_3H_7ClO_2$ and molecular weight is 110.5.

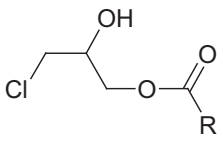
Glycidol has the IUPAC name oxiranylmethanol [CAS Number 556-52-5].



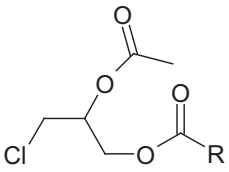
It has also been called 2,3-epoxy-1-propanol, 3-hydroxypropylene oxide, epoxypropyl alcohol, hydroxymethyl ethylene oxide, and 2-hydroxymethyl oxiran.
Glycidol has the empirical formula $C_3H_6O_2$ and molecular weight is 74.08.
Glycidol exists as the optically active isomers (*R*)-(+)-glycidol [CAS Number 57044-25-4] and (*S*)-(-)-glycidol [CAS Number 60456-23-7].

3-MCPD, 2-MCPD and glycidol esters

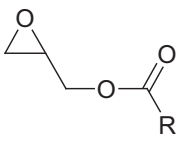
3-MCPD, 2-MCPD and glycidol can each form esters with the fatty acids commonly present in edible vegetable oils.



3-MCPD monoester



3-MCPD diester



glycidyl ester

R: Fatty acid

3-MCPD and 2-MCPD can each form monoesters in which one of the two hydroxyl groups is esterified. They can also form diesters where both hydroxyl groups are esterified with the same or with different fatty acids.

Glycidol has a single hydroxyl group and so forms only monoesters.

1.2.2. Background

Chloropropanols are chlorinated derivatives of glycerol (1,2,3-propanetriol), having one or two chlorine atoms in various configurations on the glycerol molecule. Their presence in food was discovered by Velíšek et al. (1978) during studies carried out at the Institute of Chemical Technology in Prague into the composition of acid-HVP used as a savoury flavour-enhancing food ingredient. Several chloropropanols were identified in HVP and in subsequent studies on model systems. The major volatile compounds found were 3-chloropropan-1-ol, 1,3-dichloropropan-2-ol (1,3-DCP), 2, 3-dichloropropan-1-ol (2,3-DCP). Non-volatile chloropropanols found were 3-monochloropropane-1, 2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) and their fatty acid esters (Velíšek et al., 1979, 1980; Davídek et al., 1980). Efforts to remove the simple chloropropanols from foodstuffs have led to a significant reduction in contamination; however, the extent and level of contamination of 3- and 2-MCPD fatty acid esters in foods was largely overlooked until Svejkovská et al. (2004) reported their presence in a range of foods, and the source was later identified as refined vegetable oil (Zelinková et al., 2006).

3- and 2-MCPD fatty acid esters are produced in vegetable oils on refining and they contain the fatty acids common to the parent oils and fats and in a similar ratio, although some factors such as volatility and deodorisation conditions can cause small differences. Glycidol is associated with the formation and decomposition of 3- and 2-MCPD. It forms monoesters with fatty acids (GE) during the refining of vegetable oils.

The formation, occurrence, analysis and toxicity of 3- and 2-MCPD and their esters including GE has been reviewed to varying extent in several publications (Hamlet, 2008, 2009; Hamlet and Sadd, 2009; Larsen, 2009; Bakhiya et al., 2011; Andres et al., 2013; Jala et al., 2015; Teng and Wang, 2015).

1.2.3. Previous assessments

3- MCPD and 3-MCPD fatty acid esters

The European Commission's Scientific Committee on Food (SCF) concluded that the increase in benign tumours observed in the long-term carcinogenicity assay in rats is the result of non-genotoxic mechanisms (SCF, 1994), either through chronic hormonal imbalance (mammary gland fibromas, Leydig cell tumours) or sustained cytotoxicity and chronic hyperplasia (renal tumours). This conclusion was also reached by the UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (UK COC, 2000). 3-MCPD was classified by the European SCF in 2001 as a non-genotoxic, threshold carcinogen (SCF, 2001; JECFA, 2002).

In 2004, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a risk assessment on the presence of 3-MCPD in food, which results from hydrochloric acid hydrolysis of vegetable protein and mainly occurred in soy sauce. Renal tubular hyperplasia represented the critical effect in rats exposed chronically. Data indicating a lack of genotoxicity *in vivo* led the Committee to conclude that MCPD induces neoplasia in the rat by a mechanism that does not involve DNA damage and does require exposure above a threshold dose. A provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bw per day was established based on a lowest observed effect level (LOEL) of 1.1 mg/kg bw per day for renal tubular hyperplasia and a safety factor of 500. Exposure data available to the Committee, which showed infrequent occurrences of 3-MCPD in the soy sauce, indicated that the estimated mean intake of 3-MCPD by consumers of soy sauce would be at or above the PMTDI.

In 2008, the CONTAM Panel released a statement in response to a request from the EC related to the presence of 3-MCPD fatty acid esters in food. The Panel confirmed the assessment of the Bundesinstitut für Risikobewertung (BfR) in its 2007 opinion regarding the uncertainties described by the BfR associated with the toxicity and bioavailability of 3-MCPD from its fatty acid esters and reaffirmed the need for additional toxicokinetic studies.

An opinion on the presence of 3-MCPD and its fatty acid esters in foods was published by the BfR in 2012 based on its 2007 review of 3-MCPD in food that established a TDI of 2 μg/kg bw per day and newer findings related to bioavailability of MCPD from its fatty acid esters and subchronic toxicity testing of MCPD and its dipalmitate ester in rats. Important data gaps were identified in the current exposure assessment arising from incomplete information on the content of MCPD fatty acid esters in food and the impact of minimisation strategies already implemented by the food industry on the content of MCPD fatty acid esters in food.

In 2012, Codex Alimentarius published a code of practice, based on good manufacturing practices, for the reduction of 3-MCPD during the production of acid-HVP and its products. Several approaches were recommended to reduce the formation of 3-MCPD during hydrolysis of vegetable protein products, including careful control of the temperature and heating time for the acid hydrolysis step, its subsequent neutralisation with alkali, use of sulphuric acid instead of hydrochloric acid, and substitution by fermentation.

In 2013, the International Agency for Research on Cancer (IARC) noted that there was 'no evidence to suggest that 3-MCPD is not genotoxic'. This conclusion was based on the available *in vivo* data at that time: micronucleus assay on bone marrow and unscheduled DNA synthesis in the liver (Robjohns et al., 2003).

None of the authorities have published risk assessments on 2-MCPD.

Glycidol and glycidyl fatty acid esters

Glycidol was classified by IARC as group 2A, 'probably carcinogenic to human' (IARC 2000).

A preliminary assessment of GE in refined vegetable oils was published by the BfR in 2009 based on the preliminary findings of mg/kg levels of GE in several common refined vegetable oils, particularly palm oil. A preliminary estimate of potential dietary intake, especially from margarine and commercial dairy products for infants, the genotoxic and carcinogenic properties for glycidol, and the likelihood for hydrolytic metabolism to bioavailable glycidol, led the BfR to conclude that current levels of exposure of infants and some adults could present a hazard to human health. The BfR recommended that the levels of GE in vegetable oils should be reduced as far as possible. Important data gaps were identified as analytical methodology for accurate quantification of glycidol and its fatty acid esters in edible fats and oils and a determination of the bioavailability of glycidol from its fatty acid esters.

As a part of a safety assessment of foods containing diacylglycerol (DAG) the Food Safety Commission of Japan (FSCJ, 2015) conducted a risk assessment of glycidol and GE. From the evidence available on genotoxicity of glycidol, in particular the glycidol-induced DNA damage and gene mutation, FSCJ concluded glycidol to be a genotoxic carcinogen. Carcinogenicity tests have shown increased incidences of tumours attributable to glycidol exposure in rats and mice. FSCJ noted that the data available for GE show weak genotoxicity compared to glycidol and that the data on carcinogenicity were limited. A benchmark dose (BMD) approach was applied and margins of exposure of 17,800 and 10,900 for average and maximum consumers, respectively, were derived. FSCJ concluded 'While these data suggest no apparent adverse effects due to the consumption of edible oils currently available, the genotoxic carcinogenicity of glycidol was not denied. Therefore, glycidyl ester exposure levels should be kept as low as possible according to the principle of ALARA (as low as reasonably achievable)'.

1.2.4. Chemistry

1.2.4.1. Physical properties

3-MCPD is a colourless or pale yellow viscous oily liquid at room temperature with a density of 1.32 g/cm^{-3}, a melting point of −40°C and a boiling point of 213°C at 760 mmHg. 3-MCPD is hygroscopic, and is highly soluble in water and in organic solvents of moderate to high polarity including methanol, ethanol, chloroform and ethyl acetate.

2-MCPD is similarly a colourless or pale yellow oily liquid at room temperature with a hygroscopic nature. It has a density of 1.3 ± 0.1 g/cm^{-3} and a boiling point of 213°C at 760 mmHg.

Glycidol is a colourless liquid at room temperature, soluble in water and most polar solvents.

Fatty acid esters of 3-MCPD and 2-MCPD and glycidol have similar properties (e.g. polarity and solubility) to the parent fatty acids, with slightly lower melting points (Hamlet et al., 2011). However, detailed measurements of these properties are yet to be made.

1.2.4.2. Chemical properties

3-MCPD is a chiral molecule (described in Section 1.2) and exists as a mixture of (R)- and (S)-enantiomers that are derived from prochiral L-glycerol. They are present in a ratio of 1:1 in acid-HVP. The biological activity of the enantiomers differs and is discussed in if available. It should be noted that the majority of investigations described in this report and elsewhere, across a broad variety of topics from chemistry to toxicity, are for the racemic mixture. 3-MCPD reacts readily with acids,

alcohols, aldehydes, ammonia, amino compounds, ketones and thiols (Velísek et al., 1991). The reactions of 2-MCPD with compounds of these classes are likely to be similar.

3-MCPD, 2-MCPD and glycidol can form esters with fatty acids. The reaction usually occurs under the high temperature conditions of edible oil refining. Thus the fatty acids forming esters are those commonly encountered in edible vegetable oils. The major esterifying acids depend on the type of oil but most common oils are lauric acid (dodecanoic acid C12:0), myristic acid (tetradecanoic acid C14:0), palmitic acid (hexadecanoic acid C16:0), stearic acid (octadecanoic acid C18:0), oleic acid (octadecenoic acid C18:1), linoleic acid (octadecadienoic acid C18:2) and linolenic acid (octadecatrienoic acid C18:3). 3-MCPD and 2-MCPD can each form monoesters and diesters, and in the case of diesters positional isomers exist in which the two hydroxyl groups are esterified with different acids. The esters are formed in a similar ratio to that of the acids in the parent oil, although some factors such as volatility and deodorisation conditions can cause small differences.

Fatty acid esters of 3- and 2-MCPD, and GE, are soluble in non-polar solvents and have poor solubility in water. The full solubility characteristics of individual MCPD fatty acid esters and GE have not been studied; however, it may be assumed that MCPD monoesters have a higher solubility than diesters in polar solvents and that increasing the fatty acid chain length in both mono- and diesters reduces the solubility in polar solvents and increases the solubility in non-polar solvents.

1.2.4.3. Formation mechanisms of 3- and 2-MCPD

Chloropropanols are formed in acid-HVP during the hydrochloric acid-mediated hydrolysis step of the manufacturing process. Acid-HVP is usually manufactured by treating cereal materials, including proteinaceous oilseeds, with hydrochloric acid at high temperature and pressure. The hydrochloric acid reacts with lipids such as triacylglycerols and phospholipids, and with partial acylglycerols (monoglycerols and DAG) and glycerol formed by acid hydrolysis of triacylglycerols (Collier et al., 1991). In food production, chloropropanols are formed from the reaction of endogenous or added chloride with glycerol or acylglycerols, although the precise mechanisms are yet to be fully elucidated. The mechanism for 3- and 2-MCPD formation involves a cyclic acyloxonium ion intermediate (Collier et al., 1991; Hamlet et al., 2003, 2004b). Glycerol is protonated by hydrochloric acid at the primary and secondary hydroxyl groups to form alkyloxonium ions. Water is displaced from the primary position hydroxyl alkyloxonium cations giving a racemic mixture of both 3-MCPD enantiomers. The secondary position hydroxyl group alkyloxonium ions dissociate to give water and a carbocation which reacts with chloride to give 2-MCPD.

The formation of 3-MCPD and 2-MCPD from glycerol and acylglycerols increases with increasing salt concentration and reaches a maximum with a water content of about 15%, and more is formed from triacylglycerols than from glycerol alone. In the absence of added water, there is no prior hydrolysis of the acyl group and 3-MCPD is formed by the direct substitution of the glycerol hydroxyl group by chloride ion.

1.2.4.4. Formation mechanisms of 3-MCPD and 2-MCPD fatty acid esters

Current knowledge on the factors affecting the formation of 3-MCPD fatty acid esters and GE has been summarised by Craft et al. (2013). The formation pathways of 3- and 2-MCPD fatty acid esters have been reviewed (Hamlet, 2009; Hamlet and Sadd, 2009; Hamlet et al., 2011; Zhang et al., 2013; Jala et al., 2015). The major proposed formation routes have been described in some detail by Zhang et al. (2013), Hamlet et al. (2011) and by Rahn and Yaylayan (2011).

Under high temperature conditions in the presence of some water, a triacylglycerol is hydrolysed to give a mixture of DAG isomers (1,2-diacyl- and 1,3-acylglycerol). Further hydrolysis gives a mixture of monoacylglycerol isomers (1-acylglycerol and 2-acylglycerol). The acylglycerols react through mechanisms that include formation via an intermediate acyloxonium cation involving a nucleophilic ring-opening substitution reaction by a chlorine anion, or direct nucleophilic substitution of an ester or a hydroxyl group by a chlorine anion. Alternatively, a cyclic acyloxonium free radical formed by elimination of water at high temperature might react with a chlorine radical. MCPD ester formation is accelerated at high temperatures, but the associated decomposition rates also increase (Svejkovská et al., 2006; Seefelder et al., 2008).

1.2.4.5. Formation of glycidyl fatty acid esters

The only identified source of GE in food is refined vegetable oil where they are believed to be formed during the heating of DAG or monoacylglycerols (partial glycerols) by the elimination of water or a fatty acid under the high temperature conditions of deodorisation (Hrncirik and Ermacora, 2010;

Masukawa et al., 2010; Hrncirik and van Duijn, 2011). The greatest amounts are found in oils with a high DAG content, such as palm oil. The mechanism probably involves an acyloxonium ion or an intramolecular reaction of DAG (Hamlet et al., 2002; Svejkovská et al., 2006; Hrncirik and Ermacora, 2010; Weißhaar and Perz, 2010; Rahn and Yaylayan, 2011; Craft et al., 2012; Destaillats et al., 2012).

GE formation is believed to be independent from MCPD fatty acid ester formation (Destaillats et al., 2012), although they may also be formed by elimination of hydrochloric acid from MCPD monoesters that have a vicinal chlorohydrin structure. These monoesters are 3-MCPD that is esterified in the 1- or 2-position and 2-MCPD monoesters. The acid composition of GE, and the quantity produced during oil refining are much dependent on the level of partial acylglycerol precursors and temperature conditions, as loss through volatilisation accompanies formation, especially for esters of the smaller acids (Craft et al., 2012).

1.2.4.6. Formation of free and esterified MCPD and glycidyl esters during food processing

The manufacture of HVP by hydrochloric acid hydrolysis forms significant levels of free 3- and 2-MCPD; however, steps have now been incorporated that both reduce their formation and to lower their levels to below the limits prescribed in some countries by legislation. In the EU a maximum level of 20 μg/kg 3-MCPD has been set for liquid HVP and soy sauce based on a 40% dry matter content (Commission Regulation (EC) 1881/2006[4]). Reduction measures include lowering of the hydrolysis temperature and decomposition of the MCPD by alkaline hydrolysis (Velíšek, 2009). Soy sauce prepared by enzymatic hydrolysis does not contain detectable free or esterified 3- or 2-MCPD.

3-MCPD is formed in fish during smoking and curing by salting (Crews et al., 2002). The level of 3-MCPD in fish (salmon and herring) increases with the smoking time and salt concentration. In long-term stored samples such as canned fish 3-MCPD might be released from its esters by lipase activity (Reece et al., 2005).

In model food heating systems containing water, sodium chloride and glycerol or lipid precursors 3-MCPD production increases with increasing temperature once above 160°C, and with NaCl concentration up to 10% with acylglycerol precursors but at about 5% NaCl with glycerol. The optimum water content is 15–20% for 3-MCPD but higher than this for 2-MCPD. Glycerol was the best precursor of 3-MCPD on a weight basis, and monoacylglycerol was a significantly better precursors than DAG or triacylglycerol.

3-MCPD is formed in cereal (barley) when it is roasted in malt production at temperatures above 170°C. 3-MCPD is extracted from the malt during brewing, but on account of dilution is not detectable in most beers. Coffee beans have similar concentrations of fat and chloride to barley but unexpectedly do not form 3-MCPD when heated under similar conditions to malt.

Baked goods are the major source of 3- and 2-MCPD and the formation of these contaminants in model bakery systems has been studied in some detail in model systems (Hamlet et al., 2003, 2004a,b). Free glycerol produced by the action of yeast enzymes is the major precursor and the formation reactions of 3- and 2-MCPD follow zero-order kinetics. The levels of 3-MCPD and 2-MCPD formed increase exponentially with temperature up to the maximum (about 220°C) used in baking. Low (1% up to 15%) moisture promotes 3- and 2-MCPD production with levels of 2-MCPD typically 20% of that of 3-MCPD. The ratio of 3-MCPD to 2-MCPD is related to the water content, on account of either formation or degradation.

Domestic cooking procedures have been shown to increase the levels of 3-MCPD in a limited number of foods studied. The effect is particularly observed in the toasting of bread (Crews et al., 2001; Breitling-Utzmann et al., 2003, 2005; Hamlet and Sadd, 2004). The increase in the level of 3-MCPD content of bread is greater in brown and wholemeal types. It has been shown that domestic grilling can increase 3-MCPD levels in cheeses, and microwave cooking produces a lesser increase (Crews et al., 2001). The frying or grilling of meat in the form of beef burgers produces low levels of 3-MCPD, and a significant increase in 3-MCPD can occurs on frying batter, depending on the ingredient composition. Some pre-cooked or cured meats have also been shown to contain low levels of 3-MCPD (typically < 0.05 μg/kg) prior to cooking under laboratory simulated home cooking, with the highest levels in salami. The formation of 2-MCPD has not been the subject of similar studies.

3-MCPD has been detected as a product of the combustion of wood (Kuntzer and Weißhaar, 2006). It was hypothesised that smoke production was associated with the fission of saccharides to give compounds such as 3-hydroxyacetone which is known to form 3-MCPD on reaction with hydrochloric

[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5.

acid. 3-MCPD can be formed in small amounts from certain carbohydrates (Collier et al., 1991), from the sweetener sucralose (Rahn and Yaylayan, 2010), and by various routes from epichlorohydrin, used in the preparation of paper-coating resins that might come into contact with food (Boden et al., 1997). The formation of 2-MCPD and the epimeric composition of the 3-MCPD formed from the above sources have not been studied.

Esters of 3- and 2-MCPD are formed during the deodorisation step of edible oil refining either from partial acylglycerols (Svejkovská et al., 2006; Hrncirik and Ermacora, 2010; Hamlet et al., 2011; Matthäus, 2012; Shimizu et al., 2012; Freudenstein et al., 2013) or from triacylglycerol (Destaillats et al., 2012). Partial acylglycerols form MCPD fatty acid esters more readily than triacylglycerol (Svejkovská et al., 2006; Matthäus et al., 2011; Shimizu et al., 2012; Freudenstein et al., 2013). The quantity of 3- and 2-MCPD fatty acid esters formed in the oil is not clearly correlated with the level of partial acylglycerols fatty acid ester. The DAG content of oils in fresh fruit pulps such as olive and palm is usually low (1–3%) but in the oil of fruits of poor quality and in stored and transported oils the quantity of DAG, particularly 1,3-DAG increases. Where the DAG content exceeds 3–4% there is an exponential increase in GE formation during deodorisation (Craft et al., 2012). The partial acylglycerol content of seed oils is generally lower. Partial acylglycerols are also formed from the action of heat and steam during cooking operations such as frying (Hamlet et al., 2011).

The acylglycerols react with chlorine released from organic compounds present in the oil naturally, levels of which may be enhanced by uptake of chloride from inorganic fertilisers added to soil (Nagy et al., 2011). The chlorine is released under high temperatures, particularly during deodorisation, forming MCPD fatty acid esters and other compounds from reactants that might compete for the available chlorine (Matthäus et al., 2011, 2012; Shimizu et al., 2012, 2013a,b; Freudenstein et al., 2013).

The quantity of available chlorine is the limiting factor of MCPD fatty acid ester formation in edible oil refining. The chlorine source comprises a number of organic and inorganic compounds that have not been characterised. Those of higher polarity form the major chloride source through either their greater abundance or higher reactivity (Ermacora and Hrncirik, 2014).

Glycidyl esters are formed mainly from DAG on heating vegetable oils to temperatures in excess of 200°C (Masukawa et al., 2010; Hrncirik and van Duijn, 2011; Destaillats et al., 2012; Craft et al., 2012) for example during the deodorisation stages of chemical or physical refining, and are therefore a particular problem in palm oil, which can have a high (4–12%) DAG content. The formation rate and level is related to the availability of precursors, the deodorisation time and temperature (Weißhaar and Perz, 2010; Hrncirik and van Duijn, 2011; Craft et al., 2012), and is independent of the formation of MCPD fatty acid esters. GE can also be formed by the dehydration of monoacylglycerol, but as levels of monoacylglycerol are naturally low and reduced further during deodorisation they are not significant contributors to GE contamination (Craft et al., 2012). The formation mechanism from DAG is likely to proceed via an acyloxonium ion or an intramolecular SN2 reaction (Hamlet et al., 2002; Weißhaar and Perz, 2010; Rahn and Yaylayan, 2011; Destaillats et al., 2012).

There is no evidence of significant change in the levels of MCPD fatty acid esters or GE during the cooking of non-cereal foods (cheeses, salami, cooking oils and potato products). No MCPD fatty acid esters were seen to be formed or lost under conditions that simulated biscuit baking, but in dough containing a commercial lipase, free 3- and 2-MCPD isomers were readily released from isotopically labelled added 3-MCPD fatty acid esters.

1.2.5. Methods of analysis

1.2.5.1. Free 3- and 2-MCPD

Methods for the analysis of free 3- and 2-MCPD were targeted initially at the first identified food source of 3-MCPD, acid-HVP and then at foods found to be frequently contaminated (soy sauce, processed meats and fish, and baked cereals). More recently, the analytical methods for free 3- and 2-MCPD have been included in indirect methods for the measurement of ester-bound MCPD. Descriptions of the methods have been included in various review publications, notably by Wenzl et al. (2007, 2015), Hamlet (2008), Hamlet and Sadd (2009) and Teng and Wang (2015).

Methods are based on the use of gas chromatography with mass spectrometric detection (GC-MS). Derivatisation is required prior to the determination of free 3- and 2-MCPD to improve volatility and the mass spectrometric response.

The first method used for the routine determination of 3-MCPD in acid-HVP was that applied to acid-HVP by Van Bergen et al. (1992). Acid-HVP, which was originally produced as a liquid of high salt

content, was first absorbed on to a solid phase extraction (SPE) column. Volatile and less polar chloropropanols such as 1,3-dichloropropanol (1,3-DCP) were eluted with a mixture of hexane and diethyl ether, and 3-MCPD was eluted separately with diethyl ether. The extracts were combined and derivatised with heptafluorobutyrylimidazole (HFBI) and resulting heptafluorobutyrate (HFB) ethers determined by GC with electron capture detection (GC-ECD).

More sensitive methods were developed from the van Bergen procedure. HVP powders (which replaced earlier liquid products) were dissolved in sodium chloride solution to replicate the liquid HVP. The diethyl ether extract was concentrated to low volume and the 3-MCPD derivatised to the di-(HFB) prior to GC-MS. Deuterated internal standards were also introduced. Food samples were also analysed in this way (after removal of fat) by Hamlet (1998) who used GC with tandem mass spectrometric determination (GC-MS/MS), and by Brereton et al. (2001) who used GC-MS in selected ion monitoring (SIM) mode. The latter method was validated by collaborative trial and was accepted as official methods by the Association of Official Analytical Chemists (AOAC) and by the European Committee for Standardization (CEN, 2004) and has found widespread use (Nyman et al., 2003; Leòn et al., 2008).

Ethyl acetate has frequently been used as an alternative extraction solvent to diethyl ether (Wittmann, 1991; Spyres, 1993; Hamlet, 1998; Chung et al., 2002) and it has been reported to give higher recoveries of 3-MCPD from cereal products such as flour and bread (Hamlet, 1998; Rétho and Blanchard, 2005).

Alternative derivatisation reagents have been applied including *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) (Kissa, 1992; Cao et al., 2009; Racamonde et al., 2011), and heptafluorobutyric anhydride (HFBA) (Chung et al., 2002; Abu-El-Haj et al., 2007).

The most popular current method for determining 3-MCPD is to use a combined extraction and derivatisation procedure by adding an organic solution of phenylboronic acid (PBA), which binds across hydroxyl groups that are in close proximity to form dioxaborolanes (Rodman and Ross, 1986; Pesselman and Feit, 1988). In the PBA procedure an aqueous extract of foodstuff is shaken with a solution of PBA in acetone made basic with pyridine, sodium chloride is added to promote partition and the derivative formed is extracted into hexane. A high concentration of PBA is required for quantitative reaction and the reagent causes some damage to the GC column, but the use of a low temperature injection (< 180°C) can reduce the transfer of reagent to the column (Breitling-Utzmann et al., 2005). The method has been applied widely with minor variations (Ushijima et al., 1995; Breitling-Utzmann et al., 2003; Divinová et al., 2004).

The PBA method as described by Plantinga et al. (1991) has been validated and accepted as the German official methods for 3-MCPD in food (LMBG, 1995). Solvent extraction for removal of fat from some foods prior to implementation of the PBA method has been reported by Divinová et al. (2004).

Alternative derivatisation methods based on bonding across the MCPD hydroxyl groups have been based on the formation of the dioxolane derivative by reaction with acetone or cyclohexanone (Meierhans et al., 1998; Dayrit and Niñonuevo, 2004; Rétho and Blanchard, 2005; Becalski et al., 2013) (Figures 1 and 2).

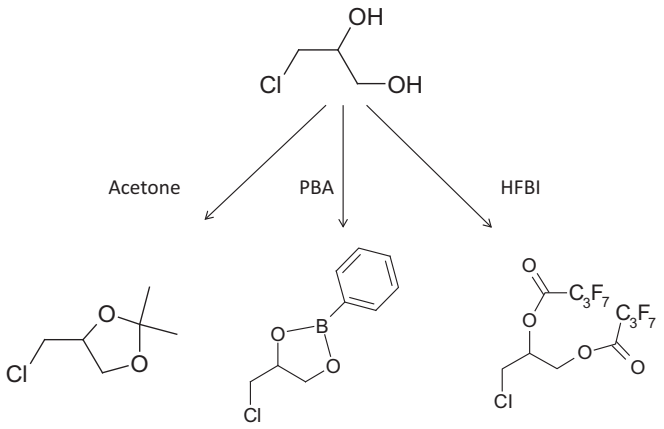


Figure 1: Derivatives formed from reaction of 3-monochloropropane-1,2-diol (3-MCPD) with acetone, phenylboronic acid (PBA) and heptafluorobutyrylimidazole (HFBI)

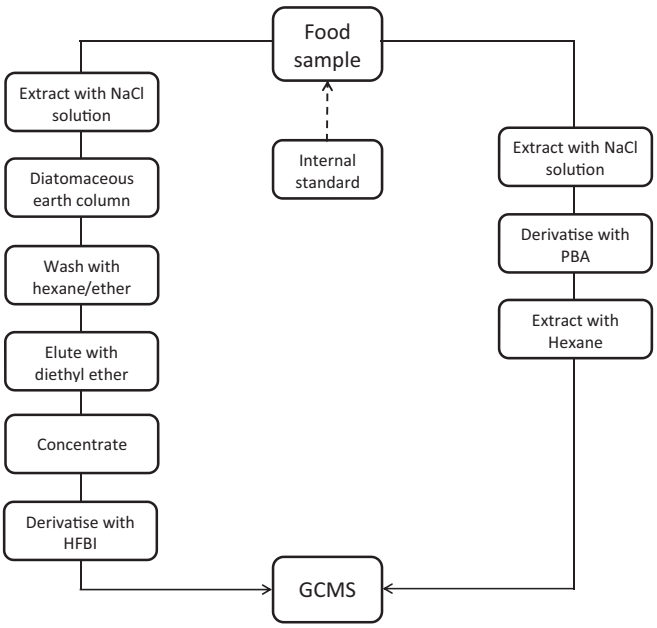


Figure 2: Flow chart showing solid phase extraction/heptafluorobutyrylimidazole (HFBI) and boronate methods

These methods all have advantages and disadvantages. HFBI derivatises many co-extracted compounds and gives relatively clean chromatograms and better GC column life. The mass spectra of HFB derivatives have relatively low intensity in electron impact (EI) mode but peak identity can be confirmed by the ratio of particular masses. MCPD isomers have a low molecular weight, and the addition of the relatively bulky HFB groups reduces the effect of background mass spectral noise from co-extracted compounds. Unfortunately, the reagent is very sensitive to moisture and relatively unstable, making it difficult to handle. HFBA is less expensive than HFBI and easier to handle, but its efficiency has been questioned and catalysis with triethylamine has been recommended (Xu et al., 2006). The trimethylsilyl derivatives of 3- and 2-MCPD have ions of such low intensity in EI mode that limits of detection are unsatisfactory (Wenzl et al., 2007). The EI mass spectra of HFB derivatives of 3- and 2-MCPD do not show the molecular ion at *m/z* 502. The ions normally used for quantitative determination of 3-MCPD di-HFB are at *m/z* 453 [M-CH2Cl]+, 289 [M-C3F7CO2]+, 275 [M-C3F7CO2CH2]+ and 253 [M-C3F7CO2HCl]+. The di-HFB derivative of 2-MCPD has a similar mass spectrum but lacks the ions at *m/z* 453 and *m/z* 275. The characteristic ions in NCI modes are *m/z* 502 (molecular ion), 482 [M-HF]− and 446 [M-HF-HCl]−.

2-MCPD can readily be determined by using reagents (HFBI, HFBA, BSTFA) that derivatise the –OH groups individually. Although boronate reagents can act across pairs of –OH groups that are not on adjacent carbon atoms, their quantitative reaction with 2-MCPD has not been assured, and the dioxolane forming reagents do not react with 2-MCPD.

Quantification of 3-MCPD is always achieved by use of a calibration graph and isotope dilution with d5-labelled 3-MCPD. Quantification of 2-MCPD has been approximated by comparison with the 3-MCPD calibration graph (Meierhans et al., 1998; Chung et al., 2002) but reference standards are now available.

GC-MS/MS has been used less routinely for 3-MCPD detection, Hamlet (1998) used an ion trap instrument in multiple reaction monitoring (MRM) mode, and Kuballa and Ruge (2004) showed that sensitivity was improved by using a triple quadrupole instrument operated in the selected reaction monitoring mode.

There is only a single report of the separation of the (R)- and (S)- isomers of 3-MCPD, this was achieved by GC with flame ionisation and GC-MS of the PBA derivatives on a stationary phase of γ-cyclodextrin after pentylation of the 2,6-hydroxyl groups and trifluoroacetylation of the 3-position hydroxyl groups (Reece, 2005).

Method validation

Reference standards of both 3-MCPD and 2-MCPD are commercially available, along with their deuterated analogues. Reference materials containing 3-MCPD are commercially available only for soy sauce. The HFBI method has been validated for 3-MCPD in acid-HVP, stocks and soups, soy sauce, salami, fish, cheese, cereals and bread by an international collaborative trial (Brereton et al., 2001). The PBA method has been validated in-house for 3- and 2-MCPD using samples of biscuits, potato crisps, cereals, bread, meat and fish (Wenzl et al., 2015) and found to have good agreement with results obtained with HFBI. Limits of quantification (LOQs) are typically 15 and 10 μg/kg for the free forms of 3-MCPD and 2-MCPD, respectively.

1.2.5.2. Esters of 3- and 2-MCPD and of glycidol

The determination of fatty acid esters of 3- and -2-MCPD is complicated by their great variety and structural diversity. Ester bonds can be formed at either or both of the free hydroxyl positions of MCPD with any of the fatty acids naturally present in the sample. Taking into account the number of possible positional isomers of MCPD the formation of about 100 different ester compounds is possible. For GE the number is much reduced on account of the single hydroxyl group and lack of positional isomers. However, in practice, because of the relative abundance of the fatty acids only a core of six or seven esters (lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids) needs to be considered in food analysis (Dubois et al., 2011).

Wenzl et al. (2015) have provided a comprehensive overview of the development and testing of methods for both free and ester-bound 3- and 2-MCPD.

Two approaches are used for the determination of the fatty acid esters of 3-MCPD, 2-MCPD and glycidol; these are direct methods that quantify the targeted intact esters, and indirect methods that quantify the chloropropanol or glycidol (as MCPD) released from the ester bond.

For the analysis of the large numbers of food samples required to obtain data for exposure estimates the indirect methods have predominated.

Direct analysis of 3- and 2-MCPD fatty acid esters

Direct analysis of 3-MCPD fatty acid esters by GC has not proceeded beyond initial investigation (Reece 2005) but several LC-MS approaches have evolved. In the direct methods based on LC-MS a sample of oil or of lipid extracted from a foodstuff with solvents such as mixtures of hexane with t-butylmethyl ether, acetone or diethyl ether is diluted and injected into the LC column either with or without prior clean-up or fractionation. SPE clean-up typically involves the use of a dual cartridge system using first a C18 SPE cartridge with acetonitrile elution, followed by a silica SPE cartridge (MacMahon et al., 2013a).

3- and 2-monoesters and diesters of MCPD can be separated from each other under the protocols of direct methods provided that esters of the same molecular mass and product ion spectra do not coelute from the LC column.

Indirect analysis

For the indirect determination of ester-bound 3- and -2-MCPD oil samples are transmethylated after dissolving in solvent. For food samples the fat or oil fraction is first isolated by solvent extraction and the residue discarded.

Separation of monoester from diesters prior to analysis by indirect methods has been achieved using silica, diol or amino-phase SPE cartridges, with diol and amino phases being most effective (Dubois et al. 2011; Hamlet et al., 2014).

The indirect determination of ester-bound 3- and 2-MCPD in oils involves the cleavage of the ester bonds by transmethylation (methanolysis) and the measurement of the released 3- and 2-MCPD by one of the methods described above for free MCPD. The MCPD can be released by the use of lipase enzymes (Hamlet and Sadd, 2004; Chung and Chan, 2012; Chung et al., 2013), but the use of acid (Divinová et al., 2004; Zelinková et al. 2006) or alkali (Weißhaar, 2008, Küsters et al., 2010) is much more common. GE are cleaved in the same reaction. In an alternative procedure described below esterified glycidol is brominated prior to cleavage of the monobromopropanediol (3-MBPD) produced (Figures 3 and 4).

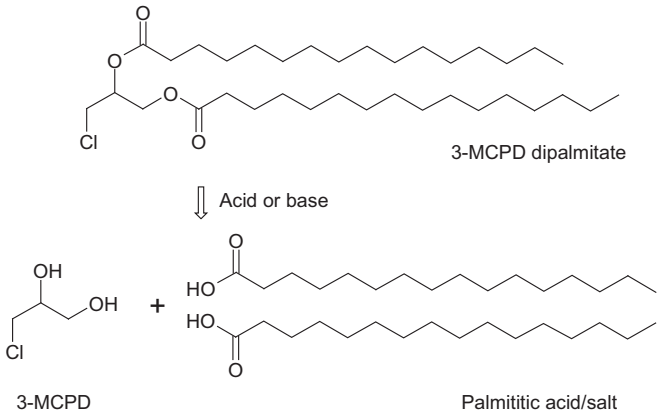


Figure 3: Acid and base methanolysis of monochloropropanediol (MCPD) fatty acid esters

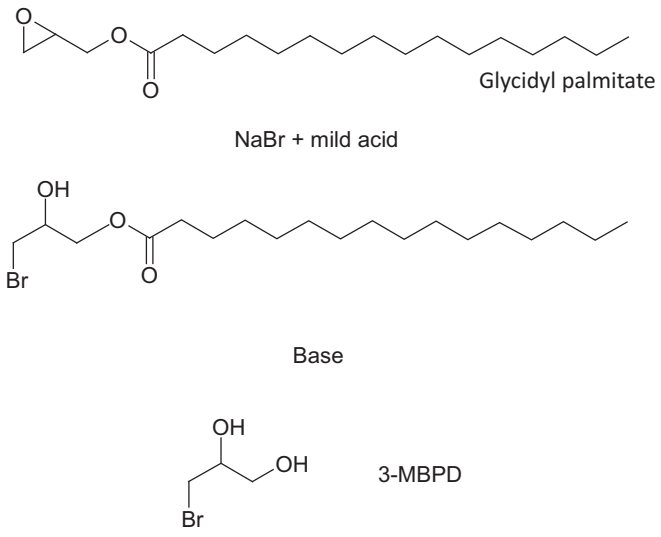


Figure 4: Formation of monobromopropanediol (3-MBPD) fatty acid ester from glycidyl fatty acid ester, and base methanolysis to 3-MBPD

For the extraction of 3- and 2-MCPD fatty acid esters (with or without GE) from food samples for indirect determination a variety of solvents have been used. They generally need to be more polar than simple alkanes such as hexane for complete recovery, particularly for 3-MCPD monoesters. The solvents commonly used are t-butyl methyl ether or mixtures of t-butylmethyl ether with hexane or petroleum ether, or mixtures of hexane with diethyl ether (Hamlet and Asuncion, 2011; Wenzl et al., 2015). The inclusion of acetone is particularly useful for the extraction of MCPD fatty acid esters from dry infant formula.

The BfR compared extraction methods for MCPD fatty acid esters in infant formula, sweet spread and chocolate cream, each containing vegetable fats, plant-based onion lard, and mayonnaise (BfR 2013; Fry et al. 2013). For a collaborative trial an extraction step based on accelerated solvent extraction (ASE, also known as pressurised liquid extraction, PLE) with a solvent mixture of petroleum ether/iso-hexane/acetone (2/2/1 v/v) using two extraction cycles at a temperature of 125°C was used. The ASE method had very good repeatability within the laboratories and reproducibility between the laboratories for all of the matrices. It was compared with Soxhlet extraction, which was less reproducible, possibly on account of variations in the procedures used, and with several cold solvent extraction methods, which had poorer recovery. The accuracy and precision were better for hot extraction methods than for cold ones, and they varied with type of food product analysed.

Problems with and pitfalls of the methods

Early applications and trials of the methods for 3-MCPD based on acid and alkali transesterification revealed that there were both discrepancies between the methods and irregular results for the application of the same (acid or alkali) approach to oils analysis.

If acid methanolysis is carried out without due care, 3- and 2-MCPD can be formed from the reaction of chloride with various precursors, of which glycidol is the major compound identified (Weißhaar 2008, Weißhaar and Perz, 2010 Haines et al., 2011). This unwanted formation reaction has been mitigated by pre-treatment with acid to destroy glycidol, and to a lesser extent by washing the lipid extract with water to remove chloride (Hamlet and Asuncion, 2011; Chung et al., 2013).

Alkaline methanolysis suffers from the instability of 3- and 2-MCPD under basic conditions and the temperature and time of the reaction must be very carefully controlled. Data produced before the introduction of these controls in 2013 must be considered suspect.

The current situation – AOCS methods

Early standardisation of methods based on alkali methanolysis was carried out by the German Society for Fat Science (DGF), the methods underwent frequent revision and comparison with other procedures (Fiebig, 2011).

Well-characterised procedures for the determination of MCPD fatty acid esters and GE in edible oils have now been presented (Karasek et al., 2013) by the EU Joint Research Centre (JRC) and have been adopted by the American Oil Chemists' Society (AOCS). The methods can determine fatty acid esters of 3- and 2-MCPD and glycidol. The methods have been validated in trials involving 20 participants from eight countries (AOCS 2013). These three methods (AOCS Cd 29a-13, Cd 29b-13 and Cd 29c-13), which are described below, are today considered to be the most reliable in giving a true result.

AOCS Method Cd 29a-13 determines fatty acid esters of 3- and 2-MCPD and glycidol based on the procedure of Ermacora and Hrncirik (2013). The sample is incubated with sodium bromide in acid solution to convert GE to 3-MBPD monoesters. The reaction is stopped by the addition of dilute alkali and the oil phase containing the esters is extracted with n-heptane. The residue, containing 3-MBPD esters, together with 3- and 2-MCPD fatty acid esters present in the original sample, is subjected to sulphuric acid methanolysis at 40°C for 16 h, after which the released free 3-MBPD, 2-MCPD and 3-MCPD are determined by the PBA method. For quantification deuterated internal standards (d5-3-MCPD dipalmitate and d5-glycidyl palmitate) are added to the oil sample.

AOCS Method Cd 29b-13 determines glycidol together with the total 2-MCPD and 3-MCPD present in both bound and free forms. The method is based on alkaline-catalysed hydrolysis prior to the conversion of released glycidol to MBPD as published by Kuhlmann (2011). Parallel analyses of two aliquots of the same sample are carried out. One aliquot (A) is spiked with an isotopically labelled GE and a second aliquot (B) is spiked with isotopic labelled 3- and 2-MCPD fatty acid esters as standards. Free MCPD and free glycidol are released from both assays by hydrolysis with dilute sodium hydroxide in methanol at low temperature. The reaction is stopped by the addition of sodium bromide in dilute phosphoric acid whereby free glycidol is converted into 3-MBPD with a trace amount of 2-MBPD which are determined after PBA derivatisation. GE is quantified against the isotopically labelled GE in aliquot (A) and bound 3- and 2-MCPD against the labelled MCPD fatty acid esters in aliquot (B).

AOCS Method Cd 29c-13 determines the sum of bound 3-MCPD and bound glycidol. In a first assay, free 3-MCPD and free glycidol are released by dilute sodium hydroxide or sodium methoxide in methanol, and the reaction is stopped by the addition of an acidic chloride salt solution. Glycidol reacts with the chloride to form additional 3-MCPD and a small amount of 2-MCPD. The released 3- and 2-MCPD are determined as their PBA derivatives as before. In a second assay, the base-catalysed transesterification is followed as above but the reaction is stopped by the addition of acid under chloride-free conditions in which the free glycidol does not generate additional 3-MCPD. The two assays allow the calculation of the sum of bound MCPD and GE and a second determination of bound glycidol, the latter assuming that glycidol is the only precursor of 3-MCPD present.

Application of both direct and indirect methods to the determination of the esters in food products other than oil has been limited, and restricted mainly to indirect methods applied to small surveys (Svejkovská et al., 2004; Zelinková et al., 2009; Chung and Chan, 2012; Becalski et al., 2013).

Validation

In addition to validation for oils and fats carried out for the AOCS methods, method performance data for the determination of MCPD fatty acid esters and glycidyl esters in processed foods has been reported by Küsters et al. (2010, 2011). A BfR proficiency test of methods for MCPD fatty acid esters in infant formulae, mayonnaise, and spreads (Fry et al., 2013) showed that about 75% of results for 3-MCPD fatty acid esters and 78% for 2-MCPD fatty acid esters were satisfactory (z-score < 2).

The indirect methods applied to foods typically have LOQs of about 15 μg/kg MCPD released from esters on a fat basis for both 3- and 2-MCPD. The LOQ for glycidol released from esters on a fat basis is about 30 μg/kg. Only limited data are available for other parameters such as precision, Wenzl et al. (2015) reporting values of 5–15% for oil spiked at about 50 μg/kg with 3- and 2-MCPD fatty acid esters and GE and similar values for potato crisps.

A comprehensive range of chemical standards of individual 3- and 2-MCPD fatty acid esters and GE is now commercially available, including isotopically labelled forms. Certified reference materials are however not yet available.

Summary

Analytical methods for free 3- and 2-MCPD in foods are well characterised, validated for a suitable range of foods and fit for purpose. There are no suitable methods for the unstable free glycidol.

Indirect methods for ester-bound 3- and 2-MCPD and glycidol in foods are well characterised for the important range of foods. The critical stage of the methods is the fission of the MCPD from the ester in the oil extract. This has been validated for all three AOCS-adopted methods, which should provide directly comparable results.

The methods for free MCPD do not include MCPD released from esters, and the methods for ester-bound 3- and 2-MCPD and glycidol do not provide data for the free compounds. Thus the two approaches were applied independently to obtain the exposure data.

1.2.6. Legislation

In this scientific opinion, where reference is made to European legislation (Regulations, Directives, Decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.

In order to protect public health, Article 2 of the Council Regulation (EEC) No 315/93[5] stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Thus, a number of maximum levels for contaminants, natural plant toxicants as well as the process contaminant 3-MCPD are currently laid down in Commission Regulation (EC) No 1881/2006[6]. According to the ANNEX, Section 4 of this Regulation, the maximum levels for 3-MCPD in HVP and soy sauce are each 20 μg/kg. The maximum level is given for the liquid product containing 40% dry matter, corresponding to a maximum level of 50 μg/kg in the dry matter. The level needs to be adjusted proportionally according to the dry matter content of the products. In contrast to 3-MCPD, no maximum levels are laid down for 2-MCPD, 2-MCPD fatty acid esters, 3-MCPD-esters, glycidol and its esters.

In order to gain more occurrence data on the presence of MCPD fatty acid esters and GE in food, and to enable a more accurate exposure assessment, the EU Commission enacted Recommendation 2014/661/EU[7] on the monitoring of the presence of 2 and 3-MCPD, 3- and 2-MCPD fatty acid esters and GE in food. The Recommendation inter alia specifies details on the type of food to be analysed, sampling procedures and analytical methods, including requirements for LOQs.

[5] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–5.

[6] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

[7] Commission Recommendation 2014/661/EU of 10 September 2014 on the monitoring of the presence of 2 and 3-monochloropropane-1,2-diol (2 and 3-MCPD), 3- and 2-MCPD fatty acid esters and glycidyl fatty acid esters in food. OJ L 271, 12.9.2014, p. 93–95.

2. Data and methodologies

2.1. Data

2.1.1. Occurrence data

In September 2013, EFSA published a scientific report analysing the occurrence data on 3-MCPD (both in free and ester-bound form) in food in Europe based on results on samples collected by EU Member States' authorities in the years 2009–2011 and submitted to the EFSA chemical occurrence database (EFSA, 2013). The report highlighted uncertainties affecting the occurrence data, in particular in relation to the analytical methods applied for bound forms (no validated method for 3-MCPD from esters was available at that time) and to the coverage of the relevant food categories (the available data did not cover many food categories potentially contaminated with 3-MCPD). In August 2013, three analytical methods for ester-bound MCPDs and glycidol were validated by the AOCS in a collaborative study involving 20 laboratories from eight countries. The three new methods for the analysis of 3- and 2-MCPD from esters and glycidol from esters are those described in Section 1.2.5 (AOCS Cd 29a-13, Cd 29b-13 and Cd 29c-13).

In order to address the requirement of Commission Recommendation 2014/661/EU (see Section 1.2.6) and reduce the uncertainties identified in the above mentioned EFSA report, EFSA activated two new data collection initiatives on 3- and 2-MCPD and glycidol; the collection was restricted (when originating from esters) to results obtained with methods corresponding or strictly related to the three new methods. Data produced with one of these methods before the AOCS validation were also considered acceptable for the data collection.

The first initiative was a Service Level Agreement (SLA) with the JRC of the European Commission, Institute for Reference Materials and Measurements (JRC-IRMM) (SLA/EFSA-JRC/DCM/2013/01) for the development and in-house validation of analytical methods for the analysis of 3-, 2-MCPD (both free and from esters) and glycidol from esters in various food matrices (Wenzl et al., 2015). It was requested that the method for the analyses from esters be derived from one of the three validated methods. The project included the performance of an *ad hoc* survey on specific food groups to test the analytical methods and provide a minimum database on levels of 3- and 2-MCPD and glycidol in the food groups indicated in Table 1. The analyses of 3- and 2-MCPD covered both, those originally present in food in free form and those from esters of fatty acids. The analyses of glycidol were for glycidol derived from esters only.

Table 1: Food groups addressed by the survey included in the JRC Project

Food group
Bread and rolls
Fine bakery wares
Smoked fish products
Smoked meat products
Fried or roast meat (all possible types, including grilled and griddled)
Chips, crisps, fries and dough-based analogues (both, potato- or cereal-based)
Margarine
Infant and follow-on formulae

JRC: Joint Research Centre.

The second initiative was to publish (in October 2014) an open call for data for MCPDs (free and from esters) and glycidol from esters with a deadline of December 2014.[8] The call for data was addressed to all potential data providers, including food business operators and academia. The specific requirements for the analysis of the ester-bound forms were substantially aligned with those of Recommendation 2014/661/EU and with those of the SLA with the JRC. Several food groups were indicated as highest priority for the data collection, as summarised in Table 2.

[8] Available on the EFSA website at the address http://www.efsa.europa.eu/en/data/call/141111.htm (consulted on 11/05/2015)

Table 2: Food groups indicated as highest priority for the data collection in the call for data published by EFSA in October 2014

Food group
Bread and similar products
Leavened bread and similar
Unleavened or flat bread and similar
Crackers and breadsticks
Crisp bread
Rusk
Fine bakery wares
Biscuits
Cakes
Yeast-leavened pastry
Shortcrust (pies-tarts)
Puff pastry
Smoked fish products
Canned/jarred smoked fish in oil
Smoked fish
Smoked meat products
Charcuterie meat products (when smoked, particularly hot smoked)
Fried or roast meat (all possible types, including grilled and griddled)
Chips, crisps, fries and dough-based analogues (both, potato- or cereal-based)
Chips/crisps
Puffs/curls-type extruded snack
Fries (finger chips)
Infant and follow-on formulae
Infant formulae
Follow-on formulae
Traditional margarine
Vegetable fats and oils, edible
Animal fats and oils (processed fat from animal tissue)

By the end of April 2015, EFSA collected different sets of data submitted for the purpose of the present assessment of the occurrence of 3- and 2-MCPD and glycidol.

- A total of 2,535 results on the concentration of 3- and 2-MCPD both free and from esters and of glycidol from esters in 507 food samples were produced in the framework of the SLA with the JRC. For all the samples, both the MCPD forms (i.e. free and from esters) were measured separately. The total occurrence levels for both 2- and 3-MCPD were then calculated during the data analysis as the sum for each sample of the results obtained for the free and the ester-bound form. The data cover the food groups listed in Table 1;
- A total of 210 results on the concentration of 3- and 2-MCPD and glycidol from esters in 70 samples of infant formulae were submitted by the BfR of Germany; these data only covered the three contaminants present in ester-bound form; they did not include the potential presence of 3- and 2-MCPD in free form; therefore, the occurrence levels calculated from these data may be underestimated;
- A total of 4,503 results on 3- and 2-MCPD- and glycidol from esters from vegetable fats and oils were submitted by the Association of the EU Vegetable Oil & Proteinmeal Industry (FEDIOL); these data only refer to 3-, 2-MCPD and glycidol present in ester-bound form and did not cover the potential presence of 3- and 2-MCPD in free form; this might imply underestimation of the total occurrence levels; however, the presence of 2- and 3-MCPD in free form in fat and oils is expected to be negligible respect to the ester-bound form (Zelinková et al., 2006);

- A total of 420 results on 3- and 2-MCPD- and glycidol from esters in margarines and similar products were submitted by the European Margarine Association (IMACE); as in the case of the data on fats and oils, these data did not cover the potential presence of 3- and 2-MCPD in free form, but this is expected not to be relevant in comparison with the ester-bound form.

At the beginning of September 2015, two additional data sets were submitted and included in the assessment:

- A group of results on the concentration of 3- and 2-MCPD from esters and glycidol from esters in 35 samples including 'olive oil' (9 samples), and fried or baked fish (26 samples), were submitted by the JRC; these data did not include the potential presence of 3- and 2-MCPD in free form; while the lack of data on 3- and 2-MCPD in free form is expected not to be relevant for estimating the occurrence in olive oil, underestimation of the occurrence in the fried or baked fish samples is possible;
- A total of 344 results on concentration of 3-MCPD from esters (138 results), 2-MCPD from esters (68 results) and glycidol from esters (138 results) in sunflower seed oil samples were submitted by the Lebensmittelchemisches Institut des Bundesverbandes der Deutschen Süßwarenindustrie (Köln); these data do not include the potential presence of 3- and 2-MCPD in free form; however, the presence of 3- and 2-MCPD in free form in fat and oils is expected to be negligible with respect to the ester-bound form.

In order to also include the food groups addressed by Commission Regulation (EC) No 1881/2006, analytical results on 3-MCPD in free form in soy sauce, HVP and related food products such as condiments and soup preparations were retrieved from the EFSA chemical occurrence database. Overall, 708 data were obtained by selecting results submitted by the national competent authorities of the EU Member States on the mentioned food groups in the last 5 years (sampling year from 2009 to now) in the framework of the annual data collection on chemical contaminants. The data were retrieved on 6 April 2015. These data only refer to 3-MCPD in free form and do not include the potential presence of 3- and 2-MCPD and glycidol from esters, therefore the occurrence levels calculated from these data may be underestimated. Data on 3-MCPD in free form in food groups already covered by the previously mentioned data collection initiatives (which included the contribution from both, free and ester-bound forms) were not retrieved from the database, because they would have increased the uncertainty in the assessment. For the same reason, data on 3- and 2-MCPD from esters not submitted to the above mentioned call for data and not generated with the analytical methods defined in the call were not considered in this assessment. Data submitted after beginning of September 2015 could not be considered in the assessment.

2.1.2. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at the individual level. It was first built in 2010 (EFSA, 2011b; Huybrechts et al., 2011; Merten et al., 2011) and then updated in 2015[9]. Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a).

The database used to estimate dietary exposure in the present opinion included the 17 surveys from 2014 and surveys conducted between 2005 and 2012, with two exceptions (DIPP 2001–2009, Finland; VELS 2001–2002, Germany - see Table B.2, Appendix B). The database contains data from 41 surveys in 23 different European countries for a total of 78,990 participants (Appendix D). Data from six surveys were available for 'Infants' (< 12 months old), 11 for 'Toddlers' (≥ 12 months to < 36 months old), from 19 surveys for 'Other children' (≥ 36 months to < 10 years old), from 19 surveys for 'Adolescents' (≥ 10 years to < 18 years old), from 21 surveys for 'Adults' (≥ 18 years to < 65 years old), from 15 surveys for the 'Elderly' (≥ 65 years to < 75 years old) and from 13 surveys for the 'Very elderly' (≥ 75 years old).

In the surveys above, consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Owing to the differences in the methods used for data collection, direct country-to-country comparisons must be taken with caution.

[9] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database (consulted on 01/02/2016)

2.2. Methodologies

2.2.1. Management of occurrence data

2.2.1.1. Data cleaning

The reported concentration values were checked in order to identify duplicate data (based on the sample code) and possible errors in the unit of measurement. The check of the unit of measurement was performed by comparing the order of magnitude of the values provided for the analytical results, the limit of detection (LOD) and the LOQ across the database. One result was identified where LOD and LOQ were reported wrongly (by a factor of 1,000) and this was corrected.

2.2.1.2. Sampling method and sampling strategy

All data were from analysis of individual samples. With respect to the sampling strategy,[10] almost all of the samples were from objective or selective sampling. A total of six results on free 3-MCPD were reported as from suspect sampling and were removed from the data set.

2.2.1.3. Left-censored results

Left-censored results are analytical results reported either as $<$ LOD or $<$ LOQ. The limit (LOD or LOQ) applied for each specific result is the left-censoring limit. Depending on the scope of the analysis (free form, ester-bound form), the analytical method and the left-censored limits may vary. For the analysis of free 3-MCPD in food, left-censored limits have been established in some foods (the LOQ must be $\leq$ 10 μg/kg on dry matter basis (LOD $\leq$ 5 μg/kg) for HVP and soy sauce) as defined in Commission Regulation (EC) No 333/2007. The left-censored limits for the analytical results on HVP and soy sauce were at or below the prescribed limits. For the analysis of 3-MCPD-, 2-MCPD- and glycidol from esters, the limit indicated in the call for data was 100 μg/kg (referred to fat). No exclusion criterion was applied based on left-censored limits.

2.2.1.4. Management of left-censored results

Left-censored results were treated by the substitution method (EFSA, 2010b). This approach, based on the consideration that the true value for left-censored results may actually be any value between 0 and the left-censored limit, compares the two extreme scenarios. The lower bound (LB) scenario assumes that the substance is absent; thus, to left-censored results a value of 0 is input. The upper bound (UB) scenario assumes that the substance is present at the level of the limit; thus, to results reported as $<$ LOD or $<$ LOQ the value of the respective left-censored limit is assigned. Additionally, as a point estimate between the two extremes, the middle bound (MB) scenario is calculated by assigning a value of LOD/2 or LOQ/2 to the left-censored results.

2.2.1.5. Food classification

The analytical results were classified according to the FoodEx1 food classification system. FoodEx1 is a provisional food classification system developed by the EFSA's Dietary and Chemical Monitoring Unit in 2009 with the objective to link occurrence and food consumption data at a detailed level to assess exposure to hazardous substances. It contains about 1,800 food descriptors (food codes) which can be grouped according to the needs of a specific analysis (EFSA, 2011b). For the purpose of the present assessment, based on the FoodEx1 codes and additional information present in the chemical occurrence database, the classification of the analytical results was revised and some *ad hoc* food groups were created to allow a more detailed analysis of the results.

The *ad hoc* food group 'Vegetable fats and oils' was created, aggregating the FoodEx1 groups A.01.001362 'Vegetable fat' and A.01.001367 'Vegetable oil'. An *ad hoc* group 'Special fats' was also created to collect particular fat compositions used as ingredients in food processing. Within the FoodEx1 group A.01.001632 'Seasoning or extracts' the *ad hoc* group 'Other seasoning products' was

[10] Objective sampling is based on the selection of a random sample from a population on which the data are reported; Selective sampling is based on the selection of a random sample from a subpopulation (or more frequently from subpopulations) of a population on which the data are reported. The subpopulations are often determined on a risk basis; Convenient sampling is based on the selection of a sample for which units are selected only on the basis of feasibility or ease of data collection; Suspect sampling is based on samples taken repeatedly from the same site as a consequence of evidence or suspicion of (illegal) contamination. Suspect samples are usually taken as a follow-up of demonstrated non-compliance with legislation.

created, to aggregate seasoning or extracts other than stock cubes. Within the FoodEx1 group A.01.001649 'Condiments', the *ad hoc* group 'Other condiment sauces' was created to aggregate condiments other than soy sauce and similar.

Some *ad hoc* groups were created to classify occurrence data referred to specific food categories for which only FoodEx1 groups at less detailed level were available. Within the food group A.01.000876 'Fish and other seafood' the *ad hoc* group 'Fish meat (smoked)' was created, as subgroup of the FoodEx1 group A.01.000877 'Fish meat' containing only smoked fish meat. Within the food group A.01.000727 'Meat and meat products (including edible offal)' the *ad hoc* group 'Preserved meat (smoked)' was created, as subgroup of the FoodEx1 group A.01.000795 'Preserved meat' containing only smoked products. Within the FoodEx1 group A.01.001757 'Protein and amino acids supplements' the *ad hoc* group 'Hydrolysed vegetable proteins' was created. Within the FoodEx1 group A.01.001789 'Composite food' the *ad hoc* group 'Dry preparations for soups (to be reconstituted)' was created, a subgroup of the FoodEx1 group A.01.001856 'Ready-to-eat soups' including only dry products to be reconstituted. An overview of the food groups used to evaluate occurrence in this report is provided in Table B.1 in Appendix B.

2.2.1.6. Substances

All analytical results were expressed as free moiety (3-MCPD, 2-MCPD or glycidol) from one of the forms listed in Recommendation 2014/661/EU. They are available in the PARAMCODE catalogue of the standard sample description (SSD), the EFSA standard for collecting data (EFSA, 2010a); the reported substance codes are summarised in Table 3.

Table 3: Substance codes reported in the data set for MCPDs and glycidol

PARAMCODE	Description
RF-00000377-ORG	3-MCPD free
RF-00000380-ORG	3-MCPD esters [expressed as 3-MCPD moiety]
RF-00000378-ORG	3-MCPD total [expressed as 3-MCPD moiety]
RF-00002832-PAR	2-MCPD free
RF-00002833-PAR	2-MCPD esters [expressed as 2-MCPD moiety]
RF-00002834-PAR	2-MCPD total [expressed as sum of 2-MCPD free and 2-MCPD esters expressed as 2-MCPD moiety]
RF-00001344-PAR	Glycidyl esters [expressed as glycidol moiety]

The codes refer to the PARAMCODE catalogue of the EFSA standard sample description.

For the analyses of the JRC data set where both forms (free and from ester) of 3- and 2-MCPD were determined, LB, MB and UB values for 3-MCPD or 2-MCPD were calculated as their respective sums; the calculated sum was used for the assessment of occurrence.

2.2.1.7. Expression of reported analytical results

The analytical results were expressed on different bases: whole weight (w.w.), 40% dry matter, dry matter or fat weight.

- In the case of analytical results reported on 40% dry matter, the values were transformed into whole weight dividing by 40 and multiplying by the dry matter of the sample expressed as a percentage (i.e. 100% of moisture);
- In the case of analytical results reported on dry matter, the values were transformed into whole weight dividing by 100 and multiplying by the dry matter of the sample expressed as a percentage (i.e. 100% of moisture).
- In the case of analytical results reported on fat weight, the values were transformed into whole weight dividing by 100 and multiplying by the fat content of the sample expressed as a percentage.

Among the data submitted for the present assessment, a total of 205 results on free 3-MCPD were reported as 40% dry matter or dry matter; a total of 210 results on infant formulae were reported as fat weight; all the remaining results were reported as whole weight.

2.2.1.8. Recovery rates

Commission Regulation (EC) No 333/2007 defines methods of analysis to be applied to 3-MCPD in soy sauce and HVP as needing to have recovery rates in the range of 75–110%. No prescription is on

place for other food categories. Recovery rates were only reported for 639 results. Although recovery rates were not reported for a majority of analytical results, no data were excluded based on this criterion since the analytical methods for both free and bound forms include the use of isotope-labelled internal standards which correct automatically for recovery losses.

2.2.2. Statistical analysis

All analyses were run using the SAS® Statistical Software (SAS software, 1999). Frequency tables per sampling year, sampling country and food group were produced to describe the 3-MCPD, 2-MCPD and glycidol data collection. Descriptive summary statistics of concentration levels per food group were calculated. The Guidance on the use of the Comprehensive Food Consumption Database indicates that the 95th percentile estimates obtained with less than 60 observations may not be statistically robust (EFSA, 2011c) and therefore they should be considered with caution.

2.2.3. Methodology used for hazard identification and characterisation

2.2.3.1. Methodology literature search

Strategy for literature search

For the present evaluation the CONTAM Panel considered literature made publicly available until 10 February 2015. A comprehensive search for literature was conducted for peer-reviewed original research pertaining to the occurrence of 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) fatty acid esters and glycidyl fatty esters in food and its adverse health effects on humans, experimental animals and in vitro systems. The search strategy was designed to identify scientific literature dealing with chemical analysis, chemistry, occurrence, exposure, toxicity, mode of action, toxicokinetics and epidemiology of 3- and 2-MCPD fatty acid esters and glycidyl fatty esters.

Additionally, research or reports on chloropropanols in food were considered. Articles with other meanings of the acronym MCPD and articles on other esters were excluded. The literature search was not restricted to publications in English language; however, literature in other languages was only considered if an English abstract was available. The first literature search was performed in September 2014 and has since been updated in November 2014, December 2014, February 2015 and January 2016. Web of Science[11] and PubMed[12] were identified as databases appropriate for retrieving literature for the present evaluation. The references resulting from the literature search were imported and saved using a software package (EndNote[13]), which allows effective management of references and citations. Additionally, reviews, relevant scientific evaluations by national or international bodies were considered for the current risk assessment, i.e. previous evaluations of SCF (1994, 2001), FAO/WHO (2002), FSA (2008, 2009, 2010) and BfR (2007, 2009, 2012). Two scientific opinions by the BfR (2007, 2012), available only in the original language, were translated into English by the Translation Centre for the Bodies of the European Union.

Appraisal of studies

Information retrieved has been reviewed by the CONTAM Working Group on MCPD and GE in food and used for the present assessment using expert judgement. Any limitations of the information used are clearly documented in this opinion.

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 (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by WHO/IPCS (2009), the principles in the EFSA guidances on risk assessment (EFSA SC, 2012a) and the applicability of a margin of exposure (MoE) approach to safety assessment of impurities which are both genotoxic

[11] Web of Science (WoS), formally ISI Web of Knowledge, Thomson Reuters. Available online: http://thomsonreuters.com/thomson-reuters-web-of-science/

[12] PubMed, Entrez Global Query Cross-Database Search System, National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), Department of the National Institutes of Health (NIH), United States Department of Health and Human Services. Available online: http://www.ncbi.nlm.nih.gov/pubmed/

[13] EndNote X5, Thomson Reuters. Available online: http://endnote.com/

and carcinogenic (EFSA SC, 2012b) have been applied for the present assessment. In brief, the EFSA guidance covers the procedures currently used within EFSA for the assessment of dietary exposure to different chemical substances and the uncertainties arising from such assessments (EFSA, 2006). For details on the specific EFSA guidances applied, see Appendix A.

3. Assessment

3.1. Occurrence of 3- and 2-MCPD and glycidol in food

The available occurrence data (see Section 2.1.1) were divided in three groups and considered separately:

- 3-MCPD (in free form) in soy sauce, HVP and related products;
- 3- and 2-MCPD from esters and glycidol from esters in oils/fats;
- 3- and 2-MCPD (free and from esters) and glycidol (from esters) in food groups other than those mentioned above. In most cases, the contribution to the total 3- and 2-MCPD from the free form was included, while the results on glycidol were only from esters.

3.1.1. Occurrence of free 3-MCPD in soy sauce, HVP and related products

The number of analytical results on free 3-MCPD in soy sauce, HVP and related products are summarised per sampling year in Table 4 and per sampling country in Table 5.

Table 4: Distribution of results on free 3-MCPD in soy sauce, HVP and related products per sampling year

Sampling year	N[(a)]	%
2009	78	11
2010	150	21
2011	185	26
2012	217	31
2013	72	10
Total	702	100

3-MCPD: 3-monochloropropane-1,2-diol; HVP: hydrolysed vegetable protein.
(a): N = number of analytical results reported.

Table 5: Distribution of results on free 3-MCPD in soy sauce, HVP and related products per sampling country

Sampling country	N[(a)]	%
Belgium	91	13
Czech Republic	112	16
Denmark	17	2
Finland	137	20
France	18	3
Germany	114	16
Greece	15	2
Ireland	19	3
Lithuania	4	< 1
Luxembourg	25	4
Malta	15	2
Poland	85	12
Spain	50	7
Total	702	100

3-MCPD: 3-monochloropropane-1,2-diol; HVP: hydrolysed vegetable protein.
(a): N = number of analytical results reported.

All the data referred to individual samples and no aggregated data were present in the data set. More than 80% of the results reported the analytical method and in all cases this was based on GC-MS techniques.

The sampling strategy was reported as 'Objective sampling' in 58% of the results, and 'Selective sampling' in 35% of the results and in the remaining cases it was not reported. The two reported strategies (objective and selective sampling) are substantially equivalent in this case, considering that the food categories included in the assessment were selected based on the potential presence of 3-MCPD in free form.

Most of the data were expressed on whole weight, while some of them were reported on 40% dry matter (21% of the results) or on dry matter (8% of the results); in the last two cases, the occurrence value was recalculated as whole weight.

The data set included several left-censored results (i.e. reported as < LOD or < LOQ). The left censoring value ranged from 1 to 28 μg/kg with a median of 10 μg/kg. The number and percentage of left-censored results is shown in Table 6. For the evaluation of occurrence, the left-censored results were considered following the approach explained in Section 2.2.2.

Table 6: Number of results and number and percentage of left-censored values in the free-3-MCPD data set, by level 1 food categories

Food group	N[(a)]	LC[(b)]	%LC[(b)]
Composite food	26	13	50
Herbs, spices and condiments	660	525	80
Protein and amino acid supplements	16	11	69
Total	702	549	78

3-MCPD: 3-monochloropropane-1,2-diol.
(a): N = Number of results.
(b): LC = Number of left-censored results; %LC = percentage of left-censored results in each food group.

The mean, median and 95th percentile of concentrations (μg/kg) of free 3-MCPD in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on free 3-MCPD are presented in Table 7. The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

Table 7: Mean, median and 95th percentile of concentrations (μg/kg) of free 3-MCPD by food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones). The data set on free 3-MCPD covers soy sauce, HVP and related products. The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported

Food groups[(a)] levels 1–3[(b)]	N[(c)]	% LC[(d)]	LOQ (min–max) (μg/kg)	Mean[(e)] MB (LB–UB) (μg/kg)	Median[(e)] MB (LB–UB) (μg/kg)	P95[(e)] MB (LB–UB) (μg/kg)
Herbs, spices and condiments	660	80	(1–30)	7.1 (3.8–10)	5 (0–9.3)	23 (23–24)
Herb and spice mixtures	46	43	(5–10)	23 (19–27)	12 (8.3–24)	–
Seasoning or extracts	97	65	(3–10)	11 (9.3–14)	5 (0–10)	54
Stock cubes (bouillon cube)	69	62	(3–10)	10 (8.5–13)	5 (0–10)	43
Other seasoning products*	28	71	(7–10)	14 (11–17)	5 (0–10)	–
Condiment	497	86	(1–30)	4.7 (1.3–8.2)	3.92 (0–6.6)	10 (8–20)
Soy sauce	469	85	(1–30)	4.5 (1.1–7.9)	3.75 (0–6.2)	10 (7.6–18)
Other condiment sauces*	28	93	(5–22)	8.8 (4.2–13)	5 (0–10)	–
Dressing	5	40	10	8.8 (7–10)	–	–
Savoury sauces	15	93	(5–10)	5.4 (1.3–9.4)	5 (0–10)	–
Protein and amino acid supplements	16	69	(10–13)	25 (22–28)	5 (0–10)	–
Hydrolysed vegetable proteins*	16	69	(10–13)	25 (22–28)	5 (0–10)	–

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Composite food	26	50	10	10 (8.7–12)	5.2 (2.7–9.7)	–
Dry preparations for soups (to be reconstituted)*	26	50	10	10 (8.7–12)	5.2 (2.7–9.7)	–

3-MCPD: 3-monochloropropane-1,2-diol; HVP: hydrolysed vegetable protein; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC% = percentage of left-censored results; the values are rounded to the nearest integer.
(e): Mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported).

The food groups having higher mean values are 'Hydrolysed vegetable proteins' with levels MB (LB–UB) of 25 (22–28) μg/kg and 'Herb and spice mixtures' with 23 (19–27) μg/kg.

As anticipated in Section 2.1.1, food groups such as seasonings, condiments, savoury sauces and dry preparations for soups may contain fats and oils and the presence of 3-MCPD in the form of fatty acid esters is possible. As this potential presence was not covered by the available data, the occurrence in these food groups summarised in Table 7 may underestimate the total 3-MCPD occurrence.

3.1.2. Occurrence of 3- and 2-MCPD from esters and glycidol from esters in fats and oils

Data on the levels of 3- and 2-MCPD from esters and glycidol from esters in refined fats and oils were available from sampling year 2010. All of them were generated with one of the methods mentioned previously in this section; those generated before August 2013 applied the same methods but before the AOCS validation. The original set of data on oils and fats was qualitatively examined to investigate whether all of them should be used in this assessment. To this end, the yearly average per type of oil was reported in a graph for 3-MCPD, 2-MCPD and glycidol. The graphs are shown in Figure 5.

The qualitative evaluation of the data shows a decrease in the average levels of 3-MCPD and glycidol from esters in the years between 2010 and 2012 for palm oil. The same observation is not applicable to other oils. Based on this finding it was decided to limit the data analysis in fats and oils to the years 2012–2015 and exclude the previous data, as it did not reflect the present situation.

The number of analytical results on 3- and 2-MCPD from esters and glycidol from esters in fats and oils used the present assessment are summarised per sampling year in Table 8.

Table 9 shows the distribution of the results per sampling country. Many of the results in this data set were classified as sampled in the European Union and do not allow tracing the actual country of sampling.

All the samples were analysed individually and no aggregated results were included in the data set. Industry sampling (samples collected during industrial processing) accounted for 63% of the results.

All the results were obtained with GC-MS based methods of Ermacora and Hrnčiřík (2013) (corresponding to AOCS cd 29a-13), Kuhlmann (2011) (corresponding to AOCS cd 29b-13), DGF C-VI 18 (10) (corresponding to AOCS cd 29c-13) and JRC (proposed, 2014) (based on AOCS cd 29a-13).

The sampling strategy was reported as 'Objective sampling' in 93% of the results, and 'Selective sampling' in 6% of the results and in the remaining cases it was not reported; no 'Suspect sampling' was reported. It can therefore be assumed that the sampling inside each food group was random. Selective and objective sampling are substantially equivalent in this case, considering that the food categories included in the assessment were selected based on the known possible presence of these substances.

All results were reported as whole weight. The data set included variable proportions of left-censored results (i.e. reported as < LOD or < LOQ), depending on the food group. The left censoring value ranged from 13 to 172 μg/kg with a median at 100 μg/kg. The number and percentage of left-censored results is shown in Table 10. For the evaluation of occurrence, the left-censored results were considered following the approach explained in Section 2.2.2.

The mean, median and 95th percentile of concentrations (μg/kg) of 3- and 2-MCPD and glycidol (all from esters) by food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on oils/fats are presented in Tables 11, 12 and 13. The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

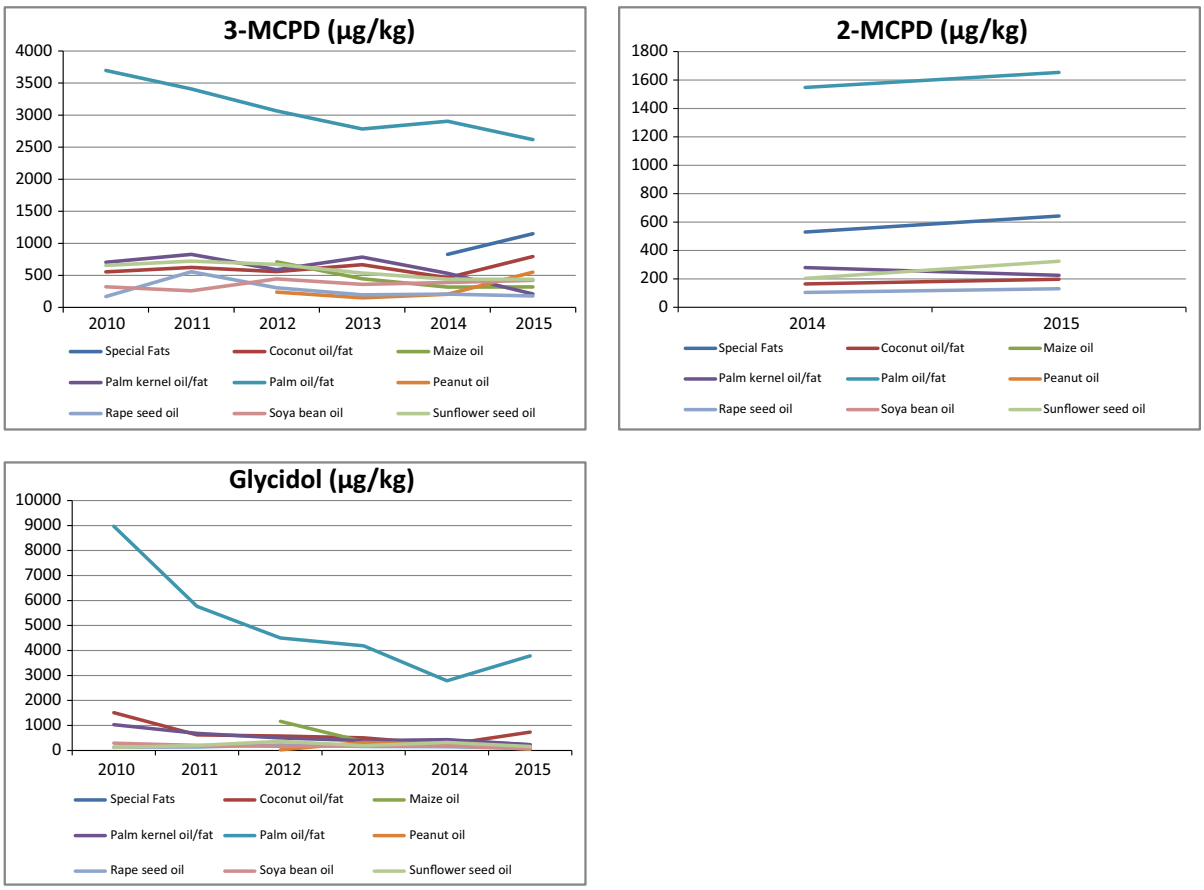


Figure 5: Graphs showing the evolution across the years 2010–2015 of the average level (µg/kg) of 3- and 2-monochloropropanediol (MCPD) from esters and glycidol from esters (all expressed as free moiety) in different types of oils and fats

Table 8: Distribution of results on 3- and 2-MCPD from esters and glycidol from esters in fats and oils per sampling year

Sampling year	Substance analysed			N[(b)]	%
	3-MCPD (from esters)[(a)]	2-MCPD (from esters)[(a)]	Glycidol (from esters)[(a)]		
2012	499	–	482	981	21
2013	664	–	648	1,312	27
2014	808	425	754	1,987	42
2015	179	116	179	474	10
Total	2,150	541	2,063	4,754	100

3-MCPD: 3-monochloropropane-1,2-diol; 2-monochloropropane-1,3-diol.
(a): Number of analytical results reported per substance analysed.
(b): Total number of reported analytical results.

Table 9: Distribution of results on 3- and 2-MCPD and glycidol (all from esters) in fats and oils per sampling country

Sampling country	N[(a)]	%
Belgium	48	1
Croatia	3	< 1
Denmark	3	< 1
Germany	292	6
Netherlands	18	< 1
Unspecified country of the European Union	4,357	92
Total	4,754	100

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol.
(a): N = number of analytical results reported.

Table 10: Number of results and number and percentage of left-censored values by level 1 and 2 food categories in the 3- and 2-MCPD from esters and glycidol from esters data set on fats and oils

Food group	N[(a)]	LC[(b)]	%LC[(b)]
Animal and vegetable fats and oils	4,754	584	12
Margarine and similar products	510	36	7
Special fats	113	7	6
Vegetable fats and oils	4,131	541	13

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol.
(a): N = Number of results.
(b): LC = Number of left-censored results; %LC = percentage of left-censored results in each food group.

For 3-MCPD, the food groups having higher mean values were 'palm oil/fat' with levels MB (LB–UB) of 2,912 μg/kg (LB and UB were equal), 'margarine, normal fat' with 668 (667–669) μg/kg, 'palm kernel oil' with 624 μg/kg and 'coconut oil/fat' with 608 μg/kg. 'Special fats' also had a relatively high level (867 μg/kg) but their practical use in foodstuffs could not be established.

Table 11: Mean, median and 95th percentile of concentrations (μg/kg) of 3-MCPD (from esters) in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on fats and oils

Food groups[(a)] levels 1–3[(b)]	N[(c)]	% LC[(d)]	LOQ (min-max) (μg/kg)	Mean[(e)] MB (LB–UB) (μg/kg)	Median[(e)] MB (LB–UB) (μg/kg)	P95[(e)] MB (LB–UB) (μg/kg)
Animal and vegetable fats and oils	2,150	5	(13–150)	1,034 (1,032–1,037)	490	3,900
Margarine and similar products	170	2	(16–150)	408 (406–409)	244 (240–246)	1,150
Margarine, normal fat	73	–	(16–150)	668 (667–669)	430	1,640
Margarine, low fat	82	4	(22–100)	218 (215–220)	180 (177–180)	430
Fat emulsions	15	–	(50–100)	181	150	–
Special Fats*	41	–	(100–150)	867	750	–
Vegetable fats and oils*	1,939	5	(13–150)	1,093 (1,090–1,095)	510	4,020
Maize oil	38	3	(100–150)	503 (502–505)	430	–
Olive oil	9	11	13	48 (48–49)	32	–
Palm kernel oil	97	–	(100–150)	624	590	1,410
Peanut oil	8	–	(13–150)	229	235	–
Rapeseed oil	294	16	(100–150)	232 (224–239)	180	630
Soya bean oil	191	4	(100–150)	394 (392–396)	330	914

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min-max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Sunflower seed oil	596	7	(90–150)	521 (517–524)	410	1,510
Walnut oil	1	–	13	236	–	–
Coconut oil/fat*	204	–	(100–150)	608	590	1,050
Palm oil/fat*	501	< 1	(100–150)	2,912	2,920	5,210

3-MCPD: 3-monochloropropane-1,2-diol; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC % = percentage of left-censored results; the values are rounded to the nearest integer.
(e): Mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

Table 12: Mean, median and 95th percentile of concentrations (μg/kg) of 2-MCPD (from esters) in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set fats and oils

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min-max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Animal and vegetable fats and oils	541	20	(15–150)	341 (330–352)	160	1,510
Margarine and similar products	170	14	(18–150)	159 (152–166)	100 (100–107)	498 (494–500)
Margarine, normal fat	73	22	(18–150)	236 (224–248)	180	550
Margarine, low fat	82	7	(25–100)	104 (101–107)	80	230
Fat emulsions	15	7	(50–100)	80 (77–84)	70	–
Special Fats*	31	–	(100–150)	544	460	–
Vegetable fats and oils*	340	25	(15–150)	414 (400–427)	184	1,830
Maize oil	6	–	(100–150)	233	170	–
Olive oil	9	22	(15–15)	86 (85–88)	27	–
Palm kernel oil	25	28	(100–150)	270 (249–291)	180	–
Peanut oil	4	25	15	102 (90–115)	–	–
Rapeseed oil	48	56	(100–150)	109 (78–140)	75 (0–100)	–
Soya bean oil	12	17	100	167 (159–175)	130	–
Sunflower seed oil	153	22	(90–150)	218 (207–229)	200	530
Walnut oil	1	–	15	127	–	–
Coconut oil/fat*	27	37	(100–150)	169 (143–194)	150	–
Palm oil/fat*	55	4	(100–150)	1,565 (1,563–1,566)	1,510	–

2-MCPD: 2-monochloropropane-1,3-diol; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC% = percentage of left-censored results; the values are rounded to the nearest integer.
(e): Mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

For 2-MCPD, the food groups having higher mean values were 'palm oil/fat' with levels MB (LB–UB) of 1,565 (1,563–1,566) μg/kg, 'palm kernel oil' with 270 (249–291) μg/kg, 'margarine, normal fat' with 236 (224–248) μg/kg and 'sunflower seed oil' with 218 (207–229) μg/kg. 'Special fats' also had a relatively high level (544 μg/kg) but their practical use in foodstuffs could not be established.

Table 13: Mean, median and 95th percentile of concentrations (μg/kg) of glycidol (from esters) in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on fats and oils

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Animal and vegetable fats and oils	2,063	18	(31–172)	1,176 (1,167–1,184)	261	6,070
Margarine and similar products	170	6	(38–172)	361 (358–364)	175 (175–176)	1,280
Margarine, normal fat	73	3	(38–150)	582 (580–584)	270	1,946
Margarine, low fat	82	0	(50–172)	209 (204–213)	140 (140–142)	500
Fat emulsions	15	–	(50–100)	114	100	–
Special Fats*	41	17	(100–150)	386 (373–399)	360	–
Vegetable fats and oils*	1,852	19	(31–150)	1,268 (1,259–1,277)	280	6,260
Maize oil	36	6	(100–150)	650 (647–654)	475	–
Olive oil	9	100	31	15 (0–31)	15 (0–31)	–
Palm kernel oil	95	13	(100–150)	421 (415–428)	320	1,120
Peanut oil	8	50	(31–150)	148 (133–162)	110 (85–135)	–
Rapeseed oil	290	49	(100–150)	166 (144–188)	100 (100–110)	560
Soya bean oil	189	30	(100–150)	171 (157–186)	120 (120–140)	560
Sunflower seed oil	542	20	(90–150)	269 (259–279)	200	680
Walnut oil	1	–	31	247	–	–
Coconut oil/fat*	184	8	(100–150)	476 (472–479)	426	1,065
Palm oil/fat*	498	1	(100–150)	3,955 (3,954–3,955)	3,610	9,700

LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC% = percentage of left-censored results; the values are rounded to the nearest integer.
(e): Mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

For glycidol, the food groups having higher mean values were 'palm oil/fat' with levels MB (LB–UB) of 3,955 (3,954–3,955) μg/kg, 'Maize oil' with 650 (647–654) μg/kg, 'margarine, normal fat' with 582 (580–584) μg/kg, 'coconut oil/fat' with 476 (472–479) μg/kg and 'palm kernel oil' with 421 (415–428) μg/kg. 'Special fats' also have a considerable level (386 (373–399) μg/kg) but their practical use in foodstuffs could not be established.

As highlighted in Section 2.1.1, the occurrence data in this data set were limited to the ester-bound form and the potential presence of 3- and 2-MCPD in free form (however expected to be practically negligible) was not considered.

3.1.3. Occurrence of total 3- and 2-MCPD and glycidol from esters in food other than oils/fats, soy sauce, HVP and related products

In this data set, the free and ester-bound form of 3- and 2-MCPD were separately reported for all samples except 70 infant formula samples and 26 'Fried or baked fish' samples where only the contribution from esters was reported. The total occurrence of 3- and 2-MCPD in infant formulae and fried or baked fish may thus be underestimated, while for the other food groups the contribution of both free and ester-bound forms was considered.

The number of analytical results on total 3- and 2-MCPD and glycidol from esters in food groups other than those mentioned in Sections 3.1.1 and 3.1.2 are summarised per sampling year in Table 14. The majority of the analyses were performed in 2014.

Table 15 shows the distribution of the results per sampling country. The data in this data set were produced in *ad hoc* studies. For products where a large variability across Europe was not expected the samples were taken near the laboratories performing the study (Belgium and Germany) while for products where a larger variability across Europe was expected, sampling was extended to different European areas.

Table 14: Distribution of results on total 3- and 2-MCPD and glycidol from esters in food other than oils/fats, soy sauce, HVP and related products per sampling year

Sampling year	Substance analysed			N[(b)]	%
	3-MCPD (expressed as 3-MCPD moiety)[(a)]	2-MCPD (expressed as 2-MCPD moiety)[(a)]	Glycidyl esters (expressed as glycidol moiety)[(a)]		
2012	2	2	2	6	< 1
2013	68	68	68	204	12
2014	478	478	478	1,434	83
2015	25	25	25	75	4
Total	573	573	573	1,719	100

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol.
(a): Number of analytical results reported per substance analysed.
(b): Total number of reported analytical results.

Table 15: Distribution of results on total 3- and 2-MCPD and glycidol from esters in food other than oils/fats, soy sauce, HVP and related products per sampling country

Sampling country	N[(a)]	%
Austria	57	3
Belgium	561	33
Bulgaria	12	< 1
Croatia	3	< 1
Czech Republic	9	< 1
Denmark	24	1
France	30	1
Germany	519	32
Greece	9	< 1
Hungary	15	< 1
Italy	135	8
Latvia	57	3
Netherlands	183	11
Poland	6	< 1
Portugal	6	< 1
Slovakia	3	< 1
Spain	12	< 1
Unknown	78	5
Total	1,719	100

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol.
(a): N = number of analytical results reported.

All the samples were analysed as individual samples and no aggregated sample was present in the data set.

The sampling strategy was reported as 'Objective sampling' in 83% of the results, and 'Convenient sampling' in 12% of the results (infant formulae) while in the remaining cases it was not reported; no 'Suspect sampling' was reported. The selection of the infant formula samples based on the ease of data collection (convenient sampling) introduces an uncertainty that might be partially mitigated by a relative concentration on few producers (with respect to other food groups) of the market of infant formulae.

All the results were reported on whole weight, with the exception of 210 data on infant formulae reported on fat basis. In this case, the occurrence was converted to whole weight using the fat content declared for the samples.

The data set included various left-censored results (i.e. reported as < LOD or < LOQ). The left censoring value ranged from < 0.1 to 56 μg/kg with a median of 9 μg/kg. The number and percentage of left-censored results is shown in Table 16. For the evaluation of occurrence, the left-censored results were considered following the approach explained in Section 2.2.2.

Table 16: Number of results and number and percentage of left-censored values by level 1 food categories in the data set on total 3- and 2-MCPD and glycidol from esters in food other than oils/fats, soy sauce, HVP and related products

Food group	N[(a)]	LC[(b)]	%LC[(b)]
Cereal-based products and similar	687	310	45
Fried, baked or roast meat or fish products	225	71	32
Infant formulae (powder)	210	56	27
Smoked meat or fish products	387	284	73
Snacks and potato products	210	22	10

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol; HVP: hydrolysed vegetable protein.
(a): N = Number of results.
(b): LC = Number of left-censored results; %LC = percentage of left-censored results in each food group.

The mean, median and 95th percentile of concentrations (μg/kg) of 3- and 2-MCPD and glycidol in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on food other than oils/fats, soy sauce, HVP and related products are presented in Tables 17, 18 and 19. The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

Table 17: Mean, median and 95th percentile of concentrations (μg/kg) of total 3-MCPD in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on food other than oils/fats, soy sauce, HVP and related products

Food groups[(a)] levels 1–3[(b)]	N[(c)]	% LC[(d)]	LOQ (min–max) (μg/kg)	Mean[(e)] MB (LB–UB) (μg/kg)	Median[(e)] MB (LB–UB) (μg/kg)	P95[(e)] MB (LB–UB) (μg/kg)
Infant formulae (powder)	70	1	200	108 (108–109)	105	147
Infant formula, milk-based, powder[(f)]	70	1	200	108 (108–109)	105	147
Cereal-based products and similar*	229	35	14	83 (77–90)	16 (11–20)	405 (398–412)
Bread and bread rolls	75	55	14	29 (23–36)	7.1 (0–14)	125 (118–132)
Wheat bread and rolls	21	48	14	31 (24–37)	11 (4–18)	–
Rye bread and rolls	12	83	14	8.5 (1.9–14)	7.1 (0–14)	–
Mixed wheat and rye bread and rolls	20	75	14	11 (5.2–17)	7.1 (0–14)	–
Multigrain bread and rolls	12	50	14	19 (13–25)	8.1 (1–14)	–
Unleavened bread, crispbread, rusk	10	–	14	101 (95–108)	59 (52–66)	–
Breakfast cereals	66	45	14	26 (19–33)	8.7 (1–17)	75 (68–82)
Cereal flakes	27	48	14	12 (6.1–19)	8 (1–15)	–
Muesli	8	38	14	95 (88–102)	8.5 (1.5–15)	–
Cereal bars	10	30	14	21 (14–29)	12 (5.5–19)	–
Popped cereals	11	18	14	29 (23–35)	20 (13–27)	–
Porridge	10	90	14	8.8 (0.6–17)	8.4 (0–16)	–

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Fine bakery wares	88	11	14	172 (167–178)	104 (97–110)	518 (511–525)
Cookies	36	11	14	200 (194–206)	128 (123–135)	–
Fatty cake products*	13	8	14	138 (132–145)	66 (59–73)	–
Hot surface cooked pastries*	9	–	14	247 (242–253)	257	–
Puff pastry*	7	29	14	106 (100–112)	20 (19–25)	–
Shortcrusts*	7	–	14	154 (148–160)	116 (109–123)	–
Yeast leavened pastries*	16	19	14	133 (127–138)	59 (52–66)	–
Fried, baked or roast meat or fish products*	75	15	(0.03–14)	30 (26–34)	17 (12–22)	119
Fried or baked fish*[f]	28	–	(0.03–14)	42 (42–43)	22	–
Fried or roast meat*	47	23	14	23 (17–29)	16 (10–22)	–
Smoked meat or fish products*	129	47	14	21 (15–28)	9.1 (1–17)	57 (54–61)
Smoked fish*	60	43	14	18 (12–24)	9 (1.5–17)	59 (55–65)
Smoked meat products*	69	51	14	24 (17–30)	9.4 (0–18)	49 (45–56)
Snacks and potato products*	70	4	14	130 (123–137)	63 (56–70)	502 (495–509)
Miscellaneous snack products*	8	13	14	119 (112–126)	100 (93–107)	–
Potato products*	62	3	14	132 (125–138)	61 (54–68)	502 (495–509)
French fries	8	13	14	57 (51–63)	37 (30–44)	–
Potato croquettes	10	–	14	30 (23–37)	23 (16–30)	–
Potato crisps	32	–	14	216 (210–223)	158 (151–165)	–
Oven baked potato products (include also home-made products like pan fried potato pieces or Roesti)*	12	8	14	40 (33–47)	31 (24–38)	–

3-MCPD: 3-monochloropropane-1,2-diol; HVP: hydrolysed vegetable protein; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound; P95: 95th percentile.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC % = percentage of left-censored results; the values are rounded to the nearest integer.
(e): mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.
(f): 'Infant formula, milk-based, powder' and 26 samples of 'Fried or baked fish' do not include the contribution from free 3-MCPD

Table 18: Mean, median and 95th percentile of concentration (μg/kg) of total 2-MCPD in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on food other than oils/fats, soy sauce, HVP and related products

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Infant formulae (powder)	70	53	200	44 (31–58)	28 (0–55)	73
Infant formula, milk-based, powder[f]	70	53	200	44 (31–58)	28 (0–55)	73
Cereal-based products and similar*	229	48	9	42 (38–47)	6.5 (1–12)	219 (215–224)

Food groups[a] levels 1–3[b]	N[c]	% LC [d]	LOQ (min–max) (µg/kg)	Mean[e] MB (LB–UB) (µg/kg)	Median[e] MB (LB–UB) (µg/kg)	P95[e] MB (LB–UB) (µg/kg)
Bread and bread rolls	75	68	9	14 (9.8–19)	4.7 (0–9.3)	59 (55–64)
Wheat bread and rolls	21	57	9	15 (10–19)	4.7 (0–9.3)	–
Rye bread and rolls	12	100	9	4.6 (0–9.3)	4.7 (0–9.3)	–
Mixed wheat and rye bread and rolls	20	85	9	6.1 (1.5–10)	4.7 (0–9.3)	–
Multigrain bread and rolls	12	67	9	7.3 (2.7–11)	4.7 (0–9.3)	–
Unleavened bread, crispbread, rusk	10	20	9	49 (44–54)	29 (24–33)	–
Breakfast cereals	66	65	9	15 (10–20)	5.6 (0–11)	82 (78–87)
Cereal flakes	27	81	9	8.7 (3.8–13)	4.8 (0–9.6)	–
Muesli	8	63	9	50 (46–55)	5.6 (0–11)	–
Cereal bars	10	30	9	12 (7.3–16)	6.50 (2–11)	–
Popped cereals	11	27	9	17 (12—22)	8.50 (4–13)	–
Porridge	10	100	9	5.9 (0–11)	5.70 (0–11)	–
Fine bakery wares	88	18	9	87 (82–92)	48 (43–52)	268 (264–273)
Cookies	36	11	9	103 (98–107)	66 (61–70)	–
Fatty cake products*	13	8	9	71 (66–75)	30 (29–32)	–
Hot surface cooked pastries*	9	22	9	123 (118–128)	126 (122–131)	–
Puff pastry*	7	57	9	47 (42–53)	6.8 (0–13)	–
Shortcrusts*	7	14	9	79 (75–84)	62 (58–67)	–
Yeast leavened pastries*	16	25	9	65 (60–70)	31 (26–35)	–
Fried, baked or roast meat or fish products*	75	57	(0.07–9)	10 (7–14)	6.5 (0–11)	46 (42–51)
Fried or baked fish*[f]	28	46	(0.07–9)	14 (13–15)	3 (2–3.5)	–
Fried or roast meat*	47	64	9	8.38 (3–13)	6.7 (0–13)	–
Smoked meat or fish products*	129	98	9	6.2 (0.5–11)	5.5 (0–11)	7.60 (0–15)
Smoked fish*	60	98	9	6.1 (0.8–11)	5.2 (0–10)	7.10 (0–14)
Smoked meat products*	69	99	9	6.2 (0.2–12)	6.1 (0–12)	7.70 (0–15)
Snacks and potato products*	70	9	9	79 (75–84)	32 (28–37)	285 (281–290)
Miscellaneous snack products*	8	13	9	67 (62–71)	55 (50–59)	–
Potato products*	62	8	9	81 (76–85)	31 (26–35)	285 (281–290)
French fries	8	25	9	23 (19–28)	13 (8.5–17)	–
Potato croquettes	10	–	9	17 (12–21)	17 (12–21)	–
Potato crisps	32	–	9	135 (131–140)	67 (63–72)	–
Oven baked potato products (include also home-made products like pan fried potato pieces or Roesti)*	12	25	9	28 (23–32)	16 (12–21)	–

2-MCPD: 2-monochloropropane-1,3-diol; HVP: hydrolysed vegetable protein; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound; P95: 95th percentile.

(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.

(b): The names are provided in indented form to show the hierarchical relationship of the food groups.

(c): N = number of analytical results reported.

(d): LC % = percentage of left-censored results; the values are rounded to the nearest integer.

(e): mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

(f): 'Infant formula, milk-based, powder' and 26 samples of 'Fried or baked fish' do not include the contribution from free 2-MCPD.

Table 19: Mean, median and 95th percentile of concentrations (μg/kg) of glycidol (from esters) in food groups related to the Comprehensive Food Consumption groups (including some *ad hoc* ones) in the data set on food other than oils/fats, soy sauce, HVP and related products

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Infant formulae (powder)	70	26	200	87 (80–94)	68	220
Infant formula, milk-based, powder	70	26	200	87 (80–94)	68	220
Cereal-based products and similar*	229	52	(0.03–15)	51 (50–51)	3 (0–4.8)	318
Bread and bread rolls	75	76	(0.1–8.9)	8 (7.8–8.3)	0.3 (0–0.6)	62
Wheat bread and rolls	21	67	(0.1–8.9)	12 (11–12)	0.3 (0–0.6)	–
Rye bread and rolls	12	92	(0.3–0.6)	0.4 (0.08–0.6)	0.3 (0–0.6)	–
Mixed wheat and rye bread and rolls	20	90	(0.1–0.6)	0.8 (0.8–1)	0.3 (0–0.6)	–
Multigrain bread and rolls	12	92	(0.2–4.6)	4.1 (3.8–4.3)	0.3 (0–0.6)	–
Unleavened bread, crispbread, rusk	10	30	(0.3–7.4)	28 (27–28)	11	–
Breakfast cereals	66	67	(0.03–14)	17 (16–18)	2 (0–2.9)	64
Cereal flakes	27	67	(0.03–7.3)	6.3 (5.5–7.1)	1 (0–1.2)	–
Muesli	8	75	(0.1–4.9)	84 (83–85)	1.8 (0–3.5)	–
Cereal bars	10	60	(0.1–8.1)	12 (11–12)	2.1 (0–3.1)	–
Popped cereals	11	36	(0.6–7.8)	15 (14–16)	3.9 (3–7.2)	–
Porridge	10	100	(1.8–14)	3 (0–6)	2.5 (0–5)	–
Fine bakery wares	88	20	(0.1–15)	112 (112–113)	39	585
Cookies	36	8	(0.3–15)	134 (134–135)	67	–
Fatty cake products*	13	15	(0.1–7.2)	102 (102–103)	16	–
Hot surface cooked pastries*	9	44	(0.3–8.1)	137 (136–138)	56	–
Puff pastry*	7	57	(3.8–9.5)	21 (19–23)	4.8 (0–9.5)	–
Shortcrusts*	7	14	(0.2–8.1)	149 (148–149)	58	–
Yeast leavened pastries*	16	25	(0.6–9.4)	81 (81–82)	13	–
Fried, baked or roast meat or fish products*	75	23	(0.08–13)	38 (38–39)	19	144
Fried or baked fish*	28	–	(0.08–13)	30	12	–
Fried or roast meat*	47	36	(0.3–11)	43 (42–44)	22	–
Smoked meat or fish products*	129	74	(0.2–17)	17 (15–19)	3.4 (0–6.4)	18
Smoked fish*	60	57	(0.2–17)	5.8 (4.7–6.8)	3 (0–4.8)	18
Smoked meat products*	69	90	(0.2–17)	27 (24–30)	3.5 (0–7)	13 (13–17)
Snacks and potato products*	70	19	(0.01–17)	58 (58–59)	19	249
Miscellaneous snack products*	8	63	(4.7–17)	15 (12–17)	6 (0–12)	–
Potato products*	62	13	(0.01–10)	64	22	249
French fries	8	25	(1.9–4.5)	41 (40–41)	14	–
Potato croquettes	10	30	(0.2–7.4)	5 (4.8–5.2)	6	–
Potato crisps	32	–	(2–10)	110	44	–

Food groups[a] levels 1–3[b]	N[c]	% LC[d]	LOQ (min–max) (μg/kg)	Mean[e] MB (LB–UB) (μg/kg)	Median[e] MB (LB–UB) (μg/kg)	P95[e] MB (LB–UB) (μg/kg)
Oven baked potato products (include also home-made products like pan fried potato pieces or Roesti)*	12	25	(0.01–4.4)	6.4	4.5	–

HVP: hydrolysed vegetable protein; LOQ: limit of quantification; MB: middle bound; LB: lower bound; UB: upper bound; P95: 95th percentile.
(a): Some *ad hoc* groups created for the purpose of this assessment are present together with FoodEx1 groups; *ad hoc* groups are flagged with an asterisk * after the name.
(b): The names are provided in indented form to show the hierarchical relationship of the food groups.
(c): N = number of analytical results reported.
(d): LC % = percentage of left-censored results; the values are rounded to the nearest integer.
(e): Mean = arithmetic mean; median = 50th percentile (when the number of data points is less than 11, the median is not reported); P95 = 95th percentile (when the number of data points is less than 60, the value of P95 is not reported). The values are reported as MB followed by the range LB–UB; when the values are coincident, the range is not reported.

For 3-MCPD, the food groups showing higher mean values were 'Hot surface cooked pastries' with levels MB (LB–UB) of 247 (242–253) μg/kg, 'Potato crisps' with 216 (210–223) μg/kg, 'Cookies' with 200 (194–206) μg/kg and 'Shortcrusts' with 154 (148–160) μg/kg.

For 2-MCPD, the food groups showing higher mean values were 'Potato crisps' with levels MB (LB–UB) of 135 (131–140) μg/kg, 'Hot surface cooked pastries' with 123 (118–128) μg/kg, 'Cookies' with 103 (98–107) μg/kg and 'Shortcrusts' with 79 (75–84) μg/kg.

For glycidol, the food groups showing higher mean values were 'Shortcrusts' with 149 (148–149) μg/kg, 'Hot surface cooked pastries' with 137 (136–138) μg/kg, 'Cookies' with 134 (134–135) μg/kg and 'Potato crisps' with 110 (LB and UB coincident) μg/kg.

3.1.4. Correlation of total 3-MCPD and 2-MCPD levels in food

The possible correlation between total 3-MCPD and total 2-MCPD levels was analysed in the data on food submitted in the framework of the JRC survey. The data on the samples where both 3-MCPD and 2-MCPD were quantified were used. A linear least squares fitting of first order was performed using 2-MCPD level as dependent variable and 3-MCPD level as independent variable.

The fitting is shown in Figure 6.

The straight line of best fit has a slope of 0.53 and an R^2 value of 0.88. Among the food data, the food group with the best correlation is 'Cereal-based products and similar' with a slope of 0.5 and an R^2 value of 0.98. The model suggests therefore an expected level of 2-MCPD at about (or slightly higher than) half of the 3-MCPD level.

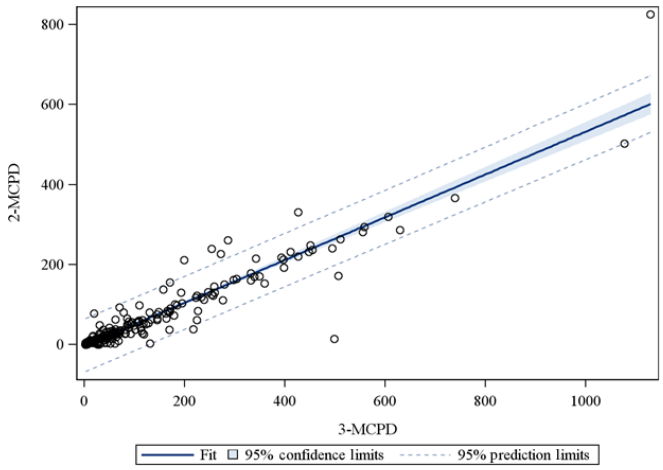


Figure 6: Linear first-order fitting (least squares method) of total 3-monochloropropane-1,2-diol (3-MCPD) level versus total 2-monochloropropane-1,3-diol (2-MCPD) level in the different samples of the Joint Research Centre data set on food

3.2. Exposure assessment of 3-, 2-MCPD and glycidol in humans

The exposure to 3- and 2-MCPD was based upon the level of exposure to the parent compounds regardless of their original form (i.e. as free or as ester of fatty acids), and referred to as 3-MCPD and 2-MCPD. Likewise, exposure to glycidol refers to the parent compound, but in this case the original form was exclusively as fatty acid esters.

3.2.1. Occurrence data used for exposure assessment

The assessment of dietary exposure to MCPDs, and glycidol from esters required the combination of occurrence values for all relevant food groups with food consumption data from the EFSA Comprehensive Food Consumption Database (as described in Section 2.1.2).

The Foodex1 groups used to estimate exposure are those listed in Table B.3. For many of those food groups occurrence data were available as described in Sections 3.1.1, 3.1.2 and 3.1.3. Occurrence data were not available for some relevant food groups, but could be calculated from models based on estimates of the amount of oil in the products:

- Vegetable oils not included in the data set;
- Mayonnaise, emulsion sauces and salad dressings;
- Chocolate spreads and similar spreadable products.

For the first two food groups an occurrence value was calculated based on the occurrence data measured in vegetable oils using models making assumptions on the type and proportion of oils in the food. For mayonnaise, emulsified sauces and salad dressings, the occurrence of 3-MCPD in free form was also taken into account.

The occurrence value for an 'average edible vegetable oil' was calculated considering the market share expressed as a percentage for the oils included in the data set (from the data on edible oil consumption in the EU in 2011 made available by FEDIOL as summarised in Table 20), and the mean occurrence values calculated for these oils. Palm oil, coconut oil and palm kernel oil were excluded from the model representing the 'average edible vegetable oil', because they often have different uses than the other oils. Olive oil was also excluded because it is not often used in the products modelled.

Table 20: Data on edible oil consumption in the EU in 2011 (data provided by FEDIOL); the amount in 1,000 tons and the corresponding percentage are reported

Oil source	EU27 oil consumption 2011 (× 1,000 tons)	%
Sunflower oil	2,963	23
Palm oil	2,751	22
Rapeseed oil	2,665	21
Olive oil	2,045	16
Soybean oil	1,150	9
Coconut oil	415	3
Palm kernel oil	398	3
Maize germ oil	196	2
Peanut oil	88	1
Total	12,671	

EU: European Union; FEDIOL: Association of the EU Vegetable Oil & Proteinmeal Industry.

The occurrence values calculated for the 'average edible vegetable oil' were used to attribute an occurrence value to edible oils not included in the occurrence data set and also to calculate the occurrence values for products such as mayonnaise, emulsion sauces and salad dressings. The calculation is described in Table B.3 in Appendix B.

No data were available on the occurrence of MCPDs and glycidol from esters in chocolate spreads and similar spreadable products. To identify a model, the ingredient labels of the products of this type were consulted in the Global New Products Database (GNPD) published by Mintel;[14] the fat content and fat

[14] Product launches in European countries in August-September 2015, accessed at: http://www.mintel.com/global-new-products-database

type in the products varied, and many products declared the presence of palm oil alone or in combination with other vegetable oils. Choosing the most often featured brand and several similar products produced under distribution labels, variability in fat content between 29% and 31% was observed. In many cases, hazelnuts was declared on the label as ingredient at about 13%; therefore, a contribution of roughly 8% fat from the hazelnuts was subtracted; consequently, a proportion of vegetable oil of 22% was estimated. The model assumed the use of only palm oil; this may represent a worst-case scenario.

No model was possible for some additional food groups not covered by the available data and where 3- and 2-MCPD and glycidol were potentially present but sufficient information on the fat source or on the proportion of fat in the food was not available. These included for example chocolate and related products, ice cream, meat specialties (such as terrine or paté), meat and dairy imitations and fish oil. The absence of these food groups may lead to underestimation to some extent of the dietary exposure.

Table B.3 in Appendix B describes the occurrence values used for calculating exposure and for the groups where a model was applied it also briefly describes the calculation applied.

In fish and fish products the Foodex1 code does not record the treatment, therefore the occurrence value for 'Fried or baked fish' was applied, because the occurrence levels in this group were either higher (more conservative choice) or similar to those in 'Smoked fish'. It is assumed here that fish were either fried or smoked which leads to overestimation of occurrence. No occurrence data were available for potential combination of the two treatments. In the case of meat and meat products, the occurrence values in 'Fried or roast meat' were used for unprocessed meat food groups, while the occurrence values for 'Smoked meat products' were used for sausages and similar charcuterie products. These assumptions are conservative and might overestimate the exposure.

3.2.2. Mean and high chronic dietary exposure to 3- and 2-MCPD and glycidol

For calculating the chronic dietary exposure to 3- and 2-MCPD or glycidol, food consumption and body weight data at the individual level were accessed from the Comprehensive Food Consumption Database. The occurrence data described in the previous section and consumption data were linked at FoodEx1 level. In addition, different but related food commodities were grouped in food categories to better explain their contribution to the total dietary exposure to these substances. For each country, exposure estimates were calculated per dietary survey and age class. Chronic exposure estimates were calculated for 41 different dietary surveys carried out in 22 different European countries. Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one consumption survey; the dietary surveys available for the different age classes are reported in Table B.2 in Appendix B. The mean and the high (95th percentile) chronic dietary exposures (in μg/kg bw per day) were calculated by combining the mean occurrence values as shown in Table B.3 in Appendix B with the average daily consumption for each food at individual level and choosing the mean and P95 of exposure for each age class in each dietary survey.

The chronic dietary exposure was calculated separately for 3-MCPD, 2-MCPD and glycidol. The tables present the minimum, median and maximum exposure across surveys for each age class. As the occurrence values were calculated as LB, MB and UB scenarios, the exposure corresponding to the three occurrence values is presented in the form MB (LB–UB). Considering the relatively narrow range between exposure estimates based on LB and UB occurrence, the Panel decided to focus on the exposure estimates corresponding to MB occurrence. The full range of estimated exposure based on LB, MB and UB occurrence data is shown in Tables 21–26.

3.2.2.1. 3-MCPD

The mean and P95 of chronic dietary exposure to 3-MCPD across dietary surveys from different European countries for the different age classes are summarised in Tables 21 and 22, respectively.

The median across dietary surveys of mean chronic exposure to 3-MCPD was below 1 μg/kg bw per day (MB) in all age classes, with 'Infants', 'Toddlers' and 'Other children' in the range 0.7–0.9 μg/kg bw per day (MB). The minimum across dietary surveys of the mean exposure in the same age classes was 0.5–0.6 μg/kg bw per day (MB). The maximum of mean exposure across dietary surveys in the age classes 'Toddlers' and 'Other children' reached a value of 1.4–1.5 μg/kg bw per day (MB). In adolescents and adult population groups (adults, elderly, very elderly) the mean exposure to 3-MCPD ranged from 0.2 to 0.7 μg/kg bw per day (MB).

Considering the P95 of exposure (high exposure), the median across dietary surveys for 'Infants', 'Toddlers' and 'Other children' was in the range 1.4–1.7 μg/kg bw per day (MB). The minimum across dietary surveys in the same age classes was in the range 1.1–1.5 μg/kg bw per day (MB) and the maximum was in the range 2.4–2.6 μg/kg bw per day (MB).

Table 21: The minimum, median and maximum values for the mean chronic exposure to 3-MCPD (μg/kg bw per day) across dietary surveys from different countries

Age class[a]	Min MB (LB–UB) (μg/kg bw per day)	Median MB (LB–UB) (μg/kg bw per day)	Max MB (LB–UB) (μg/kg bw per day)
Infants	0.5	0.9 (0.8–0.9)	1.0 (0.9–1.0)
Toddlers	0.6 (0.5–0.6)	0.8 (0.8–0.9)	1.4 (1.3–1.5)
Other children	0.5 (0.5–0.6)	0.7 (0.7–0.8)	1.5 (1.4–1.6)
Adolescents	0.2 (0.2–0.3)	0.4 (0.4–0.5)	0.7 (0.6–0.7)
Adults	0.2	0.3	0.4 (0.4–0.5)
Elderly	0.2	0.3 (0.3–0.4)	0.4
Very elderly	0.2	0.3	0.5 (0.4–0.5)

3-MCPD: 3-monochloropropane-1,2-diol; bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.

Table 22: The minimum, median and maximum values for the P95 chronic exposure to 3-MCPD (μg/kg bw per day) across dietary surveys from different countries. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported

Age class[a]	Min MB (LB–UB)[b] (μg/kg bw per day)	Median MB (LB–UB)[b] (μg/kg bw per day)	Max MB (LB–UB)[b] (μg/kg bw per day)
Infants	1.5 (1.5–1.6)	1.7 (1.7–1.8)	2.5 (2.5–2.6)
Toddlers	1.4 (1.4–1.5)	1.7 (1.6–1.9)	2.4 (2.3–2.6)
Other children	1.1 (1.1–1.2)	1.4 (1.4–1.6)	2.6 (2.5–2.7)
Adolescents	0.5 (0.5–0.6)	0.9 (0.8–0.9)	1.3 (1.3–1.4)
Adults	0.4 (0.4–0.5)	0.7 (0.6–0.7)	0.9 (0.8–0.9)
Elderly	0.4 (0.3–0.4)	0.6 (0.6–0.7)	0.8 (0.8–0.9)
Very elderly	0.3 (0.3–0.4)	0.7 (0.6–0.7)	0.9 (0.8–0.9)

P95: 95th percentile; 3-MCPD: 3-monochloropropane-1,2-diol; bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

For 'Adolescents' and adult population groups (adults, elderly, very elderly), the median across dietary surveys of P95 of exposure (high exposure) was ≤ 0.9 μg/kg bw per day (MB) with a minimum in the survey with lowest exposure of 0.3 μg/kg bw per day (MB) and a maximum in the survey with highest exposure of 1.3 μg/kg bw per day (MB).

3.2.2.2. 2-MCPD

The mean and P95 of chronic dietary exposure to 2-MCPD for the different age classes are summarised in Tables 23 and 24, respectively.

The median across dietary surveys of mean chronic exposure to 2-MCPD was below 0.5 μg/kg bw per day (MB) in all age classes, with 'Infants', 'Toddlers' and 'Other children' in the range 0.3–0.4 μg/kg bw per day (MB). The minimum of mean exposure across dietary surveys in these age classes was 0.2–0.3 μg/kg bw per day (MB). The maximum of mean exposure across dietary surveys was similar to the median in 'Infants', while in 'Toddlers' and 'Other children' reached a value of 0.6–0.7 μg/kg bw per day (MB).

In adolescents and adult population groups (adults, elderly, very elderly) the mean exposure to 2-MCPD ranged from 0.1 to 0.3 μg/kg bw per day (MB).

Considering the P95 of exposure (high exposure), the median across dietary surveys for 'Infants', 'Toddlers' and 'Other children' was in the range 0.7 – 0.8 μg/kg bw per day (MB); the minimum across dietary surveys in the same age classes was in the range 0.5 – 0.7 μg/kg bw per day (MB). The maximum for the same age classes was in the range 1.0 – 1.2 μg/kg bw per day (MB).

Table 23: The minimum, median and maximum values for the mean chronic exposure to 2-MCPD (μg/kg bw per day) across dietary surveys from different countries

Age class[a]	Min MB (LB–UB) (μg/kg bw per day)	Median MB (LB–UB) (μg/kg bw per day)	Max MB (LB–UB) (μg/kg bw per day)
Infants	0.2 (0.2–0.3)	0.4 (0.3–0.5)	0.4 (0.3–0.5)
Toddlers	0.3 (0.2–0.3)	0.4 (0.3–0.4)	0.6 (0.6–0.7)
Other children	0.3 (0.2–0.3)	0.3 (0.3–0.4)	0.7 (0.6–0.7)
Adolescents	0.1	0.2	0.3
Adults	0.1	0.1 (0.1–0.2)	0.2
Elderly	0.1	0.1 (0.1–0.2)	0.2 (0.1–0.2)
Very elderly	0.1	0.1 (0.1–0.2)	0.2

2-MCPD: 2-monochloropropane-1,3-diol; bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.

Table 24: The minimum, median and maximum values for the P95 chronic exposure to 2-MCPD (μg/kg bw per day) across dietary surveys from different countries

Age class[a]	Min MB (LB–UB)[b] (μg/kg bw per day)	Median MB (LB–UB)[b] (μg/kg bw per day)	Max MB (LB–UB)[b] (μg/kg bw per day)
Infants	0.7 (0.5–0.8)	0.8 (0.6–1)	1.0 (0.8–1.4)
Toddlers	0.6 (0.5–0.7)	0.8 (0.7–0.9)	1.1 (1–1.2)
Other children	0.5 (0.4–0.5)	0.7 (0.6–0.8)	1.2 (1.1–1.3)
Adolescents	0.3 (0.2–0.3)	0.4 (0.4–0.5)	0.6 (0.6–0.7)
Adults	0.2 (0.2–0.3)	0.3 (0.3–0.4)	0.4 (0.3–0.4)
Elderly	0.2	0.3 (0.2–0.3)	0.4 (0.3–0.4)
Very elderly	0.2	0.3	0.4 (0.3–0.4)

P95: 95th percentile; 2-MCPD: 2-monochloropropane-1,3-diol; bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

In adolescents and adult population groups (adults, elderly, very elderly) the high exposure (P95) ranged from 0.2 to 0.6 μg/kg bw per day (MB) across dietary surveys.

3.2.2.3. Glycidol from esters

The mean and P95 of chronic dietary exposure to glycidol for the different age classes are summarised in Tables 25 and 26, respectively.

Table 25: The minimum, median and maximum values for the mean chronic exposure to glycidol from esters (μg/kg bw per day) across dietary surveys

Age class[a]	Min MB (LB–UB) (μg/kg bw per day)	Median MB (LB–UB) (μg/kg bw per day)	Max MB (LB–UB) (μg/kg bw per day)
Infants	0.4 (0.3–0.4)	0.7	0.8 (0.7–0.8)
Toddlers	0.4 (0.4–0.5)	0.6	0.9
Other children	0.3	0.6 (0.5–0.6)	0.9 (0.9–1)
Adolescents	0.2	0.3	0.5
Adults	0.2 (0.1–0.2)	0.2	0.3
Elderly	0.1	0.2 (0.2–0.3)	0.3
Very elderly	0.1	0.2 (0.2–0.3)	0.3

bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.

Table 26: The minimum, median and maximum values for the P95 chronic exposure to glycidol from esters (μg/kg bw per day) across dietary surveys

Age class[a]	Min MB (LB–UB)[b] (μg/kg bw per day)	Median MB (LB–UB)[b] (μg/kg bw per day)	Max MB (LB–UB)[b] (μg/kg bw per day)
Infants	1.2 (1.2–1.3)	1.4 (1.3–1.5)	2.1 (2.1–2.2)
Toddlers	1	1.1 (1.1–1.2)	2.0 (2.0–2.1)
Other children	0.8	1.1	1.7
Adolescents	0.4	0.6 (0.6–0.7)	1.1
Adults	0.3	0.5	0.7 (0.6–0.7)
Elderly	0.3 (0.2–0.3)	0.5	0.6
Very elderly	0.2	0.5	0.7 (0.7–0.8)

P95: 95th percentile; bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): Details on the dietary surveys in the different age classes are in Table B.2 in Appendix B. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

The median across dietary surveys of mean chronic exposure to glycidol was at 0.7 μg/kg bw per day (MB) in 'Infants', and at 0.6 μg/kg bw per day (MB) in 'Toddlers' and 'Other children'; for 'Adolescents' and older age classes it was in the range 0.2–0.3 μg/kg bw per day (MB). The range of mean exposure to glycidol across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children' was 0.3–0.9 μg/kg bw per day (MB). In 'Adolescents' and adult population groups ('Adults', 'Elderly', 'Very elderly') the mean exposure to glycidol ranged from 0.1 to 0.5 μg/kg bw per day (MB).

Considering the P95 of exposure (high exposure), the median across dietary surveys was 1.4 μg/kg bw per day (MB) for 'Infants', and 1.1 μg/kg bw per day (MB) for 'Toddlers' and 'Other children'. The minimum across dietary surveys in the same age classes was only slightly lower, with 'Infants' at 1.2 μg/kg bw per day (MB) and 'Toddlers' and 'Other children' in the range 0.8–1 μg/kg bw per day (MB). The maximum for the same age classes reached values in the range 1.7–2.1 μg/kg bw per day (MB); for 'Adolescents' the maximum exposure in this scenario (high exposure) was 1.1 μg/kg bw per day (MB). For 'Adults' and older age classes, the median across dietary surveys of P95 of exposure (high exposure) was 0.5 μg/kg bw per day (MB) with maximum in the surveys with highest exposure in the range 0.6–0.7 μg/kg bw per day (MB). The range of P95 of exposure (high exposure) to glycidol across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children' was 0.8–2.1 μg/kg bw per day (MB). The high exposure (P95) in 'Adolescents' ranged from 0.4 to 1.1 μg/kg bw per day (MB) and in adults and older population groups ranged from 0.2 to 0.7 μg/kg bw per day (MB).

3.2.3. Contributions of different food groups to 3- and 2-MCPD and glycidol exposure

The contribution of the different food groups to the MB mean exposure to 3- and 2-MCPD and glycidol by population groups is summarised in Tables B.5, B.6 and B.7 in Appendix B. The tables cover different ranges of % contribution to the MB mean exposure (< 1%, 1–5%, 5–10%, 10–20%, 20–30%, 30–40%, 40–60%, > 60%) presenting for each age class and each food group the number of dietary surveys where the % contribution of the specific food group falls into a specific range. Food groups where a higher number of surveys are listed on the right-hand columns of the tables are those more contributing to the overall mean exposure of the respective age class. In the large majority of the cases, while the % contribution of a particular food group is high in one or more national dietary survey, it may be low or even very low in others. The considerations on these tables represent therefore a warning of potential higher contribution to the exposure in specific areas or age classes.

3.2.3.1. 3-MCPD

The food group with higher contribution to the mean MB chronic dietary exposure to 3-MCPD in 'Infants' was 'infant and follow-on formulae' (around or more than 50% contribution), followed by 'vegetable fats and oils' and 'cookies'. As stated previously, these data must be considered with caution, because the age class of 'Infants' is not homogeneous and the diet is diversified only after weaning.

The most relevant contributors to the mean MB chronic dietary exposure to 3-MCPD in 'Toddlers' were 'vegetable fats and oils', 'cookies', 'pastries and cakes' and 'infant and follow-on formulae'. In the class 'Other children', the foods having higher contribution were 'pastries and cakes', 'margarine and similar', 'vegetable fats and oils' and 'cookies'.

In the age class 'Adolescents', 'margarine and similar', 'fried or baked potato products' and 'pastries and cakes' were important contributors to the mean MB chronic dietary exposure to 3-MCPD, followed by 'bread and bread rolls', 'fried or roast meat' and other food groups in lower proportion. In 'Adults', relevant contributors to the mean MB chronic dietary exposure to 3-MCPD were 'margarine and similar' and 'pastries and cakes' and 'vegetable fats and oils'; however, 'bread and bread rolls' and 'fried or roast meat' were also important contributors in many surveys, though at a lower degree.

'Margarine and similar', 'pastries and cakes', 'vegetable fats and oils' and 'bread and bread rolls' were the major contributors to the mean MB chronic dietary exposure to 3-MCPD in the classes 'Elderly' and 'Very elderly'.

3.2.3.2. 2-MCPD

The food groups with the higher contribution to the mean MB chronic dietary exposure to 2-MCPD in 'Infants' were 'infant and follow-on formulae' (around or more than 50% contribution), together with 'vegetable fats and oils' and 'cookies'. These data must be considered with caution, because the age class of 'Infants' is not homogeneous and the diet is diversified only after weaning. Regarding the consumption of vegetable fats, they also include the fats considered in the disaggregation of composite food.

The most relevant contributors to the mean MB chronic dietary exposure to 2-MCPD in 'Toddlers' were 'vegetable fats and oils', 'cookies', 'pastries and cakes' and in some surveys 'infant and follow-on formulae'. In the class 'Other children', the foods having higher contribution in some surveys were 'pastries and cakes', 'margarine and similar', 'vegetable fats and oils' and 'cookies'.

'Margarine and similar', 'fried or baked potato products', 'cookies' and 'pastries and cakes' were important contributors to the mean MB chronic dietary exposure to 2-MCPD in 'Adolescents', but different other food groups contribute in somewhat lower proportions. 'Adults' showed a relevant contribution from 'margarine and similar', 'vegetable fats and oils' and 'pastries and cakes'; at lower level, 'bread and bread rolls' and 'fried or roast meat' were relevant contributors in many surveys.

'Margarine and similar', 'pastries and cakes', 'vegetable fats and oils' and 'bread and bread rolls' were the major contributors to the mean MB chronic dietary exposure to 2-MCPD in the classes 'Elderly' and 'Very elderly'.

3.2.3.3. Glycidol from esters

The food group with higher contribution to the mean MB chronic dietary exposure to glycidol in 'Infants' was 'infant and follow-on formulae' (around or more than 50% contribution), followed by 'vegetable fats and oils' and 'cookies'. As stated previously, these data must be considered with caution, because the age class of 'Infants' is not homogeneous and the diet is diversified only after weaning.

The most relevant contributors to the mean MB chronic dietary exposure to glycidol in 'Toddlers' were 'vegetable fats and oils', 'cookies', 'fried or roast meat' and 'pastries and cakes'. Only one survey with very high contribution (40–60%) was observed for 'margarine and similar'. In the class 'Other children', the foods having higher contribution were 'margarine and similar', 'pastries and cakes', 'fried or roast meat', 'cookies' and in some surveys 'chocolate spread and similar'.

In the age class 'Adolescents', 'margarine and similar', 'fried or roast meat', 'pastries and cakes' and in some surveys 'chocolate spreads and similar' were the major contributor to the exposure to glycidol.

Important contributors to the mean MB chronic dietary exposure of 'Adults' to glycidol were 'margarine and similar', 'fried or roast meat' and 'pastries and cakes'; in some surveys, 'vegetable fats and oils' were also relevant contributors. The pattern of contributors to the mean MB chronic dietary exposure to glycidol of 'Elderly' and 'Very elderly' was similar.

3.2.4. Dietary exposure to 3- and 2-MCPD and glycidol for infants receiving formula only

In the previous sections, exposure estimates were presented for 3-, 2-MCPD and glycidol including the age class 'Infants'. Actually, this class contains individuals before and after weaning and are

therefore not homogeneous in terms of diet. For this reason, a specific scenario was calculated for exposure from infant formula, assuming exclusive formula feeding and this was compared with the exposure calculated from the consumption surveys. To calculate occurrence values, the mean occurrence values in infant formulae were used. The occurrence data for infant formula were reported as powdered dry product; therefore, the value was converted to ready-to-use liquid formula with a dilution factor of 7.7.

In addition to the average scenario, a worst-case scenario was also calculated to take into account brand loyalty or bulk single-lot purchases in case of highly contaminated brands or production lots. To this end, the P95 values of occurrence in powdered infant formula were used. The correction to ready-to-eat liquid infant formulae was performed as in the previous scenario using a dilution factor of 7.7.

As consumption figures, an average consumption per kg body weight was calculated based on the dosage suggested on the label of seven different infant formulae randomly chosen between products featured in the GNPD database of new products published by Mintel. As body weight, the mean between males and females for the classes 1–2 months, 2–3 months and 3–4 months from the WHO growth standards[15] was used. The calculated average consumption of infant formula (diluted, ready to eat) over the period from 1 to 4 months is 170 g per kg bw per day.

3.2.4.1. 3-MCPD

The mean occurrence of 3-MCPD in infant formula (powder) was 108 (108–109) μg/kg (MB (LB–UB)). The calculated value for the diluted formula was 14.03 (14.03–14.16) μg/kg. The P95 of occurrence of 3-MCPD in infant formulae (powder) was 147 μg/kg (MB = LB = UB). The calculated value for the diluted formula was 19.1 μg/kg. The resultant exposure scenarios for infants receiving formula only are presented in Table 27.

Table 27: Scenarios[(a)] for exposure to 3-MCPD of infants receiving formula only

Consumer	Exposure estimate (μg/kg bw per day)
Infants consuming 170 g/kg bw per day of liquid formula at the mean occurrence values	2.4
Infants consuming 170 g/kg bw per day of liquid formula at the P95 occurrence values	3.2

3-MCPD: 3-monochloropropane-1,2-diol; P95: 95th percentile; LB: lower bound; MB: middle bound; UB: upper bound; bw: body weight.

(a): The scenarios use the mean and P95 occurrence values calculated for infant formulae. The exposure estimates corresponding to LB, MB and UB occurrence scenarios are coincident

In the scenarios for 3-MCPD, no difference was observed between LB, MB and UB exposure estimates. The dietary exposure to 3-MCPD corresponding to mean occurrence is 2.4 μg/kg bw per day while the dietary exposure corresponding the P95 of occurrence is 3.24 μg/kg bw per day.

3.2.4.2. 2-MCPD

The mean occurrence of 2-MCPD in infant formulae (powder) was 44 (31–58) μg/kg (MB (LB–UB)). The calculated value for the diluted formula was 5.71 (4.03–7.53) μg/kg. The P95 of occurrence of 2-MCPD in infant formulae (powder) was 73 μg/kg (MB = LB = UB). The calculated value for the diluted formula was 9.48 μg/kg. The resultant exposure scenarios for infants receiving formula only are presented in Table 28.

In the scenario based on mean occurrence, the dietary exposure to 2-MCPD based on LB occurrence data is 0.7 μg/kg bw per day. It can be observed that in this case the difference between LB and UB approach is large and the dietary exposure based on the UB approach (1.3 μg/kg bw per day) is almost the double of the one estimated in the LB approach.

In the scenario based on the P95 of occurrence, the dietary exposure is 1.6 μg/kg bw per day.

[15] Available online: http://www.who.int/childgrowth/standards/weight_for_age/en/ (accessed on 28 September 2015)

Table 28: Scenarios[(a)] for exposure to 2-MCPD of infants receiving formula only

Consumer	Exposure based on LB occurrence (μg/kg bw per day)	Exposure based on MB occurrence (μg/kg bw per day)	Exposure based on UB occurrence (μg/kg bw per day)
Infants consuming 170 g/kg bw per day of liquid formula at the mean occurrence values	0.7	1	1.3
Infants consuming 170 g/kg bw per day of liquid formula at the P95 occurrence values	1.6	1.6	1.6

2-MCPD: 2-monochloropropane-1,3-diol; P95: 95th percentile; LB: lower bound; MB: middle bound; UB: upper bound; bw: body weight.
(a): The scenarios use the mean and P95 occurrence values calculated for infant formulae. The exposure estimates corresponding to LB, MB and UB) occurrence scenarios are reported

3.2.4.3. Glycidol

The mean occurrence of glycidol in infant formula (powder) was 87 (80–94) μg/kg (MB (LB–UB)). The calculated value for the diluted formula was 11.3 (10.39–12.21) μg/kg. The P95 of occurrence of glycidol in infant formulae (powder) was 220 μg/kg (MB = LB = UB). The calculated value for the diluted formula was 28.57 μg/kg. The resultant exposure scenarios for infants receiving formula only are presented in Table 29.

Table 29: Scenarios[(a)] for exposure to glycidol of infants exclusively formula-fed

Consumer	Exposure based on LB occurrence (μg/kg bw per day)	Exposure based on MB occurrence (μg/kg bw per day)	Exposure based on UB occurrence (μg/kg bw per day)
Infants consuming 170 g/kg bw per day of liquid formula at the mean occurrence values	1.8	1.9	2.1
Infants consuming 170 g/kg bw per day of liquid formula at the P95 occurrence values	4.9	4.9	4.9

P95: 95th percentile; LB: lower bound; MB: middle bound; UB: upper bound; bw: body weight.
(a): The scenarios use the mean and P95 occurrence values calculated for infant formulae. The exposure estimates corresponding to MB (LB–UB) occurrence scenarios are reported

In the scenarios for exposure to glycidol of infants based on mean occurrence, the difference between the estimates in the LB and UB approaches is limited. The dietary exposure to glycidol using LB occurrence data is 1.8 μg/kg bw per day while the exposure estimated using UB occurrence data is 2.1 μg/kg bw per day.

In the scenario based on the P95 of occurrence, the dietary exposure is 4.9 μg/kg bw per day.

3.3. Hazard identification and characterisation

3.3.1. Toxicokinetics

3.3.1.1. 3-MCPD and 3-MCPD fatty acid esters

Abraham et al. (2013) evaluated the toxicokinetics of 3-MCPD (10 mg/kg bw) in comparison with an equimolar dose of 3-MCPD di-palmitate (53 mg/kg bw) using GC-MS measurements on both free MCPD and the ester in plasma from male Wistar rats (8–9 weeks of age) after gavage administration. Absorption of 3-MCPD was rapid with the maximal concentration (4,850 ng/mL) observed near the first sampling time (0.37 h). The appearance of 3-MCPD in plasma after gavage of the diester was slower with the maximal concentration (949 ng/mL) observed at 3.0 h. Absorption rate constants derived from fitting the data to a one-compartment model were reported as 7.0 and 0.36 L/h for 3-MCPD and its diester, respectively with half-lives of 0.10 and 1.9 h, respectively. Elimination rate constants were similarly derived from the data, and were reported as 0.31 and 0.69 L/h for 3-MCPD

and its diester, respectively. The respective elimination half-times were calculated by the CONTAM Panel to be 1 and 2.2 h. The area under the curve (AUC) values for plasma 3-MCPD were 9.03 and 7.76 microg × h/mL for the parent compound and its diester, respectively. The diester was quantified in luminal contents of small and large intestinal segments from 1.5 to 24 h and apparent transit of the diester from small to large intestine was observed; however, by 24 h, only 0.015–0.05% of the diester remained in the two intestinal segments. Concentrations of 3-MCPD were measured in liver, kidney, and adipose tissue and were similar to plasma levels in liver and kidney, but consistently lower in adipose tissue after dosing with either 3-MCPD or its diester. After administration of the diester, the parent compound itself was not observed in plasma, liver, kidney or adipose tissue at any time point (LOQ < 50 ng/g). It was reported that excretion of 3-MCPD into urine was approximately 2% of the administered dose for either compound and that 0.5–1.4% of the dose was observed in faeces. The similar AUCs for plasma 3-MCPD for the two compounds (86% for the diester compared with 3-MCPD) were interpreted as reflecting essentially complete hydrolysis of the diester during transit through the gastrointestinal (GI) tract resulting in similar bioavailability; however, a fivefold lower maximal concentration (C_{max}) was observed for the diester and was interpreted as resulting from delayed hydrolysis during transit that could affect toxicity endpoints related to maximal concentration levels.

Gavage administration of either 3-MCPD or three of its fatty acid esters produced no detectable glycidol in either the serum or gastric, duodenal and caecal contents of male F344 rats 30 min after dosing (Onami et al., 2015).

In conclusion, 3-MCPD and its dipalmitate fatty acid esters appear to be rapidly and efficiently absorbed following ingestion with extensive presystemic de-esterification occurring in the GI tract of rats. Elimination of 3-MCPD from serum was also rapid following dosing with either the parent compound or its diester. While the serum AUC values were similar for a single gavage dose of 3-MCPD and its di-palmitate diester (80%), the C_{max} value for the diester was only 20% of that for the parent compound. The lower C_{max} and longer elimination half-life of free MCPD in blood in the case of the diester is consistent with a delayed hydrolysis during transit through the GI tract. Conjugation with glutathione is one well-characterised metabolic pathway but its extent is limited. The formation of other 3-MCPD metabolites is poorly characterised. Urinary excretion of 3-MCPD and its metabolites appears to be predominant, as faecal excretion is quite limited, but is not completely characterised (see section on metabolism; Jones, 1975; Jones et al., 1978). Conversion of 3-MCPD, administered in either free or esterified forms, into glycidol was not observed *in vivo*.

2-MCPD and 2-MCPD fatty acid esters

No data were identified.

Glycidol and glycidyl fatty acid esters

The disposition of ^{14}C-labelled glycidol was evaluated in male F344 rats approximately 11 weeks of age (Nomeir et al., 1995). The single doses administered were the same as used in the National Toxicology Program (NTP) chronic bioassay for carcinogenicity, 37.5 and 50 mg/kg bw, by the oral (gavage) and intravenous (i.v.) routes. Urinary excretion of total radioactivity was predominant (40–48%), followed by exhaled CO_2 (27–32%), faeces (5–12%), and at 72 h tissues contained 7–8%. The urinary metabolite excretion profile, which was similar between oral and i.v. routes, showed one major unidentified metabolite at 14–21% of the total radioactivity and four lesser unidentified metabolites, comprising 2–8% of the total radioactivity. Based on total radioactivity recovered after gavage (91%) and i.v. (91%) dosing at 50 mg/kg bw, glycidol was quantitatively absorbed from the GI tract. There was retention of radioactivity in tissues (9–12% at 24 h and 7–8% at 72 h) with the skeletal muscle, skin, blood cells and liver containing the highest amounts (1–4% of dose each at 24 h), which could result from either covalent binding of glycidol or incorporation of radiolabel into macromolecules through entry into normal intermediary metabolism.

Wakabayashi et al. (2012) evaluated the plasma pharmacokinetics of glycidol using GC-MS after oral and i.v. administration to Sprague-Dawley rats (n = 189) and cynomolgus monkeys (n = 3). The principal dose used (75 mg/kg bw) was chosen to match the high dose from the NTP carcinogenicity bioassay (NTP, 1990). Absorption of glycidol after gavage was rapid with T_{max} observed at 0.25 h in rats and 0.83 h in monkeys. Absolute (systemic) bioavailability of glycidol was

69% in rats and 34% in monkeys, based on the ratio of glycidol AUC for oral relative to the AUC for i.v. Elimination was rapid with i.v. half-times reported to be 0.37 and 0.41 h for rats and monkeys, respectively, and oral half-times of 1.3 and 1.5 h, respectively. Some pharmacokinetic data (C_{max} and AUC) were also reported for oral dosing at 1.64 and 4.92 mg/kg bw in rats and for monkeys at 4.92 mg/kg bw.

Wakabayashi et al. (2012) also evaluated the plasma toxicokinetics for a single dose of glycidyl di-linoleate (341 mg/kg bw, which was equimolar to the dose of glycidol, using gavage administration) to Sprague-Dawley rats and cynomolgus monkeys. While the parent ester was observed in plasma from dosed rats, it was undetectable in monkeys; however, the pharmacokinetics of the glycidol derived from ester hydrolysis was measured in plasma in both species. The time to maximal plasma glycidol concentration was slower after glycidyl di-linoleate gavage than seen for glycidol (0.5 vs 0.25 h in rats and 1.8 vs 0.83 h in monkeys) and achieved lower glycidol concentrations (C_{max} values of 26 vs 34 μg/mL in rats and 1.5 vs 8.6 μg/mL in monkeys). However, while the glycidol AUC achieved after glycidyl di-linoleate gavage to rats exceeded that from glycidol itself by 28% (41.6 vs 32.4 μg/mL × hour), the glycidol AUC was 45% lower in monkeys given glycidyl linoleate (9.1 vs 16.4 μg/mL × hour). These observations were interpreted as representing important species differences in ester hydrolysis and bioavailability based on physiology (e.g. lingual vs gastric lipases, stomach pH effects) or epoxide metabolism (epoxide hydrolase or glutathione *S*-transferase), although no specific mechanism was proposed.

Appel et al. (2013) reported the relative bioavailability of glycidol after administration of equimolar doses of glycidol (50 mg/kg bw) or glycidyl di-palmitate (209 mg/kg bw) to male Wistar rats by gavage. In one experiment, the relative amounts of dihydroxypropyl-valine adducts derived from the reaction of glycidol with the N-terminal residue of haemoglobin were measured using GC-MS. Similar maximal levels of dihydroxypropyl-valine adducts were observed in blood collected 24–48 h after dosing, although the maximal adduct level achieved after glycidol dosing was achieved earlier than for the ester (approximately 4 vs 24 h). The excretion of the glycidol metabolite derived from initial glutathione conjugation, dihydroxypropyl-mercapturate (DHPMA), was also measured in these rats using LC/MS/MS. Similar total excretion of the mercapturate was observed over a 48-h period for the two groups of dosed rats (14% of the administered glycidol dose vs 13.7% of the glycidyl di-palmitate dose). These similar apparent blood AUC values for glycidol, derived from either formation of a haemoglobin adduct or the similar urinary excretion for an important glycidol metabolite, after ingestion of either glycidol or its di-palmitate ester were interpreted as resulting from quantitative ester hydrolysis during transit through the GI tract in rats. In a second experiment, the disposition of [^{14}C]-glycidyl-[^{3}H] di-palmitate was evaluated over a 7-day period. Excretion of the ^{14}C label occurred primarily through the urine (41%) whereas excretion of the ^{3}H label occurred primarily through the faeces (51%). It was also reported that 9% of the ^{14}C label and 23% of the ^{3}H label were associated with the tissues, although it was not determined whether this was due to covalent binding or metabolic incorporation.

Gavage administration of either glycidol or two of its fatty acid esters produced concentrations of 3-MCPD in serum of male F344 rats that exceeded those of glycidol at 30 min after dosing (Onami et al., 2015). Formation of 3-MCPD after gavage of two glycidyl mono-esters was observed in duodenal and caecal contents of male F344 rats, but at levels near the LOQ and inconsistently among the replicate animals.

In conclusion, glycidol and its fatty acid esters are efficiently absorbed following ingestion. Substantial presystemic hydrolysis of GE occurs, although the de-esterification process appears to be more extensive in rats than in monkeys based on different measures of glycidol internal exposure; however, the physiological basis for this observation is uncertain. Metabolism of the glycidol moiety proceeds by several enzymatic pathways, including glutathione conjugation and mercapturate formation. The glycidol moiety is predominantly excreted in urine as poorly described metabolites, with smaller amounts exhaled through the breath as CO_2, and smaller amounts excreted through the faeces. In addition, the glycidol moiety can bind covalently to cellular macromolecules (e.g. DNA and haemoglobin) by virtue of the electrophilic nature of the epoxide ring. Binding of the glycidol moiety to DNA may result in genotoxicity and mutations (see Section 3.3.5.; Mode of Action Carcinogenicity, Section 3.3.2. Genotoxicity). Alternatively, the carbon atoms in the glycidol moiety can also become incorporated into cellular macromolecules via normal intermediary metabolism after conversion to precursor molecules, including glycerol and CO_2. Extensive conversion of glycidol and its monoesters into 3-MCPD has been observed *in vivo*.

3.3.2. Metabolism

3-MCPD

The metabolic pathways of 3-MCPD (as well as of glycidol) are depicted in Figure 7. Two biotransformation routes have been postulated for 3-MCPD metabolism. One leads to conjugation with glutathione and the formation of mercapturic acids (DHPMA), and the other terminates in oxalic acid (Figure 7).

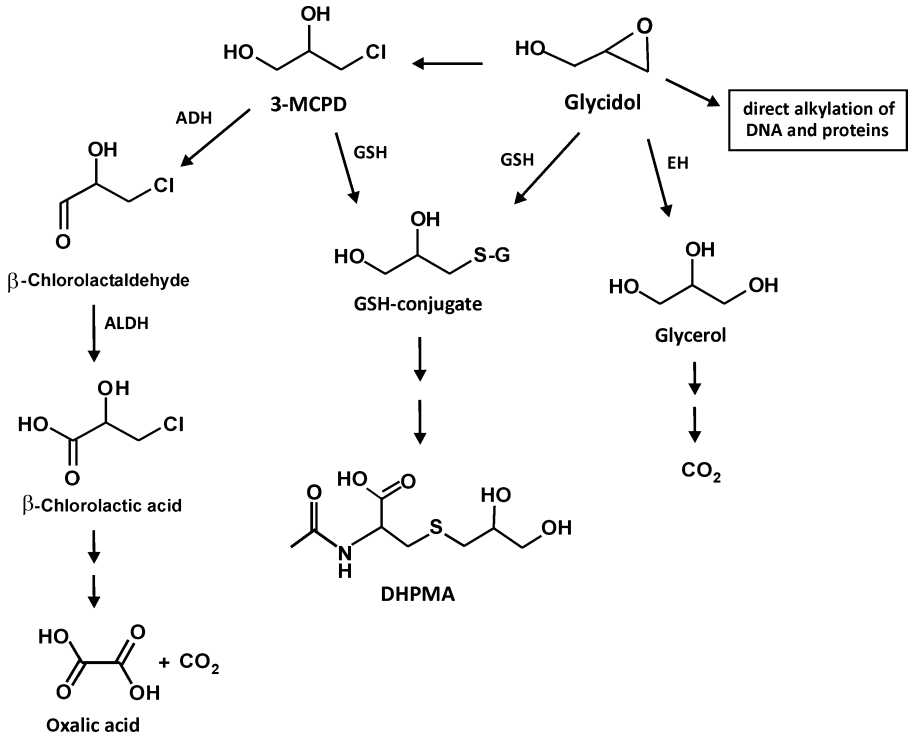


Figure 7: Overview of the metabolic pathways of 3-monochloropropane-1,2-diol (3-MCPD) and glycidol. EH: epoxide hydrolase; ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; DHPMA: N-acetyl-S-(2,3-dihydroxypropyl)cysteine or 2,3-dihydroxypropyl mercapturic acid. (adapted from Appel et al., 2013)

The ability of the microbial enzyme halohydrin dehalogenase to dehalogenate haloalcohols to produce glycidol has been demonstrated by Van den Wijngaard et al. (1989) using microbial strains isolated from freshwater sediments. The authors supposed that microbes containing enzymes capable of catalysing the dehalogenation reactions can utilise 3-MCPD as an exclusive carbon source (microbial cultures that grow on halogenated organic compounds). Whether other bacterial strains, including those present in the human gut microbiome, are able to convert 3-MCPD into glycidol is unknown; however, conversion of orally administered 3-MCPD or it esters into glycidol was not observed in rats (Onami et al., 2015).

Based on data from *in vivo* studies in rats with radiolabelled 3-MCPD (Jones et al., 1978), the oxidation to β-chlorolactaldehyde with further conversion to β-chlorolactic acid and oxalic acid was considered to be the main metabolic route of 3-MCPD in mammals (Lynch et al., 1998).

About 30% of a single dose of [^{14}C]-labelled 3-MCPD (100 mg/kg bw) administered intraperitoneally was exhaled as CO_2 and 8.5% was eliminated as unchanged 3-MCPD in the urine within 24 h post-dosing of male rats (Jones, 1975). After a single intraperitoneal (i.p.) injection of [^{36}Cl]-labelled 3-MCPD to rats (100 mg/kg bw), 23% of the total urinary radioactivity recovered was β-chlorolactate (Jones et al., 1978). Tissue distribution studies revealed no tissue-specific retention of radioactivity in rats treated with [^{36}Cl]-3-MCPD.

3-MCPD can undergo conjugation with glutathione, yielding S-(2,3-dihydroxypropyl)cysteine and the corresponding mercapturic acid (DHPMA) (Jones, 1975). Data from a recent biomonitoring study in rats (Barocelli et al., 2011) indicate that the conjugation with glutathione may play a more important role than was considered before. After a single oral application of 1.8 mg/kg bw 3-MCPD (the lowest

dose in this study), about 10.5% and 27.5% of the dose was found as mercapturic acid in urine of female and male rats, respectively. At the middle (7.37 mg/kg bw) and high (29.5 mg/kg bw) 3-MCPD doses, urinary mercapturic acid excretion amounted to 5.4% and 7.3% of the dose in female rats, and 11.5% and 12.5% of the dose in male rats, respectively. Thus, at least in rats, the urinary excretion of 3-MCPD mercapturate shows dose-dependence (higher rates at lower doses) as well as gender differences (higher proportion of the dose found in urine from male rats). Furthermore, only trace amounts of β-chlorolactate (less than 1% of the administrated dose) were recovered in urine of 3-MCPD-treated animals in a study of Barocelli et al. (2011), which contradicts earlier findings of Jones et al. (1978).

3-MCPD fatty acid esters

Based on studies described above, it can be assumed that 3-MCPD-esters are effectively hydrolysed in the gut after ingestion, liberating 3-MCPD. It has been shown *in vitro* that pancreatic lipase accepts 3-MCPD fatty acid esters as substrates and that ester hydrolysis takes place within minutes (Seefelder et al., 2008). In two *in vivo* studies, it was shown that after oral administration of 3-MCPD-1, 2-dipalmitate to rats, the bioavailability of 3-MCPD originating from the diester was 70% based on the measurement of 3-MCPD metabolites in urine (Barocelli et al., 2011) and 86% based on the determination of 3-MCPD in blood (Abraham et al., 2013), respectively.

In vitro experiments have shown that fatty acid esters of 3-MCPD and of 2-MCPD are hydrolysed by a model of small intestinal juice containing pancreatic lipase. In these experiments, 2-MCPD fatty acid esters were more efficiently cleaved than their 3-MCPD counterparts (Schilter et al., 2011). In studies with the Caco-2 cell line as a model for the human intestinal cell wall, fatty acid esters of 3-MCPD as well as 2-MCPD were efficiently hydrolysed by Caco-2 cells within hours, although there was no pancreatic lipase present in the experimental set-up (Burke et al., 2011, 2015). The authors suggested that the membrane-bound lipolytic activity of the Caco-2 cells could be responsible for the observed de-esterification of MCPD-esters. It was concluded that under *in vivo* conditions the activity of pancreatic lipase in the gut lumen as well as lipolytic activities in the membranes of intestinal enterocytes will contribute to the hydrolysis of 2-MCPD and 3-MCPD fatty acid esters in the gut (Burke et al., 2015).

2-MCPD and 2-MCPD fatty acid esters

No data were identified; however, the difference in the structural localisation of the chlorine within the molecule makes it unlikely that the metabolic patterns of 2- and 3-MCPD overlap.

Glycidol

Toxicokinetics of glycidol was studied in male Fischer 344 rats following oral or i.v. administration of [^{14}C]-labelled compound at single doses of 37.5 and 75 mg/kg bw (Nomeir et al., 1995). Approximately 87–92% of orally administered glycidol was absorbed from the GI tract. About 40–48% of radioactivity was excreted in the urine, 5–12% in faeces and 26–32% was exhaled as CO_2. At both doses, 7–8% of the dose remained in tissues 72 h following administration. The highest concentrations of radioactivity were observed in blood cells, thyroid, liver, kidney and spleen (Nomeir et al., 1995). The extensive distribution and persistence of glycidol in the tissues was attributive to the property of the epoxide group of glycidol to react with cellular nucleophiles. High performance liquid chromatography (HPLC) analysis of the urine revealed extensive metabolism: 15 different metabolites were detected. There was one major (14–21% of the dose) and four lesser metabolites (each representing 2–8%); the others were minor, each representing 1% or less of the dose. The structure of these metabolites was not clarified (Nomeir et al., 1995).

Glycidol can be conjugated with glutathione or hydrolysed to form glycerol (Figure 4). The formation of glycerol from glycidol, catalysed by epoxide hydrolases, was confirmed *in vitro* by incubation with rat liver and pulmonary microsomes (Jones and O'Brien, 1980; Patel et al., 1980). Urinary excretion of S-(2,3-dihydroxypropyl)cysteine and *N*-acetyl-S-(2,3-dihydroxypropyl)cysteine (2,3-dihydroxypropyl mercapturic acid, DHPMA) in rats and mice after i.p. application of glycidol was first demonstrated by Jones (1975). In the recent study of Appel et al. (2013), about 14% of orally administrated glycidol dose (50 mg/kg bw) was recovered as DHPMA in the urine of rats within 48 h after dosing. A single dose by gavage of 500 μL/kg (560 mg/kg) bw glycidol to male rats led to significant decreases in hepatic glutathione content between 30 min and 12 h after treatment (Montaldo et al., 1984).

S-(2,3-Dihydroxypropyl)glutathione, DHPMA, as well as β-chlorolactic acid were reported as major metabolites isolated from rat urine after i.p. administration of glycidol (NTP, 1990). Oral glycidol doses of 100 mg/kg bw were given to male rats 48, 54 and 72 h after i.p. injection of [^{36}Cl]saline (Jones and O'Brien, 1980). The only radioactively labelled metabolite found in the urine 80 h after glycidol administration was β-chlorolactic acid. It was suggested that in the presence of acid in the lumen of the stomach, glycidol could be partially converted to 3-MCPD, which is then further metabolised and excreted in urine as β-chlorolactic acid (Jones and O'Brien, 1980). However, Nomeir et al. (1995) found that only a small amount of urinary radioactivity was co-eluted with authentic β-chlorolactic acid following oral or i.v. glycidol administration (37.5 or 75 mg/kg bw), suggesting that the conversion of glycidol to 3-MCPD is quantitatively insignificant at the doses studied (0.02% of pooled radioactivity). However, Onami et al. (2015) showed that significant amounts of glycidol were converted into 3-MCPD in serum from male F344 rats dosed orally at a dose of 510 μmol/kg bw (37.7 mg/kg bw) using a GC-MS-based method with potentially better sensitivity.

Glycidyl fatty acid esters

In the study on relative bioavailability of glycidol from glycidyl esters (Appel et al., 2013), similar amounts, about 14% of the dose was recovered as DHPMA the urine of rats 48 h after oral administration of 50 mg/kg bw glycidol or equimolar amount of glycidyl palmitate.

3.3.3. Toxicity in experimental animals

3.3.3.1. 3-MCPD

The acute toxic effects of 3-MCPD treatment in the 24 h following dosing has not been extensively studied possibly because the systemic effects such as death, renal toxicity and male infertility develop primarily in the week following the initiation of treatment. The toxicokinetics of 3-MCPD, described elsewhere, show that 3-MCPD is efficiently absorbed, metabolised and these substances are excreted within a few hours (Section 3.3.1). Therefore, it is likely that cellular toxicity is produced within the first few hours after administration but requires several days to be expressed pathophysiologically.

Single dose

The acute median lethal dose (LD_{50}) of a single dose of 3-MCPD administered by gavage to adult male rats was reported as 150 mg/kg bw (cited in Ericsson and Baker 1970). No change in general health of adult male rats was noted after 7 weeks treatment with 5.0 mg per day, approximately 14 mg/kg bw per day or 5.0 mg per rat per day (Ericsson and Baker, 1970). The LD_{50} for 3-MCPD in ICR mice has been reported to be 190.7 mg/kg (Qian et al., 2007).

Repeated dose

In a 90-day study, 3-MCPD dissolved in corn oil was administered by daily gavage to groups (n = 10) of adult male and female Wistar rats at doses of 1.8, 7.37 or 429.5 mg/kg bw per day (Barocelli et al., 2011). Substantial mortality occurred as early as 8 days treatment with the highest dose, 429.5 mg/kg, of 3-MCPD. General morbidity was noted in the animals receiving the lower doses of 3-MCPD especially in the first 10 days of treatment. BMD analysis by the authors of female rat mortality after 3-MCPD gave a BMD_{10} and $BMDL_{10}$ of 7.4 and 2.3 mg/kg per day, respectively. In F344 rats MCPD was lethal (40 mg/kg approx. LD_{50}) when administered for 5 days a week to female but not male rats (Onami et al., 2014a,b).

No clinical symptoms were observed short term or later in a 104-week study in which Fischer 344 rats were administered 3-MCPD in drinking water at concentrations of 20, 100 or 500 mg/L (Sunahara et al., 1993; described in long-term toxicity below). This was estimated to produce mean daily intakes of 1.1, 5.2 and 28.3 mg/kg bw per day in males and 1.4, 7.0 and 35.3 mg/kg bw per day in females. However, pathological examination revealed systemic toxicity resulting from this sustained treatment.

Renal and hepatic toxicity

Toxicity has been reported only to be observed with the R isomer of 3-MCPD (Porter and Jones, 1982; Morris and Williams, 1980; Jones and Cooper, 1999; Barocelli et al., 2011). 3-MCPD at a single i.p. dose of 120 mg/kg administered to male rats causes kidney failure and death usually in the week

following administration. A lower i.p. dose (100 mg/kg bw) produces diuresis accompanied by high urinary protein and glucose; these effects persist for about 7–15 days. No effect was seen with a single dose of 80 mg/kg bw, or multiple doses of 10 mg/kg bw for 5 days. Renal injury, tubule focal necrosis, regeneration and dilatation were documented 75 days after treatment of male rats with a single sub cutaneous dose of 75 mg/kg bw 3-MCPD (Kluwe et al., 1983).

Renal and hepatic effects have been documented in Sprague-Dawley rats given 3-MCPD at doses of 30 or 60 mg/kg bw per day by gavage for 5 days a week for 4 weeks (Marchesini and Stadler, 1983). On the second day of treatment with 60 mg/kg bw per day the serum concentrations of alanine aminotransferase, creatinine, urea, and glucose were increased and these remained elevated until the end of the study and increased levels were often seen in the 30 mg/kg bw per day-treated rats. Kidney and liver weight were increased and the renal histopathology showed progressive nephropathy and renal tubule dilatation. Similar changes were seen when 3-MCPD was administered via the drinking water for 90 days at estimated daily intakes of between 9 and 46 mg/kg bw per day (Marchesini et al., 1989). It is notable that renal toxicity is a common observation in the chronic carcinogenicity studies described here below.

In the carcinogenicity study reported by Sunahara et al. (1993) in which Fischer 344 rats were exposed to 3-MCPD in drinking water for 104 weeks, chronic progressive nephropathy accompanied by hypertrophy of the kidney was reported at terminal necropsy in the dose groups receiving 100 or 500 mg/kg which was more pronounced in male rats (dose range 5.2–35.3 mg/kg bw per day).

At the end of a 90-day repeated dose study of 3-MCPD in Wistar rats (1.84, 7.37, or 29.5 mg/kg bw per day), the combined weights of the kidney and adrenal gland were increased in both sexes. (Barocelli et al., 2011). The weight increases were dose dependent such that they were about 70% higher after the highest daily dose of 3-MCPD (29.5 mg/kg bw per day). As the adrenal gland is relatively small, it can be assumed that this change in combined weights was primarily due to renal changes. 3-MCPD also increased water consumption in male and female rats but only towards the end of the 90-day study. In spite of no significant changes being observed in the volume of urine output, after 3-MCPD (29.5 mg/kg bw per day) administration, the urine was acidified and the concentrations of several substances were changed, notably the proteins Nag, B2M, albumin and α-GST. The large variation in the clinical biochemistry measurements did not allow rigorous statistical analysis of these results. Serum urea was not changed but serum creatinine concentrations were reduced.

The study reported an extensive array of degenerative changes in the renal tubules after 90 days treatment across the range of doses for 3-MCPD and in both sexes (Barocelli et al., 2011). When examined quantitatively these pathological effects were restricted to the highest dose of 3-MCPD (29.5 mg/kg bw per day). Histopathology scores were slightly lower in the female compared to male rats.

At the end of a 91-day study in F344 rats, kidney weight was increased by 3-MCPD (Onami et al., 2014b). These weight changes reflect a similar but not such a definitive pattern as seen for the increases in liver weight. A fall in serum creatinine concentration was observed. Severe tubular necrosis was confirmed in the rats which died after 3-MCPD treatment. In rats surviving 91 days of 3-MCPD treatment, the only histopathological changes seen were in the epididymis.

Haematology

Decreased levels of haemoglobin and erythrocyte volume fraction have been reported after treatment of rats with 3-MCPD at 30 mg/kg per day for 5 days per week for 4 weeks (Marchesini and Stadler, 1983). In female rats, the erythrocyte count was also reduced by the highest dose (60 mg/kg per day). In another study, anaemia was reported at 30 and 90 days treatment of female rats with 3-MCPD administered in drinking water (Marchesini et al., 1986). The estimated intake was 31 or 46 mg/kg per day. There was no histopathological evidence of impaired haemopoiesis.

Severe haematological toxicity was seen in 50% of a group of rhesus monkeys (n = 6) given an oral dose of 3-MCPD for 6 weeks. The dose, 30 mg/kg per day, was administered primarily in drinking water (as 3-MCPD absorbed in a sugar cube was unpalatable). Two of the affected group died towards the end of treatment displaying symptoms of severe bone marrow depression. The lack of toxicity in the three with apparently normal haematological parameters was unexplained (Kirton et al., 1970). No toxicity was noted in an unpublished study (Kirton; cited in Kirton et al., 1970) in which rhesus monkeys were given oral doses of 25 mg/kg bw per day 3-MCPD for 8 weeks.

Neurotoxicity

Exposure of CD-1 mice to 3-MCPD by daily gavage at doses of 50 or 100 mg/kg resulted in hind-limb paralysis within a few days (50 mg/kg bw per day: 4.4 ± 0.2 days; 100 mg/kg per day: 2.6 ± 0.2 days, mean ± SEM, n = 8; Ford and Waites, 1982). In a study by Kim et al. (2004) there was no observable neurobehavioural toxicity in male and female Sprague-Dawley rats after administration of 3-MCPD (each group n = 10; 10, 20 or 30 mg/kg bw, control groups received either saline or acrylamide).

Long-term toxicity and carcinogenicity

In a long-term toxicity study Jeong et al. (2010) investigated the carcinogenicity of 3-MCPD in B6C3F$_1$ mice by administration in drinking water over a period of 104 weeks. Three groups, each comprising 50 male and 50 female mice received 3-MCPD at levels of 30, 100 or 300 mg/L up to day 100, and 200 mg/L onwards (4.2, 14.3 and 33.0 mg/kg bw per day for males; 3.7, 12.2, and 31.0 mg/kg bw per day for females). At least 80% of males and 72% of females in each group survived over 104 weeks. Body weights and body weight gain were decreased in males and females receiving drinking water with 300/200 mg/L. Water and food consumptions of both sexes at 300/200 mg/L were lowered. An emaciated appearance or adoption of a crouching position was observed in animals of both sexes exposed to 300/200 mg/L per day. There were no consistent, dose-dependent differences in haematology and serum biochemistry. Histopathological examination did not reveal any neoplastic or non-neoplastic findings attributable to treatment with 3-MCPD.

Sunahara et al. (1993) treated four groups of 50 male and 50 female F344 rats (between 5 and 6 weeks old) over a period of 104 weeks with 0, 20, 100 or 500 mg/L 3-MCPD added to the drinking water (Table 30). The calculated mean daily intakes are given in the report as 0, 1.1, 5.2 or 28.3 mg/kg bw for males and 0, 1.4, 7.0 or 35.3 mg/kg bw for females. The water which the control animals received was found not to be 'free' of 3-MCPD but to contain 2.7 mg/L. The analysed mean values for the other groups were 26.5, 105.9 and 502.8 mg/L. Although the daily dose of 3-MCPD in the 'controls' was not determined, on the basis of the average daily volume consumed in the 20 mg/L group (41.5 mL/kg bw in males, 52.8 mL/kg bw in females) the CONTAM Panel calculated the intake in controls as 0.11 mg/kg bw per day in males and 0.14 mg/kg bw per day in females.

At the highest dose, body weight gain was reduced to below 70% of the control, while no increase in mortality was observed, i.e. none of the tumour incidences was associated with early death. At necropsy, the incidences of tubular adenoma were increased at the two highest dose levels in both sexes (Table 31). These were reported to be benign tumours, while no malignant kidney tumours were observed. All adenomas were microscopic and most were classified as early adenoma, i.e. being borderline between hyperplasia and adenoma.

Furthermore, significant increases in the incidences of hyperplasia and adenoma of the Leydig cells were found.

In males, mammary gland hyperplasia and fibroadenoma were more abundant at the two highest dose levels. No significant increases in malignant mammary tumours were found.

Cho et al. (2008a) treated groups of 50 male and 50 female Sprague-Dawley rats over 2 years with 3-MCPD in drinking water containing 0, 25, 100 or 400 mg/L. These resulted in average daily intakes of 0, 2.0, 8.3, or 29.5 mg/kg bw for males and 0, 2.7, 10.3 or 37.0 mg/kg bw for females. The body weights and water consumptions of the male and female rats given water with 400 mg/L 3-MCPD were significantly lower than those of the controls. The incidences of renal tubule adenomas or carcinomas and Leydig cell tumours showed dose-related positive trends in male rats and were significantly increased in male rats having received drinking water containing 400 mg/L 3-MCPD. The incidence of renal tubule adenomas showed a positive trend in female rats, which was significant in the highest dose group.

Non-neoplastic changes such as seminiferous tubular atrophy and arteritis or periarteritis were also found in all other dose groups. The incidence of renal tubule adenomas showed a positive trend in female rats, which was significant in the highest dose group. Renal tubule neoplasms were accompanied by significant increases in the incidences of renal tubule hyperplasia and chronic progressive nephropathy. In males, this effect was seen in all dose groups, while females appeared to be less sensitive, i.e. no significant nephropathy was observed in the lowest dose group.

In mice treated with a similar range of doses, no non-neoplastic or neoplastic histopathological changes were observed (Cho et al., 2008b).

Table 30: Chronic non-neoplastic, preneoplastic and neoplastic lesions in a 2-year carcinogenicity with 3-MCPD in Fischer 344 rats (Sunahara et al., 1993, modified)

Organ/lesion	Dosage (mg/L drinking water)			
	'0'[(a)]	25	100	400
Males				
Testes				
Number examined	50	50	50	50
Leydig cell hyperplasia	39	27[(b)]	4[(d)]	0[(d)]
Leydig cell adenoma	38	43	50	47[(b)]
Leydig cell carcinoma	0	0	0	3
Kidney				
Number examined	50	50	50	50
Chronic nephropathy	36	40	45[(b)]	49[(d)]
Tubular hyperplasia	3	6	15[(c)]	34[(c)]
Tubular adenoma	0	0	1	5
Preputial glands[(d)]	5	13	16	11
	1	2	6	5
	0	0	1	2
Mammary gland				
Number examined	45	48	47	49
Glandular hyperplasia	2	6	24[(d)]	42[(d)]
Fibroadenoma	0	0	2	10[(c)]
Adenoma	0	0	1	1
Adenocarcinoma	0	0	1	1
Females				
Kidney				
Number examined	50	50	50	50
Nephropathy	24	23	42[(d)]	48
Tubular hyperplasia	2	4	20[(d)]	31[(d)]
Tubular adenoma	0	1	0	9[(c)]

3-MCPD: 3-monochloropropane-1,2-diol.
(a): The untreated animals, in the '0' dose group were estimated to receive 2.7 mg/L in drinking water.
(b,c,d): Statistically significantly different from controls at $^{b}p < 0.05$, $^{c}p < 0.01$, $^{d}p < 0.001$.
(e): Preputial glands were not examined in all animals; no statistical analysis was carried out.

Table 31: Chronic non-neoplastic, preneoplastic and neoplastic lesions in a 2-year carcinogenicity with 3-MCPD in Sprague-Dawley rats (Cho et al., 2008a, modified)

Organ/lesion	Dosage (mg/L drinking water)			
	0	25	100	400
Males				
Testes				
Number examined	50	50	50	50
Atrophy	6	16	13[(a)]	34[(a)]
Arteritis/periarteritis	3	15[(a)]	9[(a)]	11[(a)]
Leydig cell tumours	1	1	4	14[(a)]
Pituitary gland, pars distalis				
Number examined	50	50	50	50
Adenoma	25	26	24	13[(a)]

Organ/lesion	Dosage (mg/L drinking water)			
	0	25	100	400
Kidney				
Number examined	50	50	50	50
Nephropathy	15	27(a)	39(a)	41(a)
Tubular hyperplasia	1	11(a)	21(a)	36(a)
Tubular adenoma	0	0	1	4
Tubular carcinoma	0	0	0	5(a)
Tubular adenoma or carcinoma	0	0	1	7(a)
Females				
Kidney				
Number examined	50	50	50	50
Nephropathy	6	8	23(a)	42(a)
Tubular hyperplasia	1	0	1	10(a)
Tubular adenoma	0	0	1	6(a)
Tubular carcinoma	1	0	1	3
Tubular adenoma or carcinoma	1	0	2	9(a)

3-MCPD: 3-monochloropropane-1,2-diol.
(a): Statistically significantly different from controls at $p \leq 0.05$.

Reproductive toxicity

The antifertility effects of 3-MCPD in male animals are well established, initially having been considered as a non-hormonal human male contraceptive (Jones, 1983). Development was curtailed on appearance of adverse haematology in monkeys (Kirton et al., 1970). However, 3-MCPD was subsequently marketed as an indirectly acting rodenticide, Epibloc, in the USA (Ericsson, 1982). The mechanism of action of the male antifertility effects are twofold, and should not be confused when considering the testicular actions of 3-MCPD and its potential hazard. They can be considered as a low dose and high dose effects (Jones, 1983). Doses which have been reported to not affect the fertility of the male rat range from 0.5 mg/kg bw per day (Ericsson and Baker, 1970) to 5 mg/kg bw per day (Jones and Jackson, 1976).

Low-dose antifertility

After 2 or 3 days of dosing daily by oral gavage with 5–10 mg/kg bw, male rats were not able to impregnate untreated female rats in spite of normal mating, normal spermatogenesis and normal ejaculation. Infertility is reversible (Jones, 1983). Although the timing indicated an effect on the epididymis, evidence accumulated to show inhibition of sperm glyceraldehyde-3-phosphate dehydrogenase reducing the availability of ATP which completely inhibits motility and the ability to fertilise the ovum. This action occurs exclusively in the caput epididymis which also exhibits impaired sodium and water reabsorption into the systemic circulation from the intraluminal fluid containing the sperm.

High-dose antifertility

These effects develop 7–14 days after the administration of single or multiple does of 3-MCPD above 90 mg/kg (Jones, 1983) and have been likened to the ligation of the ductulii efferentes which cause accumulation of fluid in the epididymis and subsequently the testis resulting in back pressure necrosis and ablation, the spermatogenic epithelium. This effect in itself will result in infertility but in contrast to the reversibility of the low dose it is permanent.

The male antifertility effects and mechanisms of 3-MCPD have been studied in hamsters, gerbils, dogs, sheep, pigs and monkeys but was not observed the mouse or quail (cited in Jones, 1983). The common embryological origin of the kidney and epididymis could suggest a specific impairment of a mechanism involved in fluid transport shared by both tissues.

The male antifertility activity of 3-MCPD has been demonstrated in rhesus monkeys receiving 30 mg/kg per day orally for 6 weeks. During treatment pregnancies were reduced from 50% to 4% of matings. Sperm motility, morphology and production were apparently normal in the majority of monkeys.

However, the study was compromised due to severe hematologic abnormalities in 50% of the monkeys resulting in some deaths (Kirton et al., 1970).

Testis histopathology is frequent in rats treated with 3-MCPD. For instance, relatively high daily doses of 3-MCPD (29.5 mg/kg) for 90 days in rats produced complete ablation of the spermatogenic epithelium in 90% of the animals accompanied by inflammatory changes in the head of the epididymis. A 3-MCPD dose–response analysis of the testicular histopathology gave a BMD_{10} of 8.4 mg/kg bw per day and a $BMDL_{10}$ of 6.0 mg/kg bw per day (Barocelli et al., 2011).

3-MCPD inhibited the oestrous cycle of female rats when administered by subcutaneous injection at 3-MCPD (10 mg) every alternate day for 30 days (dose of approximately 60 mg/kg bw) (Lohika and Arya, 1979). The weights of the uterus and ovary were reduced and histology suggested ovulation was absent.

Developmental toxicity

3-MCPD has been administered by gavage as single doses (62.5, 75 or 90 mg/kg bw to female Wistar rats on days 1 or 6 or 14 days of pregnancy (Rahmaniah, 1999). The two highest doses (75 and 90 mg/kg) decreased implantation and increased fetal loss at all three dosing time points in pregnancy. A similar study in Sprague-Dawley rats used doses of 10, 30 and 90 mg/kg per day. The pregnant rats received daily treatment with 3-MCPD from gestational days 6 to 19. Developmental toxicity was only observed in the presence of maternal toxicity and a NOAEL was suggested to be 10 mg/kg per day for the pregnant rats and 30 mg/kg per day for embryo-fetal development. Teratogenic effects were not observed (Lee et al., 2009). In a study aimed at investigating whether testicular organogenesis was affected by 3-MCPD, pregnant Sprague-Dawley rats were given daily oral doses of either 5, 10 or 25 mg/kg on days 10.5, 15 and 17.5 of gestation and the pups autopsied on day 18. Interestingly, although the authors demonstrated that 3-MCPD and its metabolite were present in the foetus at levels similar to those observed in the mother, no testicular toxicity was seen. Maternal toxicity was recorded at 10 and 25 mg/kg bw per day; however, fetal weight, deaths and resorptions were similar to control values. (El Ramy et al., 2006).

Jones and Jackson (1976) studied the effects of 3-MCPD treatment of male rats upon their subsequent offspring after mating treated males with untreated females. Male Wistar rats were administered oral doses at 5, 10 or 20 mg/kg per day for 5 days and then mated with untreated females 3, 10, 17 or 21 days after the first treatment. In rats in which fertility was maintained there was no effect on the incidence of non-viable fetal implantations confirming a previous observation in the mouse (Epstein et al., 1972).

Genotoxicity

3-MCPD

In vitro

Bacteria

3-MCPD was positive in reverse mutations assays in S. typhimurium TA100 and TA1535 (base pair substitution strains) in the absence of an exogenous metabolic activation system. Both positive and negative results have been obtained in TA98 and negative results have been observed in TA1537 and TA1538 as well as in *Escherichia coli* WP2, TM930, TM1080. In the presence of an exogenous metabolic system, it was positive in TA100 and TA1535, but was negative in TA97, TA98, TA1537 and TA1538 as well as in E. coli strains (Stolzenberg and Hine, 1979, Stolzenberg and Hine, 1980; Silhankova et al., 1982; Zeiger et al., 1988, Ohkubo et al., 1995; All cited in FAO/WHO, 2002 and IARC, 2012).

Yeast

In a forward mutation assay in *Schizosaccharomyces pombe* 3-MCPD was positive in the absence of metabolic activation and negative in the presence of metabolic activation (Rossi et al., 1983).

Mammalian cells

The results of *in vitro* genotoxicity assays in mammalian cells, including a gene mutation test in mouse lymphoma cell tk locus (2–9 mg/mL, positive in the presence of metabolic activation, negative without metabolic activation), a gene mutation test in V79 cell hprt locus (0.033–7.7 mg/mL)

and a sister chromatid exchange test in V79 cells (0.7–2.8 mg/mL with and without metabolic activation) were reported to be generally positive. However, in V79 cells a weak mutagenic effect was observed only at cytotoxic concentrations (5.5 mg/mL) (unpublished reports by Henderson et al., 1987; Görlitz, 1991; May, 1991 cited in JECFA WHO Food Additives series 48 and in Bakhiya et al., 2011). 3-MCPD was negative in a DNA synthesis inhibition assay in HeLa cells both with and without metabolic activation (Painter and Howard, 1982). In the absence of metabolic activation, it was positive in a transformation assay in mouse fibroblast M2 clone (Piasecki et al., 1990; cited in FAO/WHO, 2002 and IARC, 2012). In an alkaline Comet assay in Chinese hamster ovary (CHO) cells) exposed for 3 h to 0.5, 1.0, 2.5 and 5.0 mg/mL 3-MCPD, a statistically significant increase in DNA damage was observed with the two highest concentrations, in the presence of minimal toxicity (El Ramy et al., 2007).

In vivo

Micronucleus tests

Robjohns et al. (2003) investigated 3-MCPD for induction of structural and/or numerical chromosomal aberrations in a standard micronucleus (MN) assay in bone marrow erythrocytes. Based on a preliminary dose range finding study, the following doses were administered by gavage: 0, 15, 30 and 60 mg/kg bw. Groups of six outbred Crl:HanWist BR (Glx:BRL) BR rats were dosed once daily for two consecutive days. Bone marrow was sampled 24 h after the last administered dose. Water was used as vehicle and negative control. Administration of 3-MCPD at the highest dose produced signs of toxicity (piloerection). In 3-MCPD-treated animals, there was a dose-related decrease in the polychromatic to normochromatic erythrocyte ratio (PCE/NCE) indicating toxicity to the target cells. There was no statistically significant increase in the group mean frequencies of micronucleated PCE compared to the control group frequencies of micronucleated PCE which fell within the laboratory historical negative control range for all test groups. The positive control, cyclophosphamide, induced a significant increase in micronucleated PCE. Under the test conditions performed, 3-MCPD was negative in this MN test.

In a study by Onami et al. (2014a) the potential of 3-MCPD to induce micronuclei in bone marrow was tested in male F344 *gpt* delta rats carrying a transporter transgene lambda EG10. The rats were dosed by intragastric administration for 4 weeks, five times a week. The rats were randomly allocated to six groups with five animals in the positive control group, six rats in the negative control group (olive oil) and test group. Only one dose was given to the test group: 40 mg/kg bw 3-MCPD. The 40 mg/kg bw dose of 3-MCPD corresponds to 26% of LD_{50}. The dose of 3-MCPD was selected on basis of a previous carcinogenicity study in Sprague-Dawley rats (Cho et al., 2008a). Olive oil was used as vehicle for the test substances. No positive control was used in this experiment. The animals were killed on day 29, 24 h after administration of the last dose and bone marrow tissues were taken for the (MN) assay. No increase in the frequency of micronucleated reticulocytes was observed in the 3-MCPD treated compared with the control group. The percentage of reticulocytes among total erythrocytes (measure of chemical-induced bone marrow toxicity) did not differ between the treated and control groups. However, while levels were not measured in the target organ, the pharmacokinetics (described in Section 3.3.1) suggest that 3-MCPD would reach the bone marrow.

Negative results were also reported in MN tests in mouse (40–120 mg/kg bw) or rat (2 days, 15, 30 or 60 mg/kg bw per day) bone marrow (Jaccaud and Aeschbacher, 1989; Marshall, 2000).

Comet assays

In a study by El Ramy et al. (2007), 3-MCPD was tested for its potential to induce DNA damage in an alkaline single cell gel electrophoresis (Comet) assay in Sprague-Dawley and Fischer 344 rats. In one study 3-MCPD was administered by gavage once daily to male Sprague-Dawley rats for 2 days at doses of 25 or 60 mg/kg bw. DNA damage was assessed in blood leucocytes, bone marrow, liver, kidney and testis 3 h after the second administration. Water (solvent) was used as negative control and methyl methanesulfonate (MMS) was used as positive control. Five rats were dosed per group. At high dose, the body weight gain of rats was slightly impaired. No microscopic abnormalities have been observed in testis and kidneys of treated rats. Dose-related increases in the incidence of hepatocellular mitotic figures were observed in the livers of treated rats. 3-MCPD did not induce DNA lesions in the various organs at any tested dose. The validity of the test was demonstrated by the statistically

significant increases in both olive tail moment (OTM) and percentage of DNA in the tail in cells isolated from organs and tissues (except the bone marrow) of rats treated with MMS.

In the second study, 3-MCPD was administered by gavage once daily to male F344 rats for 2 days at a dose of 60 mg/kg bw. DNA damage was assessed in leucocytes and testis 3 h after the second administration. Four rats were dosed per group. The same positive and negative controls were used as in the first study. The body weight gain of treated rats was slightly impaired. 3-MCPD did not induce DNA lesions neither in leucocytes, nor in testis. Statistically significant increases in DNA damage were observed after MMS treatment.

Gene mutation assays

Onami et al. (2014a) tested also the potential of 3-MCPD to induce Pig-a mutations in male F344 *gpt* delta rats carrying a transporter transgene lambda EG10. The same treatment protocol as described above for the MN test was applied. Peripheral blood samples from the tail vein were collected from each animal at day 0 and 15. At day 29, within 24 h after final administration, blood samples were collected from the abdominal aorta for Pig-a mutation assays. The frequency of Pig-a mutant red blood cells did not differ among groups at any time point.

This study has some limitations because it is documented that in RBC the mutant phenotype at day 29 is only modest and that additional time is required to reach the maximum value in RBCs (Dertinger et al., 2010). On the contrary, mutation responses occur earlier in reticulocytes (RET). However, there was no tendency of accumulation of mutations in RBC after 29 days.

In the study by Onami et al. (2014a), described above, genomic DNA was extracted from the kidney and testis and investigated for gene mutations in the *gpt* or *Spi* gene. No increase was observed in mutation frequencies compared to control and olive oil treated animals in either gene in the treated group. Mutation frequencies were according to the authors within negative control values described earlier by the authors (Hibi et al., 2011). It was claimed by the authors that for the mutations frequencies of DEN-treated liver performed as positive controls were significantly elevated. Only one dose was used in this study, and not a minimum of three, appropriately-spaced dose levels as recommended in OECD Test Guideline 488. In addition, there was only 1 day between the end of the administration period and the sampling time, compared to 3 days normally used in TGR gene mutation assays. For slowly proliferating tissues, like kidney, a later sampling time following the cessation of administration may be more appropriate.

Unscheduled DNA synthesis assay

Robjohns et al. (2003) investigated the induction of DNA repair in an unscheduled DNA synthesis (UDS) assay in rat liver (Table 32). Rats (Crl:HanWist (Glx:BBL) BR) were given a single administration by oral gavage of 0 (water) 40 or 100 mg 3-MCPD/kg bw. The selected doses were based on a preliminary range finding study in which 100 mg/kg bw was close to the maximum tolerated dosage. Two studies were performed. In the first study, animals (4/group) were sampled 2–4 h after administration using DMN as positive control. In the second study rats were sampled 12–14 h after administration using 2-AAF as positive control. In the 3-MCPD treated groups, the net nuclear grain count was well below zero at all doses and sampling time and no more than 0.3% of the cells were seen in repair at any dose of 3-MCPD. The positive controls were clearly active. Therefore, under the test conditions used, 3-MCPD did not induce DNA damage that is detectable by this test.

A negative result was also reported in an UDS assay in male Han Wistar rats exposed to 40 or 100 mg 3-MCPD/kg bw (Fellows, 2000).

Dominant lethal mutation assays

3-MCPD was also negative in dominant lethal mutations assays in rats exposed by gavage (5 days, 5, 10 or 20 mg/kg bw per day) and mice exposed by i.p. (125 mg/kg bw) or by gavage (5 days, 5, 10 or 20 mg/kg bw per day) to 3-MCPD (Epstein et al., 1972; Jones et al., 1969 and Jones and Jackson, 1976).

Somatic mutation in Drosophila

3-MCPD was negative in a mutation/recombination assay wing spot test in *D. melanogaster* (Frei and Würgler, 1997).

Table 32: *In vivo* genotoxicity data on 3-MCPD

Type of test	Experimental test system	End point	Substance tested	Experimental conditions	Result	Reference	Comments
Micronucleus test	Crl: Han Wist BR rats Bone marrow	Chromosomal aberrations	3-MCPD	2 days oral gavage 0 (water), 15, 30 and 60 mg/kg bw per day Sampling: 24 h after last administration	Negative	Robjohns et al. (2003)	
	Male F344 *gpt* delta rats		3-MCPD	4 weeks, 5 times a week, gavage Neg. control: olive oil 40 mg/kg bw per day Sampling: 24 h after last administration	Negative	Onami et al. (2014a)	
	Mouse		3-MCPD	40–120 mg/kg bw	Negative	Jaccaud and Aeschbacher (1989)	
	Rat		3-MCPD	2 days, gavage 0, 15, 30 or 60 mg/kg bw per day	Negative	Marshall, (2000)	
Comet assay (alkaline)	Male Sprague-Dawley rats Blood leucocytes, bone marrow, liver, kidney and testis	Single strand breaks	3-MCPD	2 days, gavage 0 (water), 25 or 60 mg/kg bw per day Sacrifice 3 h after 2-day administration	Negative	El Ramy et al. (2007)	
	Male F344 rats Blood leucocytes and testis		3-MCPD	2 days gavage 0 (water) or 60 mg/kg bw per daySacrifice 3 h after 2-day administration	Negative	El Ramy et al. (2007)	
Pig-a mutation assay	Male F344 *gpt* delta rats Red blood cells	Gene mutation	3-MCPD	4 weeks, 5 times a week, gavage Neg. control: olive oil 40 mg/kg bw per day Sampling: 24 h after last administration	Negative	Onami et al. (2014a)	

Type of test	Experimental test system	End point	Substance tested	Experimental conditions	Result	Reference	Comments
Gpt gene mutation assay	Male F344 *gpt* delta rats	Gene mutation	3-MCPD	4 weeks, 5 times a week, gavage Neg. control: olive oil 40 mg/kg bw per day	Negative	Onami et al. (2014a)	
Spi gene mutation assay	Male F344 *gpt* delta rats	Gene mutation	3-MCPD	4 weeks, 5 times a week, gavage Neg. control: olive oil 40 mg/kg bw per day	Negative	Onami et al. (2014a)	
UDS	Crl: Han Wist BR rats liver	Induction of DNA repair as indirect measure of DNA damage	3-MCPD	Single gavage 0 (water), 40 or 100 mg/kg bw	Negative	Robjohns et al. (2003)	
	Male Han Wistar rats		3-MCPD	Single gavage 0 (water), 40 or 100 mg/kg bw	Negative	Fellows (2000)	
Dominant lethal mutation assay	Male Wistar rat	Chromosomal aberrations	3-MCPD	5 days gavage 0, 5, 10 or 20 mg/kg bw per day	Negative	Jones and Jackson, (1976)	
	ICR/Ha Swiss mouse		3-MCPD	Single i.p. 125 mg/kg bw	Negative	Epstein et al. (1972)	
	Male mouse		3-MCPD	5 days gavage 0, 5, 10 or 20 mg/kg bw per day	Negative	Jones et al. (1969) and Epstein et al. (1972)	
Somatic mutation (Wing spot test)	*Drosophila melanogaster*	Gene mutation	3-MCPD		Negative	Frei and Würgler, (1997)	

Summary of toxicity

The LD_{50} after a single oral dose of 3-MCPD has been reported as 150 mg/kg bw per day in rats and 191 mg/kg in mice. Death occurs several days after dosing. In a subchronic study in rats, a BMD for 10% mortality was calculated as 7.4 mg/kg bw per day. In a six-week study, The LD_{50} in rhesus monkeys was slightly above 30 mg/kg bw per day as two out of six animals died at this dose.

3-MCPD produces severe renal toxicity which persists for several weeks after single i.p. doses between 100 and 120 mg/kg bw per day. Multiple daily oral doses also result in renal toxicity and more subtle toxicity such as progressive nephropathy and renal tubule dilation can be seen after a daily dose as low as 5.2 mg/kg bw. Barocelli et al. (2011) calculated a BMD_{10} for a combined renal histopathology score of less than 1.84 mg/kg bw per day.

3-MCPD administered to rats at 30 mg/kg bw per day impairs red blood cell function by decreasing haemoglobin content and inducing volume fraction changes consistent with normocytic and normochromic anaemia. Severe bone marrow suppression was reported in 50% of male rhesus monkeys receiving 30 mg/kg bw per day.

After long-term exposure at doses as low as 2.0 mg/kg bw per day. 3-MCPD caused progressive nephrotoxicity (characterised by nephropathy and tubular hyperplasia) testicular toxicity (atrophy and arteritis) and mammary glandular hyperplasia in male rats and nephrotoxicity in female rats. Related to these effects, benign tumours of the testes (Leydig cells tumours), mammary gland (fibroadenoma) and kidney (tubular adenoma) were found to develop. In one study a significant increase in malignant kidney tumours was observed in male rats.

There are extensive data documenting the male antifertility activity of 3-MCPD. Doses of around 5 mg/kg bw day 3-MCPD administered to the rat can completely impair male fertility without changing sperm production. This effect has been demonstrated in several species and is reversible. The NOAEL of 3-MCPD on male fertility is not clear. Higher doses of 3-MCPD approximately 90 mg/kg bw per day permanently impair fertility most likely by blocking the passage of sperm from the testis, which may explain the testicular pathology seen in some studies. The BMD_{10} for testicular pathology of unknown relationship to fertility has been calculated as 8.4 mg/kg bw per day in a 90-day study (Barocelli et al., 2011).

Single and multiple doses of 3-MCPD administered to the pregnant rat decreased the numbers of implantation sites and increased fetal loss but were not teratogenic. The NOAEL for multiple dose was 10 mg/kg bw per day for maternal toxicity and 30 mg/kg bw per day for fetal toxicity. Pregnancies achieved by male rats receiving doses which partially maintained fertility were apparently normal and there was no increase in intra-uterine fetal abnormalities.

3-MCPD induces gene mutations in some strains of bacteria and DNA strand breaks and gene mutations in mammalian cells *in vitro*. It was not genotoxic in *Drosophila melanogaster*. The genotoxic potential of 3-MCPD has been investigated *in vivo* in mammalians considering various endpoints: gene mutations, chromosomal aberrations, DNA strand breakage and induction of DNA repair (UDS). Several organs were analysed: peripheral blood, bone marrow, liver and also the target organs for cancer: kidney and testis. Although some of the *in vivo* studies have limitations, the genotoxic potential observed in some *in vitro* tests could not be reproduced *in vivo*. Overall, the CONTAM Panel considered that there is no evidence indicating that 3-MCPD is genotoxic *in vivo*.

3.3.3.2. 3-MCPD fatty acid esters

Single dose

In an acute study in mice, Liu et al. (2012) gave single oral doses (1,000–5,000 mg/kg bw) of the mono- and dipalmitate esters of 3-MCPD dissolved in blended edible oil to mixed groups of adult male and female Swiss mice and examined the responses up to 14 days later. Some mice died within 48 h of dosing and the median LD_{50} of the monopalmitate was 2,676 mg/kg bw. The 3-MCPD dipalmitate was less toxic and the LD_{50} could only be estimated to be greater than 5,000 mg/kg bw. Body weight in the mice which subsequently died fell up to 50% in the 5 days after dosing of both esters. In surviving mice, body weight gain recovered suggesting these toxic changes were transient.

Li et al. (2013) assessed the acute toxicity of 3-MCPD dipalmitate in male and female Wistar rats. Rats were exposed via a single gavage administration (soybean oil as vehicle) to 100–3,160 mg/kg bw 3-MCPD dipalmitate (two rats/sex per group) and subject to a 14-days observation period. No signs of toxicity were recorded at 100 and 316 mg/kg bw. Acute locomotor toxicity was noted at doses 1,000 mg/kg and greater. All rats exposed to the top dose died within 36 h. An LD_{50} of 1,780 mg/kg bw was calculated.

Repeated dose

In a 90-day study 3-MCPD dipalmitate dissolved in corn oil was administered by daily gavage to adult male and female Wistar rats using equimolar doses over the range 9.78–156.7 mg/kg for 3-MCPD dipalmitate, equivalent to 1.84–29.5 mg/kg per day for 3-MCPD. (Barocelli et al., 2011). In contrast to treatment with the 3-MCPD general good health was retained in 3-MCPD dipalmitate-treated rats although the discomfort score was elevated after the highest dose, 156.7 mg/kg. Analysis of female rat mortality after 3-MCPD exposure gave a BMD_{10} and $BMDL_{10}$ of 7.4 and 2.3 mg/kg per day, respectively. Death was not observed after 90 days treatment with 156.7 mg/kg 3-MCPD dipalmitate.

No mortality was observed in a 90-day study in which male and female Wistar rats were exposed to 12.3 and 267 mg/kg bw per day 3-MCPD dipalmitate diluted in soybean oil. Reduced body weights were observed in the high dose group after 90 days of treatment (Li et al., 2013).

Another investigation administered either 3-MCPD mono- or dipalmitate or 3-MCPD dioleate in olive oil to adult F344 male and female rats for 5 days a week for 13 weeks. (Onami et al., 2014a,b). Doses were equimolar, again reflecting the intention of several authors to ensure the mass of the 3-MCPD given was comparable between the different esters. These doses were, 14–220 mg/kg, MCPD dipalmitate; 8–130 mg/kg 3-MCPD monopalmitate; 15-240 3-MCPD dioleate. 3-MCPD 40 mg/kg was used as a reference dose. In F344 female rats 3-MCPD was lethal after 1–4 weeks of treatment (40 mg/kg approximate LD_{50}). Deaths in the other treatment groups including the esters were low, sporadic and not dose related. There was no loss of body weight or weight gain.

Tee et al. (2001) indicated in an abstract that in Sprague-Dawley rats the esters 1-palmitoyl, 1-steroyl, 2-oleoyl or 1-palmitoyl-2-oleoyl 3-MCPD at doses between 50 and 400 mg/kg per day for 14 days were not toxic.

Renal and hepatic toxicity

At the end of a 90-day repeated dose study of 3-MCPD dipalmitate in Wistar rats the combined weight of the kidney and adrenal gland was increased in both sexes (Barocelli et al., 2011). The weight increases, probably reflecting changes in the kidney, were dose dependent such that they were about 70% higher after the highest dose of both MCPD dipalmitate (156.7 mg/kg) and 3-MCPD (29.5 mg/kg).

The highest doses of both 3-MCPD and its ester increased water consumption in male and female rats but only towards the end of the 90-day study. The lower doses of both 3-MCPD and its ester did not change water consumption throughout the 13 week study. In spite of no significant changes being observed in urine output, after both the highest does of 3-MCPD dipalmitate (156.7 mg/kg) and 3-MCPD (29.5 mg/kg) the urine was acidified and the concentrations of several substances were changed, notably the proteins Nag, B2M, albumin and α-GST. Serum creatinine concentrations were reduced by both compounds.

The study by Barocelli et al. (2011) reported an extensive array of degenerative changes in the renal tubules after 90 days treatment across the range of doses for both 3-MCPD dipalmitate and 3-MCPD and in both sexes. These included primary effects, such as glomerular lesions and tubular epithelial hyperplasia and secondary changes such as cellular infiltration in interstitial spaces and fibrosis. When examined quantitatively these pathological effects were restricted to the highest dose of both 3-MCPD and its ester. The overall scores at the highest dose of 3-MCPD and its ester were not different in the male rats. Histopathology scores were slightly lower in the female rats and the 3-MCPD dipalmitate score was slightly less severe than the 3-MCPD score. Both primary and secondary histopathological data were combined into a total renal score which was used for BMD modelling. In male rats the BMD_{10} and $BMDL_{10}$ for 3-MCPD dipalmitate for the total renal histopathology scores were 41.1 and 17.4 mg/kg, respectively, compared to the BMD_{10} and $BMDL_{10}$ for 3-MCPD as 5.6 and 2.5 mg/kg bw per day. In female rats the BMD_{10} and $BMDL_{10}$ for 3-MCPD dipalmitate for renal histopathology were 7.9 and 3.6 mg/kg, respectively. The renal toxicity of 3-MCPD was high even at the lowest dose studied, 1.84 mg/kg bw per day, BMD modelling was not reliable and the authors estimated BMD_{10} and $BMDL_{10}$ for MCPD were both between 0.1 and 1.84 mg/kg bw per day. It should be noted that primary and secondary data were combined for use in the BMD modelling, as these data are not independent from each other some bias may have been introduced into the estimates of the BMD and BMDL.

Another rat 90-day repeated dose study of 3-MCPD dipalmitate did not reveal any change in renal weight after 12.3 mg/kg bw per day; however, kidney weight increased by 46% after 267 mg/kg bw per day (Li et al., 2013). Serum urea and creatinine showed significant increases 90 days after the start of treatment confirming only the observation in serum for creatinine by Barocelli et al. (2011).

Liver weights and hepatic biomarkers were unchanged. Tubular cell degeneration, hyaline castes and inflammatory infiltration were noted after 90 days in the 3-MCPD dipalmitate 267 mg/kg treated group supporting the histopathology reported by other studies.

Profiling of endogenous metabolites in urine from rats treated with 3-MCPD dipalmitate has been achieved by principal component analysis and partial least squares discriminant analysis (PLS-DA) which when displayed in a score plot, showed in complete separation of the endogenous metabolite profiles between controls, 12.3 and 267 mg/kg bw per day MCPD dipalmitate after 90 days treatment. After 63 days treatment only the 267 mg/kg bw daily dose was different from control indicating progressive development of renal toxicity during prolonged treatment with 3-MCPD dipalmitate (Li et al., 2013).

At the end of a 90-day study in F344 rats absolute and relative kidney weights were increased by 3-MCPD (dosed at 40 mg/kg bw per day) and by mid and high doses of 3-MCPD dipalmitate (55 and 220 mg/kg bw per day), MCPD monopalmitate (32 and 130 mg/kg bw per day) or 3-MCPD dioleate (60 and 240 mg/kg bw per day). No changes in kidney weight were observed in rats treated with the lowest tested doses of the three esters (14, 8 and 15 mg/kg bw per day for 3-MCPD dipalmitate, monopalmitate and dioleate, respectively) (Onami et al., 2014b). The kidney weight increases for the highest doses of the esters were comparable to 3-MCPD alone. These weight changes reflect a similar but not so a definitive pattern for increases in liver weight as the only significant changes were seen after the highest dose of 3-MCPD dipalmitate (220 mg/kg bw per day), 3-MCPD monopalmitate (130 mg/kg bw per day) or 3-MCPD dioleate (240 mg/kg bw per day). Serum urea was decreased in 3-MCPD monopalmitate-treated female rats only. Falls in serum creatinine were more frequently observed the exception being the low dose 3-MCPD monopalmitate in males, and low dose 3-MCPD monopalmitate and 3-MCPD dioleate in females. Severe tubular necrosis was confirmed in the rats which died after 3-MCPD treatment. In rats surviving 13 weeks of 3-MCPD treatment and in others receiving 3-MCPD fatty acid esters no histopathological changes (including renal) were seen except in the epididymis.

In contrast to the rat studies of 3-MCPD fatty acid esters there were no changes in the relative kidney weights after treatment of Swiss mice with a single dose of 3-MCPD monopalmitate or dipalmitate (Liu et al., 2012). Mice receiving 3-MCPD monopalmitate (up to 4,162 mg/kg) which subsequently died displayed high serum urea and creatinine concentrations indicative of acute renal failure. In contrast both serum urea and creatinine concentrations in mice which survived 14 days after treatment were lower than control at all doses of the monopalmitate examined. No changes were reported after treatment with 3-MCPD dipalmitate.

In the mouse renal tubules focal degeneration, cell necrosis and protein casts were consistently observed after treatment with 3-MCPD monopalmitate (2,014 mg/kg and above) but were not reported in the kidneys of the 3-MCPD dipalmitate treated mice (up to 4,162 mg/kg) that survived treatment.

Haematology

Males and females Wistar rats treated with 3-MCPD dipalmitate for 90 days showed mild dose-dependent changes consistent with normocytic and normochromic anaemia (Barocelli et al., 2011). At the highest dose of both 3-MCPD (29.5 mg/kg) and 3-MCPD dipalmitate (156.7 mg/kg) there were small reductions of red blood cell number, haemoglobin concentration and haematocrit. There seems to be a discrepancy between haemoglobin concentration and haematocrit, the latter being relatively unchanged. Increased serum bilirubin was an indirect indicator that anaemia has mainly a haemolytic origin. White blood cells were raised in male rats and platelets in both sexes. In one study 90 days treatment with 3-MCPD dipalmitate, both 12.3 and 267 mg/kg, increased the weight of the spleen (Li et al., 2013). The $BMDL_{10}$ and BMD_{10} for 5% loss of RBC (5% loss) for 3-MCPD and 3-MCPD dipalmitate was 2.6, 4.5, 90.4, 187.0 mg/kg, respectively, in female rats and 3.5, 7.2, 24.8, 53.5 mg/kg in male rats (Barocelli et al., 2011).

Haematological changes consistent with anaemia were also seen in the 13 week F344 male and female rat study (Onami et al., 2014b). Serum haemoglobin fell after the highest dose of all ester groups (3-MCPD dipalmitate 220 mg/kg, 3-MCPD monopalmitate 130 mg/kg and 3-MCPD dioleate 240 mg/kg) with the exception of 3-MCPD dioleate in male rats. There was some indication of a dose–response relationship. These changes were sometimes accompanied by changes in haematocrit and red blood cell count but variation between groups was high making precise conclusions difficult.

No haematological changes were reported in 3-MCPD fatty acid ester treated mice (Liu et al., 2012).

Long-term toxicity and carcinogenicity

No long-term toxicity or carcinogenicity studies with 3-MCPD fatty acid esters were identified.

Reproductive toxicity

Robust fertility studies have not been conducted with the esters of 3-MCPD but there is evidence for reproductive toxicity. In a brief paper some mono-esters of 3-MCPD have been reported to possess male antifertility activity when administered to rats at doses between 15 and 100 mg/kg bw per day for 8 days. The esters were less potent than 3-MCPD itself, which was active at 3 mg/kg bw per day (Ericsson and Youngdale, 1970). 3-MCPD dipalmitate when administered orally to male rats at a dose of 100 mg/kg bw (0.17 mmol/kg bw) showed comparable molar potency to 3-MCPD 10 mg/kg bw (0.09 mmol/kg bw) in the inhibition of male fertility (Rooney and Jackson, 1980).

At the end of 90 days treatment of Wistar rats with 3-MCPD dipalmitate at doses up to 156.7 mg/kg bw per day the weights of both male and female gonads were not different from controls (Barocelli et al., 2011). However, degenerative changes in testes from rats receiving daily treatment MCPD dipalmitate for at equimolar doses to a testis toxic dose of 3-MCPD were present but inconsistent. No pathology was reported for the ovary although sexual cycles were not examined.

F344 Rats treated for 13 weeks with 3-MCPD dipalmitate (at 14, 55 or 220 mg/kg bw per day), monopalmitate (at 8, 32 or 130 mg/kg bw per day) or dioleate (15, 60 or 240 mg/kg bw per day) did not show any effects on testis weight or treatment-related testicular histology including the spermatogenic tubules and Leydig cells. However, dose-related apoptotic cell death was observed in the head of the epididymis and achieved statistical significance at the highest tested doses, equimolar to 3-MCPD 40 mg/kg bw per day, for the three 3-MCPD fatty acid esters (Onami et al., 2014b). In contrast, another study using Wistar rats reported that testis weight was increased after 90 days treatment with 267 mg/kg 3-MCPD dipalmitate but this was not reflected by changes in histopathology. (Li et al., 2013).

In mice, testis and ovarian relative weights were not changed 14 days after treatment with single doses of the 3-MCPD mono- and dipalmitic esters (Lui et al., 2012). There were some histopathological changes in testicular sections but these were subtle and may not have fully developed 14 days after dosing due to the length of time the spermatogenic epithelium takes to turnover. However, the changes may have indicated impairment in the production of round spermatids.

Developmental toxicity

No developmental studies with 3-MCPD fatty acid esters were identified.

Genotoxicity

In vivo

Micronucleus tests

In a study by Onami et al. (2014a) the potential of equimolar concentrations of 3-MCPD (see above) and three 3-MCPD fatty acid esters: palmitate diester, palmitate monoester and oleate diester to induce micronuclei in bone marrow was tested in male F344 *gpt* delta rats carrying a transporter transgene lambda EG10. The rats were dosed by intragastric administration for 4 weeks, five times a week. The rats were randomly allocated to six groups with five animals in the positive control group, six rats in the negative control group (olive oil) and test groups. Only one dose was given to each test group: 3-MCPD dipalmitate (220 mg/kg bw), 3-MCPD monopalmitate (130 mg/kg bw) and 3-MCPD dioleate (240 mg/kg bw). Olive oil was used as vehicle for the test substances. No positive control was used in this experiment. The animals were killed on day 29, 24 h after administration of the last dosing and bone marrow tissues were taken for the MN assay. No increase in the frequency of micronucleated reticulocytes was observed in the 3-MCPD fatty acid ester treated groups compared with the control group. The percentage reticulocytes among total erythrocytes (measure of chemical-induced bone marrow toxicity) did not differ between the treated and control groups.

Gene mutation assays

Onami et al. (2014a) tested also the potential of equimolar concentrations of 3-MCPD (see above) and three 3-MCPD fatty acid esters (palmitate diester, palmitate monoester and oleate diester) to induce Pig-a mutations in male F344 *gpt* delta rats carrying a transporter transgene lambda EG10 (Table 33). The same treatment protocol as described above for the MN test was applied. Peripheral blood samples from the tail vein were collected from each animal at day 0 and 15. At day 29, within 24 h after final administration, blood samples were collected from the abdominal aorta for Pig-a mutation assays. The frequency of Pig-a mutant red blood cells did not differ among groups at any time point.

Table 33: *In vivo* genotoxicity data on 3-MCPD fatty acid esters

Type of test	Experimental test system	End point	Substance tested	Experimental conditions	Result	Reference	Comments
Micronucleus test	Male F344 *gpt* delta rats	Chromosomal aberrations	CDP CMP CDO	4 weeks, 5 times a week, gavage Neg. control: olive oil 220 mg/kg bw per day 130 mg/kg bw per day240 mg/kg bw per day Sampling: 24 h after last administration	Negative	Onami et al. (2014a)	
Pig-a mutation assay	Male F344 *gpt* delta rats Red blood cells	Gene mutation	CDP CMP CDO	4 weeks, 5 times a week, gavage Neg. control: olive oil 220 mg/kg bw per day 130 mg/kg bw. per day 240 mg/kg bw per day Sampling: 24 h after last administration	Negative	Onami et al. (2014a)	
Gpt gene mutation assay	Male F344 *gpt* delta rats	Gene mutation	CDP CMP CDO	4 weeks, 5 times a week, gavage Neg. control: olive oil 220 mg/kg bw per day 130 mg/kg bw per day 240 mg/kg bw per day	Negative	Onami et al. (2014a)	
Spi gene mutation assay	Male F344 *gpt* delta rats	Gene mutation	CDP CMP CDO	4 weeks, 5 times a week, gavage Neg. control: olive oil 220 mg/kg bw per day 130 mg/kg bw per day 240 mg/kg bw per day	Negative	Onami et al. (2014a)	

CPD: 3-MCPD di palmitate diester; CMP: 3-MCPD monopalmitate; CDO: 3-MCPD dioleate.

In the study by Onami et al. (2014a), described above, genomic DNA was extracted from the kidney and testis and investigated for gene mutations in the *gpt* or *Spi* gene. No increase was observed in mutation frequencies compared to control and olive oil treated animals in either gene in any of the treated groups. Mutation frequencies were according to the authors within negative control values described earlier by the authors (Hibi et al., 2011).

Summary

It can be concluded that the range of toxic effects for esterified 3-MCPD are the same as those seen for the free 3-MCPD, supporting the view that the esters are cleaved and toxicity primarily exerted by 3-MPCD.

Acute LD_{50} of 2,676 mg/kg bw was calculated for 3-MCPD monopalmitate in mice. Acute LD_{50} of > 5,000 mg/kg bw and 1,780 mg/kg bw were determined for 3-MCPD dipalmitate in mice and rats, respectively. Considering 3-MCPD equivalents, 3-MCPD dipalmitate and palmitate showed lower acute toxicity than free 3-MCPD. This reduction of toxicity was also noted following repeated exposure to a variety of esters, monopalmitate, and steroyl diester, oleate diester and oleate-palmitate diester.

There are no data for single doses of 3-MCPD fatty acid esters. Multiple doses in rats of 3-MCPD dipalmitate were not as toxic as equimolar doses of free 3-MCPD for some endpoints but not for others. For instance, in one multiple administration study, a dose of 3-MCPD equivalent to the LD_{50} when given as the equimolar dipalmitate was not lethal. This reduction of toxicity was also noted after a variety of esters, monopalmitate, and steroyl diester, oleate diester and oleate-palmitate diester. In mice, 3-MCPD has an oral LD_{50} after a single dose of 191 mg/kg bw per day but when administered as the mono palmitate it is increased to 2,676 mg/kg bw per day and the LD_{50} of the dipalmitate could only be estimated to be greater than 5,000 mg/kg bw per day.

After equimolar multiple doses of 3-MCPD and 3-MCPD dipalmitate the biochemical changes associated with renal toxicity are similar in pattern and magnitude. Both compounds produce an extensive array of renal histopathology including glomerular lesions and tubular epithelial hyperplasia. An estimated BMD_{10} for a combined histopathological score in the rat was 41.1 mg/kg bw per day for 3-MCPD dipalmitate compared to 5.6 mg/kg bw per day for the free 3-MCPD. Similar patterns of toxicity are seen after the other 3-MCPD fatty acid esters were tested but dose comparisons between the different MCPD fatty acid esters are difficult to make.

Haematological changes consistent with anaemia of haemolytic origin have been seen in rats after treatment with 3-MCPD or one of several 3-MCPD fatty acid esters. The BMD_{10} for 5% red blood cell loss in male rats was 4.5 mg/kg bw per day for 3-MCPD compared to 187 mg/kg bw per day for 3-MCPD dipalmitate.

No long-term toxicity or carcinogenicity studies with 3-MCPD fatty acid esters were identified.

There is limited evidence that 3-MCPD esters possess male antifertility activity. For example 3-MCPD dipalmitate when administered orally to male rats at 0.17 mmol/kg bw showed comparable potency to 3-MCPD at 0.09 mmol/kg bw in the inhibition of pregnancy in untreated females.

Degenerative changes in testes from rats receiving prolonged daily treatment MCPD esters such as mono- and dipalmitate or the dioleate at equimolar doses to testis toxic doses of 3-MCPD have been reported but the changes are generally inconsistent. However, increases in testis weight in the absence of testicular histopathology was noted after 90 days treatment with 267 mg/kg 3-MCPD. Apoptotic cell death was observed in the head of the epididymis which appeared dose-related after the 3-MCPD fatty acid esters and was comparable to 3-MCPD 40 mg/kg bw per day. No pathology was reported for the ovaries from treated female rats.

In mice, testis and ovarian weights were not changed 14 days after treatment with single doses of the 3-MCPD mono- and dipalmitic esters although there were some histopathological changes in the testis.

There are no data describing the developmental toxicity of the esters of 3-MCPD.

The genotoxic potential of some 3-MCPD fatty acid esters has been investigated in one *in vivo* study (a MN test and in gene mutation assays). From the limited evidence available there is no indication that 3-MCPD fatty acid esters are genotoxic *in vivo*.

3.3.3.3. 2-MCPD

Single dose

2-MCPD can be lethal within 24 h when given by gavage to adult male Sprague-Dawley rats. Marchesini and Huggett (1992) used the 'up and down' protocol to estimate the LD_{50} to be between 50 and 60 mg/kg bw (n = 10). Death was accompanied by a brief series of convulsions.

Repeated dose

Only limited data are available on 2-MCPD toxicity. In contrast to 3-MCPD, a single i.p. injection of 200 mg/kg bw 2-MCPD did not cause diuresis in male Sprague-Dawley rats (Jones and Fakhouri, 1979). It should be noted that the corresponding LD_{50} dose for 3-MCPD was given as 90 mg/kg bw, which produced enlarged kidneys and severe renal failure, and the LD_{50} for 2-MCPD was approximately 200 mg/kg bw, which produced no evidence for kidney damage (Jones and Fakhouri, 1979).

The toxicity of 2-MCPD has been described in a repeated dose 28-day oral protocol in young adult male and female Sprague-Dawley rats (Perrin et al., 1994). 2-MCPD was dissolved in distilled water and administered by gavage at daily doses of 2, 16 or 30 mg/kg bw per day to groups (n = 10–15) of 4–5 week old male and female rats. There were a few deaths in the high dose group and their timings ranged from 8 to 23 days of treatment. Death was attributed to cardiac failure.

Dose-dependent lesions in striated muscle such as cytoplasmic vacuolisation and lysis of myocytes were recorded in the 16 and 30 mg/kg bw per day at the 8 day and terminal (29 day) study points. These pathological changes were present throughout the body but were most extensive and severe in the myocardium. Over-contraction of the heart was also noted at autopsy. Serum biochemistry was altered and the changes mainly reflected acute muscle damage. For example ASAT, ALAT, LDH and CK were elevated which was accompanied by raised serum phosphorus and potassium. There was some functional cardiac adaptation in the less severely affected rats after 29 days of treatment.

Renal damage in the form of increased kidney weight and cytoplasmic vacuolisation in the proximal convoluted tubules was also dose dependent occurring in the 16 and 30 mg/kg bw per day treated rats. The renal tubule changes were more prominent in the male rats. The renal pathology was accompanied by a water diuresis which was unexpected given the severity of the cardiac failure.

The effects of 2-MCPD were restricted to the 16 mg/kg per bw and the 30 mg/kg bw per day treated rats. No adverse effects were documented at the 2 mg/kg bw per day dose group which was recorded as the NOAEL.

No long-term toxicity or carcinogenicity studies with 2-MCPD were identified.

Genotoxicity

Bacteria

Very few genotoxicity studies on 2-MCPD have been identified, with most of those identified being unpublished study reports. 2-MCPD was mutagenic in a bacterial test system (metabolic system not stated) (Jones and Gant, unpublished data cited by Schilter et al., 2011).

Mammalian cells

In V79 cells 2-MCPD did not induce gene mutations at the hprt locus when tested up to extreme high concentrations (50 mM) either with or without metabolic activation (Morgenthaler, 1993, unpublished report).

Drosophila

In an *in vivo* 'wing spot test' in *Drosophila melanogaster* no genotoxic effect was observed (Frei and Würgler, 1997).

No mammalian *in vivo* genotoxicity studies have been identified for 2-MCPD.

Summary of toxicity

2-MCPD

There are limited data on the short-term toxicity of 2-MCPD. A single i.p. dose of 200 mg/kg bw per day although potentially lethal did not cause signs of renal toxicity. However, multiple doses of 16 or 30 mg/kg bw per day in a 28-day oral protocol caused severe lesions leading to cell death in striated muscle, particularly in cardiac myocytes, resulting in heart failure and the death of some animals as from treatment day 8. Renal effects consisting in increased diuresis, increased kidney weight and histopathological changes in proximal renal tubules were observed at the highest dose tested (30 mg/kg bw per day). An oral NOAEL was reported by the authors of the study as 2 mg 2-MCPD/kg bw per day.

No long-term toxicity or carcinogenicity studies with 2-MCPD were identified.

3.3.3.4. 2-MCPD fatty acid esters

There are no relevant data for the esters of 2-MCPD.

3.3.3.5. Glycidol

Single dose

No relevant data were identified.

Repeated dose

A relatively fast onset of the effects of daily doses of glycidol were seen when administered in water by gavage to groups (n = 5) of F344/N rats and B6C3F1 mice of each sex for 16 days. (NTP, 1990). Glycidol doses for rats ranged from 37.5 to 600 mg/kg bw per day. All rats that received 600 mg/kg bw per day died between days 3 and 13. All mice that received 600 mg/kg bw per day and 40% that received 300 mg/kg bw per day, died by day 4 of the studies. Glycidol-related histopathologic lesions included demyelination of brain neurones in both male and female mice that received 150 or 300 mg/kg bw per day, and renal tubular cell degeneration in male mice that received 300 mg/kg bw per day. No renal toxicity was reported in rats after 16 days of daily treatment.

However, 13 weeks after administration to groups of rats (n = 10) at doses of 25 to 400 mg/kg bw per day, renal tubular cell degeneration was seen in rats receiving 400 mg/kg bw per day. Doses for groups of mice (also given for 13 weeks, n = 10) ranged from 19 to 300 mg/kg bw per day. Renal tubular degeneration was only seen in male mice receiving 300 mg/kg bw per day. The brains of female mice that received daily doses of 300 mg/kg bw glycidol for 16 days or 91 days displayed focal demyelination in the medulla and thalamus. These histopathologic lesions were only present in the brains of male mice that received either 150 or 300 mg/kg bw per day. Cerebellar necrosis was seen in rats of both sexes after treatment with 400 mg/kg bw per day for 91 days.

In a 28-day study neurotoxicity developed in 5-week-old Sprague-Dawley rats which were given glycidol by gavage at 0, 30 or 200 mg/kg bw per day (Akane et al., 2014b). Neurotoxicity was only observed at highest dose in which rats showed progressively increasing abnormalities of the gait along with essentially the same lesions of central and peripheral nervous systems as those also observed in pregnant rats (Akane et al., 2013). The authors suggested that glycidol disrupts processes involving late-stage neurite extension in the subgranular zone of the dental gyrus.

It was found that 28-day exposure to glycidol to young adult rats by gavage at 200 mg/kg bw per day caused down regulation of genes related to the function of axon and synaptic transmission (Akane et al., 2014c). Observed loss of Arc+, Fos+ or Jun+ neurons in the dentate granule cell layer, cingulate cortex and cerebellar vermis at the end of 28-day glycidol exposure was suggested to signify suppressed neuronal plasticity (Akane et al., 2014a,c).

Long-term toxicity and carcinogenicity

The carcinogenicity of glycidol was evaluated by the US NTP, based on 14 day dose-range finding (n = 5 animals/sex), 13 week subchronic (n = 10), and 2 year chronic exposures (n = 50) via gavage for five times a week in adult (PND 53) F344 rats and B6C3F1 mice of both sexes (NTP, 1990). Dosing was started when the animals had reached the age of 53 days. Doses selected for the 16 day study ranged from 37.5 to 600 mg/kg bw per day, for the 13-week study 25–400 mg/kg bw in rats and 19–300 mg/kg bw in mice, and for the 2-year study 37.5 and 75 mg/kg bw per day in rats and 25 and 50 mg/kg bw per day in mice. Survival rates until the end of the 2-year study for rats were poor with 100% mortality in both glycidol-dosed groups of males and 96–100% mortality in glycidol-dosed females vs 68 and 44% mortality in the respective control groups. Mortality in mice was 46–50% in glycidol-dosed males (34% in controls) and 46–66% in glycidol-dosed females (42% in controls).

Clear evidence for carcinogenicity of glycidol was reported based on significantly increased incidences of a number of tumours in both sexes of both rodent species from chronic exposures. As shown in Table 34, significantly increased tumour incidences were observed in male F344 rats for: peri-testicular mesotheliomas; mammary gland fibroadenomas; brain gliomas; thyroid gland follicular cell adenomas and carcinomas; forestomach papillomas and carcinomas; intestinal adenomatous polyps and carcinomas; skin adenomas and adenocarcinomas of the sebaceous gland and basal cell tumours; and Zymbal gland carcinomas. Significantly increased tumour incidences were observed in female F344 rats for: mammary gland fibroadenomas and adenocarcinomas; thyroid gland follicular cell adenomas and carcinomas; brain gliomas; forestomach papillomas and carcinomas; oral mucosa papillomas or carcinomas; clitoral gland adenomas, adenocarcinomas or carcinomas; and leukaemia.

Significantly increased tumour incidences were observed in male mice for: Harderian gland adenomas or adenocarcinomas; forestomach squamous cell papillomas and carcinomas; skin squamous cell papillomas or carcinomas; liver adenoma or carcinomas; and lung alveolar/bronchiolar

adenomas or carcinomas. Significantly increased tumour incidences were observed in female mice for: Harderian gland adenomas or adenocarcinomas; mammary gland adenomas, fibroadenomas or adenocarcinomas; uterus carcinomas or adenocarcinomas; subcutaneous tissue sarcomas or fibrosarcomas; and skin squamous cell papillomas or carcinomas.

Table 34: Neoplasms associated with the 2-year gavage administration of glycidol to F344 rats [incidences (%)]

Site	Males			Females		
	Vehicle	37.5 mg/kg bw per day	75 mg/kg bw per day	Vehicle	37.5 mg/kg bw per day	75 mg/kg bw per day
Tunica vaginalis/ peritoneum mesothelioma	3/49 (6)	34/50 (68)	39/47 (83)			
Mammary gland Fibroadenoma Adenocarcinoma	3/45 (7)	8/39 (21)	7/17 (41)	14/49 (29) 1/50 (2)	32/46 (70) 11/48 (23)	29/44 (66) 16/48 (33)
Brain Glioma	0/46 (0)	5/50 (10)	6/30 (20)	0/49	4/46 (9)	4/46 (9)
Oral mucosa Papilloma or carcinoma	1/46 (2)	2/50 (4)	6/32 (19)	0/47 (0)	4/38 (11)	11/30 (37)
Intestine Adenomatous polyp or adenocarcinoma	0/47 (0)	1/50 (2)	4/37 (11)			
Skin Sebaceous gland adenoma, basal cell tumour, or sebaceous gland adenocarcinoma	0/45 (0)	5/41 (12)	4/18 (22)			
Zymbal gland Carcinoma	1/49 (2)	3/50 (6)	6/48 (13)			
Clitoral gland Adenoma, adenocarcinoma, or carcinoma				5/49 (10)	9/47 (19)	12/45 (27)
Thyroid gland Follicular cell adenoma or carcinoma	1/46 (2)	4/42 (10)	6/19 (32)	0/49 (0)	1/38 (3)	3/35 (9)
Haematopoeitic system Leukaemia				13/49 (27)	14/44 (32)	20/41 (49)

As shown in Table 35, significantly increased tumour incidences were observed in male B6C3F1 mice for: Harderian gland adenomas or adenocarcinomas; forestomach squamous cell papillomas and carcinomas; skin squamous cell papillomas or carcinomas; liver adenoma or carcinomas; and lung alveolar/bronchiolar adenomas or carcinomas. Significantly increased tumour incidences were observed in female B6C3F1 mice for: Harderian gland adenomas or adenocarcinomas; mammary gland adenomas, fibroadenomas or adenocarcinomas; uterus carcinomas or adenocarcinomas; subcutaneous tissue sarcomas or fibrosarcomas; and skin squamous cell papillomas or carcinomas.

The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of glycidol in 2000 and determined that it is probably carcinogenic to humans (Group 2A) (IARC, 2000). The IARC had previously reported that the carcinogenicity of glycidyl oleate and glycidyl stearate were not classifiable (Group 3, 1987 suppl 7) (IARC 1987).

The NTP evaluated the carcinogenicity of glycidol using a transgenic mouse model haploinsufficient for the p16Ink4a and p19Arf tumour suppressor genes (NIH publication 08-5962, 2007) based on results from the 1990 conventional 2 year bioassays in mice and despite a negative finding in a related transgenic haploinsufficient model, the p53 ± mouse (Tennant et al., 1999). Male and female p16Ink4a/p19Arf mice received glycidol at doses of 25, 50, 100, or 200 mg/kg bw in deionised water by gavage at five times per week for 40 weeks. The study found 'clear evidence' for carcinogenicity of glycidol in males based

on significantly increased incidences of histiocytic sarcomas and alveolar/bronchiolar adenomas and 'some evidence' in females based on the occurrence of alveolar/bronchiolar adenomas.

Table 35: Neoplasms associated with the 2-year gavage administration of glycidol to B6C3F1 mice [incidences (%)]

Site	Males			Females		
	Vehicle	25 mg/kg bw per day	50 mg/kg bw per day	Vehicle	25 mg/kg bw per day	50 mg/kg bw per day
Harderian gland Adenoma or adenocarcinoma	8/46 (17)	12/41 (29)	22/44 (50)	4/46 (9)	11/43 (26)	17/43 (40)
Mammary gland Adenoma, fibroadenoma, or Adenocarcinoma				2/50 (4)	6/50 (12)	15/50 (30)
Forestomach Squamous cell papilloma or carcinoma	1/50 (2)	2/50 (4)	10/50 (20)			
Uterus Carcinoma or adenocarcinoma				0/50 (0)	3/50 (6)	3/50 (6)
Subcutaneous tissue Sarcoma or fibrosarcoma				0/50 (0)	3/50 (6)	9/50 (18)
Skin Squamous cell papilloma or carcinoma	0/50 (0)	0/50 m (0)	4/50 (8)	0/50 (0)	0/50 (0)	2/50 (4)
Liver Adenoma or carcinoma	24/50 (48)	31/50 (62)	35/50 (70)			
Lung Alveolar/ bronchiolar adenoma or carcinoma	13/50 (26)	11/50 (22)	21/50 (42)			

Reproductive toxicity

Glycidol when administered orally to male rats at 100 or 200 mg/kg bw per day for 5 days or 100 mg/kg bw per day for 14 days produced infertility which was similar to the low dose effect of 3-MCPD (Cooper et al., 1974). The authors suggested that this effect may be related to *in vivo* conversion in the stomach of glycidol to 3-MCPD. The appearance of 3-MCPD in the serum of rats after oral administration of glycidol has recently been confirmed (Onami et al., 2015). Low doses of glycidol equivalent in molar terms to 5 mg 3-MCPD/kg bw per day and given by i.p. injection reduce sperm motility although fertility was maintained (Brown-Woodman et al., 1979).

Glycidol in water given by gavage to groups of F344/N rats and B6C3F1 mice of each sex was studied by the US national toxicology program (NTP, 1990). The glycidol used was 94% pure and contained 0.4% 3-MCPD. In rats that received 300 mg/kg per day after 16 days there was oedema and degeneration of the epididymal stroma and atrophy of the testis. Granulomatous inflammation of the epididymis occurred. However, these effects were not reported in mice. A13-week study used doses for rats ranging from 25 to 400 mg/kg per day, while doses for mice ranged from 19 to 300 mg/kg bw per day. Sperm count and sperm motility were reduced in rats. The rat lowest observable adverse effect level (LOAEL) was 25 mg/kg bw per day for epididymal sperm count which was reduced by 36%. Testicular atrophy and or degeneration occurred in rats that received 200 or 400 mg/kg bw per day.

In all mice that received glycidol the sperm count and sperm motility were reduced and accompanied by testicular atrophy. The mouse LOAEL was 75 mg/kg bw per day when the sperm count was 44% of controls.

Developmental toxicity

No evidence of teratogenicity was demonstrated in a study in which pregnant CD-1 mice received 100, 150, or 200 mg/kg glycidol by gavage during days 6–15 of gestation. The doses were maternally toxic (Marks et al., 1982). However, when glycidol was injected into the amniotic sac of pregnant Sprague-Dawley rats on day 13 of gestation there was embryo lethality and induced malformations in a significant number of fetuses (Slott and Hales, 1985). The relevance of this exposure approach is not clear.

Developmental neurotoxicity of rats to glycidol was further investigated by exposing pregnant Sprague-Dawley rats via the drinking water to 0, 100, 300, or 1,000 mg glycidol/L from day 6 following appearance of vaginal plug until weaning on postnatal day (PND) 21 (Akane et al., 2013). These concentrations resulted in maternal doses of 0, 18.5, 48.8, and 108.8 mg glycidol/kg bw per day. The highest dose of glycidol severely affected gait causing inability to support the body and spreading of extremities in some animals. These effects where accompanied by axonopathy in both central and peripheral nervous systems (Akane et al., 2013). There was a dose-dependent reduction in body weight of male pups of dams exposed to 48.8 and 108.8 mg glycidol/kg bw per day (in females significant only in the high-dose group), but the body weight recovered in pups of dams exposed to 48.8 mg/kg bw per day after the end of the lactation period. The highest dose caused a reversible loss of immature granule cells in the subgranular zone of the dental gyrus in offspring. The results suggested abnormalities of late-stage neurogenesis particularly of the hippocampal dentate gyrus (Akane et al., 2013). Doses of 48.8 and 108.8 mg/kg bw/day given to the dams resulted in an increase in NeuN- and reelin-positive cells and mature neurons in the dentate hilus of male pups. These results are indicative of an aberration in the distribution of interneuron subpopulations in the hilus. No effects were observed in dams or offspring at the lowest dose of 18.5 mg/kg bw per day.

Genotoxicity

In vitro

Glycidol can alkylate DNA directly and form adducts with purified DNA on incubation *in vitro* (Segal et al., 1990). This can result in the induction of genotoxic effects by glycidol.

Bacteria

Glycidol has a mutagenic effect in *in vitro* test systems with prokaryotes both with and without the addition of metabolic activation system (Wade et al., 1979; Thompson et al. 1981; NTP, 1990).

Glycidol was tested for its ability to induce gene mutations in bacteria (NTP, 1990). The test substance, dissolved in water, was tested up to 10,000 μg/plate in a pre-incubation assay in the following Salmonella Typhimurium strains: TA100, TA1535, TA97 and TA98. Glycidol was a potent inducer of gene mutations especially in the base pair substitution strains TA100 and TA1535 both with and without S9-mix from rats and hamsters. It was less potent in the frameshift strain TA97. Weak or equivocal results were obtained in TA98.

Glycidol and glycidol linoleate were tested in a bacteria reverse mutation test in S. typhimurium TA98, TA100, TA1535, TA1537 and *E. coli WP2uvrA* (Ikeda et al., 2012). The test was performed with and without metabolic activation. Glycidol was dissolved in water and glycidol linoleate in dimethyl sulphoxide (DMSO). For glycidol no precipitation and no bacterial toxicity was observed in any strain up to 5,000 μg/plate. Glycidol was a very potent mutagen in the base pair substation strains (TA100, TA1535) and to a lesser extent to *E. coli WP2uvrA*, but only weakly mutagenic/non mutagenic in the frameshift mutation strains TA98 and TA 1537, respectively, both with and without metabolic activation. For glycidol linoleate precipitation was observed at concentrations > 2,500 μg/plate without metabolic activation and at 5,000 μg/plate with metabolic activation. Toxicity was observed in all *Salmonella* strains especially with metabolic activation. No toxicity was observed in the *E. coli* strain, regardless of metabolic activation. Glycidol linoleate induced a concentration-dependent increase in revertants in TA100 and TA1535 both with and without S9-mix and in *WP2uvrA* only with metabolic activation, but it was less potent than glycidol. Glycidol linoleate did not induce mutagenicity in TA98 and TA1537. The test was performed according to OECD TG 471. It was concluded by the study

authors that the genotoxic response of glycidol linoleate was due to release of glycidol, and not to genotoxicity of glycidol linoleate itself.

Mammalian cells

Glycidol and glycidol linoleate were tested for the induction of chromosomal aberrations in Chinese hamster lung cells (CHL/IU) (Ikeda et al., 2012). The test was performed according to OECD TG 473. Both short-term treatment (with and without S9-mix) and long-term treatment (24 and 48 h without S9-mix) were performed. Glycidol was dissolved in saline and glycidol linoleate in DMSO. Glycidol was a very potent inducer of structural chromosomal aberrations without metabolic activation after short-term treatment and after 24 h continuous treatment, but to a much lesser extent after short-term treatment with metabolic activation and after 48 h treatment without S9-mix. No numerical chromosomal aberrations were observed at any concentration or time point analysed. Glycidol linoleate did not induce structural or numerical chromosomal aberrations either with or without metabolic activation at any time point analysed. Glycidol linoleate was less toxic to mammalian cells than glycidol.

Glycidol was tested in CHO cells for induction of primary DNA damage in the comet assay. The cells were exposed for 3 h to 0, 5, 10, 20 and 30 μg glycidol/mL. A positive and concentration-related response was obtained, with statistical significance at the two highest concentrations (El Ramy et al., 2007).

In mouse lymphoma L5178Y/TK cells glycidol was a potent inducer of mutations at low concentration levels. A clear and concentration related response was observed in the concentration range 5–40 ng/mL without S9-mix. It was not tested with S9-mix (NTP, 1990).

Glycidol induced Sister Chromatid Exchange (SCE) in CHO cells. Strong positive results were obtained without S9 in the following concentrations range: 1.11 to 15 μg/mL, at all concentrations tested. The substance was less potent with S9-mix but tested positive in the following concentration range: 11.1 to 150 μg/mL at all concentrations tested.

In various mammalian cells, glycidol has induced a wide spectrum of genotoxic effects *in vitro* (genetic mutations, chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis), in most cases both with and without the addition of a metabolic activation system (IARC, 2000; MAK, 2000).

In vivo

In an NTP study (NTP, 1990) the potential of glycidol to induce MN in the bone marrow of B6C3F1 mice was investigated. Glycidol was dissolved in phosphate buffered saline. Based on a preliminary dose range finding study the following concentrations: 0, 37.5, 75 and 150 mg/kg bw were administered by i.p. injection twice at 24 h intervals. The animals were killed 24 h after the last dose. Two trials were performed. In the first experiment a clear, dose-related and statistically significant response was observed ($p < 0.001$), in the second experiment a less clear but also statistically significant response was observed ($0.01 < p < 0.05$). Based on these results it is concluded that glycidol can induce micronucleus *in vivo* under the test conditions performed in this study.

Glycidol and glycidol linoleate were investigated for the induction of MN in the bone marrow of ICR mice (Ikeda et al., 2012). The test was performed according to OECD TG 474. Glycidol was dissolved in water and glycidol linoleate in olive oil. The test substance was administered by gavage in two doses separated by 24 h. Based on a preliminary dose range finding study the dose levels for glycidol were 50, 100 and 200 mg/kg bw and for glycidol linoleate 250, 500 and 1,000 mg/kg bw. For glycidol linoleate the frequency of micronucleated polychromatic erythrocytes was not different from the vehicle control in any of the test groups. For glycidol the number of micronucleated polychromatic erythrocytes was significantly increased in the mid-dose group compared to the control group (0.17 compared to 0.06). The frequencies were 0.07 and 0.11 at the low and high dose, respectively. Due to the lack of dose-response relationship it was concluded that glycidol did not induce MN under the test conditions performed. No toxicity was observed on the bone marrow measured as the PCE/NCE ratio.

Some publications on the induction of chromosomal aberrations in rodents *in vivo* report different results: positive results in Thompson and Gibson (1984), quoted in MAK (2000); negative results in Thompson and Hiles (1981), quoted in IARC (2000).

In a feeding study with *Drosophila melanogaster*, glycidol (1,230 mg/L feed) caused sex-linked recessive lethal mutations and reciprocal translocations in germ cells (NTP, 1990).

Summary

In both male and female mice that received daily doses of Glycidol in the range 150 – 300 mg/kg bw per day for 16 days, histopathologic CNS lesions developed including demyelination of neurones in the medulla and thalamus, and renal tubular cell degeneration was also observed. A higher dose 400 mg/kg bw per day was also associated with neuronal cell death. No renal toxicity was reported in rats after 16 days of daily treatment up to a lethal dose of 600 mg/kg bw per day but renal tubular degeneration was seen even at 400 mg/kg bw per day after 13 weeks of treatment. Neurotoxicity was observed after 28 days daily treatment of rats with 200 mg/kg bw per day which showed progressive abnormalities of the gait along with lesions of central and peripheral nervous systems. Exposure of young adult rats to glycidol downregulates genes related to the function of axon and synaptic transmission in the dentate granule cell layer, cingulate cortex and cerebellar vermis.

Clear evidence for carcinogenicity of glycidol has been reported by the US NTP based on increased incidences of tumours in both sexes of mice and rats chronically exposed to Glycidol for up to 2 years. Tumour in rats, exposed to 25–50 mg/kg bw per day, included peri-testicular mesotheliomas; mammary gland fibroadenomas; brain gliomas; thyroid gland follicular cell adenomas and carcinomas; forestomach papillomas and carcinomas; intestinal adenomatous polyps and carcinomas; skin adenomas and adenocarcinomas of the sebaceous gland and basal cell tumours; and leukaemia.

The NTP also evaluated the carcinogenicity of glycidol using a transgenic mouse model haploinsufficient for tumour suppressor genes Male and female *p16Ink4a/p19Arf* mice received glycidol doses up to 200 mg/kg bw, five times per week for 40 weeks (NTP, 2007). The study found 'clear evidence' for carcinogenicity of glycidol in males based on increased incidences of histiocytic sarcomas and alveolar/bronchiolar adenomas and 'some evidence' in females based on the occurrence of alveolar/bronchiolar adenomas.

The International Agency for Research on Cancer determined that Glycidol is probably carcinogenic to humans (Group 2A) and that the carcinogenicity of glycidyl oleate and glycidyl stearate were not classifiable.

When administered to male rats glycidol produces infertility which is similar to that observed with low doses of 3-MCPD. The authors suggested that this effect may be related to *in vivo* conversion in the stomach of glycidol to 3-MCPD. Glycidol, when administered to rats at doses that do not impair fertility, reduced sperm motility. In rats receiving more prolonged treatment with 300 mg/kg per day epididymal inflammation oedema and degeneration as well as atrophy of the testis develops. Sperm counts and sperm motility were reduced in rats. The rat lowest observable adverse effect level (LOAEL) was 25 mg/kg bw per day for epididymal sperm count which was reduced by 36%. Testicular atrophy and/or degeneration occurred in rats that received 200 or 400 mg/kg bw per day. Similar effects were seen in mice. The mouse LOAEL was 75 mg/kg bw per day when the sperm count was 44% of controls.

No evidence of teratogenicity was seen in a study in which pregnant mice received up to 100, 150, or 200 mg/kg bw glycidol during days 6–15 of gestation. The doses were maternally toxic which in some animals severely affected gait which was accompanied by axonopathy of central and peripheral neurones. In rat pups exposed throughout pregnancy and weaning to a maternal dose of up to 108.8 mg glycidol/kg bw per day during pregnancy and weaning body weight was reduced and abnormalities of neurogenesis observed in the brain from 48.8 mg glycidol/kg bw per day.

Glycidol is a strong inducer of gene mutations in bacteria, both with and without metabolic activation; it mainly induces base pair substitution mutations. In mammalian cells it also induces gene mutations without S9-mix (it was not tested with S9-mix).

Glycidol is a potent direct acting clastogen in mammalian cells *in vitro*; it is less potent when S9-mix is added, indicating a metabolic detoxification by rat liver S9 mix.

There are few *in vivo* studies on glycidol. Only chromosomal aberrations have been investigated with diverging results. A clear positive result was obtained in mice after i.p. injection. However, in a more recent oral study in mice negative results were obtained for both glycidol and glycidol linoleate. The reason for this discrepancy could be that two different exposure routes were used in the two studies. Glycidol is a highly reactive molecule, and a direct acting mutagen and clastogen. In *in vitro* studies it appears to be deactivated, at least to some degree, by liver enzymes. This may explain the negative result after oral exposure, where lower concentrations may have reached the bone marrow than after i.p. injection.

Based on consistent evidence of genotoxicity *in vitro* and some indication that it can be genotoxic *in vivo* the overall conclusion is that there is strong evidence that glycidol is a genotoxic compound.

The glycidol ester (glycidyl linoeate) that has been tested *in vitro* was found to be less potent than glycidol. The authors hypothesise that this is due to the incomplete release of glycidol.

3.3.3.6. Glycidyl fatty acid esters

Single dose

No relevant data were identified.

Repeated dose

In 1958, Walpole evaluated the carcinogenicity of several glycidyl esters, including stearate. Daily subcutaneous injections of pure glycidyl stearate to rats (550 mg total dose/100 g bw for 41 days) in peanut oil produced sarcomata at the site of injection after 454–608 days; however, the author questioned the reliability of the tumour induction model based on vehicle effects and recommended further study using other methods.

No further relevant information on toxicity of glycidyl esters were identified.

3.3.4. Observations in humans

3.3.4.1. 3-MCPD, 2-MCPD, Glycidol and their fatty acid esters

No data were identified.

3.3.5. Biomarkers of exposure/effects

3.3.5.1. Biomarkers of exposure

3-MCPD

Urinary metabolites

Urinary 3-MCPD-metabolites, including MCPD-mercapturate (2,3-dihydroxypropyl mercapturic acid, DHPMA) and β-chlorolactic acid, are discussed as potential biomarkers of exposure for MCPD fatty acid esters. Both urinary metabolites (DHPMA and β-chlorolactic acid) are stable compounds and can be quantified with high sensitivity (Eckert et al., 2010; Barocelli et al., 2011).

Urinary metabolites of 3-MCPD were investigated as potential biomarkers of 3-MCPD exposure/availability in the 90-day repeated dose study in rats with oral application of 3-MCPD diester (dipalmitate) or equimolar doses of 3-MCPD (Barocelli et al., 2011; study considered in section 'Toxicity in experimental animals'). Relevant amounts of 3-MCPD mercapturate (DHPMA) as well as free 3-MCPD, but only traces of β-chlorolactic acid and no glucuronidated metabolites, were detected in the collected (24 h) urine of the test animals. Gender and dose-dependent metabolite excretion were observed. Furthermore, 3-MCPD diester exposure was associated with urinary excretion rates of both 3-MCPD and 3-MCPD mercapturate were about 20% lower as compared to the same urinary biomarkers observed after exposure to equimolar doses of 3-MCPD (Barocelli et al., 2011). The disadvantage of mercapturate (DHPMA) as biomarker is its specificity: this metabolite may be derived not only from 3-MCPD but also from glycidol (Jones, 1975; see toxicokinetics, metabolism and uncertainty sections). Moreover, the relatively high background levels of DHPMA and a strong correlation with urinary creatinine reported in humans indicate a possible endogenous origin of DHPMA (Eckert et al., 2011, discussed in the next section).

Protein/DNA adducts

There is no evidence that 3-MCPD or its metabolites could covalently bind to proteins or DNA.

2-MCPD

No data were identified.

Glycidyl esters

Biomarkers of exposure for glycidyl esters/glycidol include its urinary metabolites (2,3-dihydroxypropyl mercapturic acid, DHPMA) and its adducts with haemoglobin (Hb). However, 2,3-dihydroxypropyl mercapturic acid is not a specific biomarker of glycidyl ester exposure, as it was

also confirmed to be a urinary metabolite of 3-MCPD (as mentioned in the previous section). Furthermore, 2,3-dihydroxypropyl mercapturic acid has been shown to be the urinary excretion product of several industrial chemicals (epichlorohydrin and several halogenated propanes or propanols, Gingell et al., 1985; James et al., 1981; Jones et al., 1974; Weber et al., 1995) which however do not normally occur environmentally. Eckert et al. (2011) reported comparatively high background levels of 2,3-dihydroxypropylmercapturic acid in urine of smokers (median levels of 206 μg/g creatinine) and non-smokers (median levels of 217 μg/g creatinine) whose origin is still unknown. Because the determined 2,3-dihydroxypropylmercapturic acid levels revealed a very strong correlation with urinary creatinine, the authors supposed a possible endogenous origin of 2,3-dihydroxypropyl mercapturic acid background levels. Which compound may serve as an endogenous precursor for 2,3-dihydroxypropyl mercapturic acid background levels has not yet been clarified (Eckert et al., 2011).

Protein/DNA adducts

Due to its electrophilic epoxide structure, glycidol has alkylating properties. It was shown to form the haemoglobin adduct N-(2,3-dihydroxypropyl)valine (diHOPrVal) which could be quantified by GCMS after detachment of the N-terminal valines in haemoglobin via the N-alkyl Erdman method (Hindsø Landin et al., 1996, 1997). Because of the long lifespan of erythrocytes (120 days) these adducts accumulate in the human body, making them a very sensitive parameter for human biomonitoring over this time period. Haemoglobin adducts level in blood enable the estimation of internal exposure as well as biochemical effects and seems to be better estimates for cancer risk than measuring the genotoxic substances or their metabolites in human body fluids (Angerer et al., 2007).

The formation of N-(2,3-dihydroxypropyl)valine as an adduct to haemoglobin after *in vitro* incubation of haemolysate with glycidol was first shown by Hindsø Landin et al. (1996). The same authors found background levels of diHOPrVal (1–2 pmol/g Hb) in blood of persons without known exposure and suggested that this background levels could originate from glycidol or related compounds found in heat-processed foods (Hindsø Landin et al., 1997). Indeed, increased levels of diHOPrVal were found in Sprague-Dawley rats fed heat-processed (fried) diet (Hindsø Landin et al., 2000).

Appel et al. (2013) monitored the levels of diHOPrVal adducts in blood of rats administered equimolar doses of glycidyl palmitate or glycidol to address the question of relative bioavailability of glycidol from glycidol esters *in vivo*. The extent of N-(2,3-dihydroxypropyl)valine adduct formation was comparable in groups treated with glycidol or glycidyl palmitater, suggesting a similar bioavailability of glycidol in both groups. This conclusion was also supported by the similar quantities of urinary excreted 2,3-dihydroxypropylmercapturic acid (Appel et al., 2013).

Previous biomonitoring studies have shown that humans are continuously exposed to exogenous (and possibly also endogenous) sources of glycidol or glycidol-like compounds forming N-(2,3-dihydroxypropyl)valine adducts in blood (Hindsø Landin et al., 1997). Honda et al. (2012) investigated levels of N-(2,3-dihydroxypropyl)valine adducts in blood of donors who consumed over a 4-month period a DAG-rich oil, previously reported to contain significantly higher levels of GE than regular cooking oils. While background adducts were measurable in all donors, no significant difference was observed between the DAG oil consumers and non-consumers (Honda et al., 2012).

Recently the same group (Honda et al., 2014) investigated the kinetics of N-(2,3-dihydroxypropyl) valine formation and its elimination *in vitro* and *in vivo*. A linear correlation between glycidol and N-(2,3-dihydroxypropyl)valine levels at 24 h after oral administration of glycidol (0–75 mg/kg bw) was observed in rats, indicating that glycidol was rapidly absorbed and bound to haemoglobin in a dose-dependent manner. Furthermore, a linear decrease in N-(2,3-dihydroxypropyl)valine levels over the following 40 days was observed, which was also similar to the normal turnover of rat erythrocytes (60 day half-life), suggesting that the N-(2,3-dihydroxypropyl)valine adduct is chemically stable (Honda et al., 2014). Further, *in vitro* kinetic measurements for the reaction of glycidol with N-terminal valine in rat and human haemoglobin showed a comparable binding kinetics between the species. The authors concluded that N-(2,3-dihydroxypropyl)valine is a useful biomarker for quantification of glycidol exposure and for risk evaluation (Honda et al., 2014).

3.3.5.2. Biomarkers of effect

3-MCPD

Metabolomic studies of 3-MCPD dipalmitate were carried out based on a 90-day repeated dose study in male Wistar rats with oral application of 3-MCPD dipalmitate (0, 12.3 and 267 mg/kg bw per day).

UPLC–MS (ultra-performance liquid chromatography–mass spectrometry) analysis of the urine samples revealed the differences in metabolic profiles between control and treated rats, which were clearly distinguished by partial least squares-discriminant analysis (PLS-DA, a measure of component change) of the chromatographic data. Five endogenous metabolites used as biomarkers which had earlier and significant variations have been identified – xanthurenic acid, phenylacetylglycine, taurine, indoxyl sulfate and nonanedioic acid. Of these markers, xanthurenic acid and phenylacetylglycine levels were significantly increased and taurine level was significantly decreased on the 28th day while nonanedioic acid and indoxyl sulfate levels were significantly increased on the 35th day of treatment. Authors suggest these metabolites as candidates for the early and sensitive biomarkers in evaluating the effect of 3-MCPD dipalmitate exposure (Li et al., 2013).

3.3.6. Mode of action

3-MCPD

The kidney and testis were found to be the main target organs for 3-MCPD-induced toxicity in animal studies. Both specific toxic effects (renal and testicular toxicity) were associated with oxidative metabolism of 3-MCPD to β-chlorolactaldehyde and β-chlorolactic acid (Lynch et al., 1998). The analogous embryological origins of the kidney and the testis – epididymis complex suggest that both nephropathy and epididymal sperm granuloma formation in the rat could arise by the same (or similar) mechanism (Jones, 1983).

Mode of action for nephrotoxicity

The inhibition of glycolysis by metabolites associated with the β-chlorolactate pathway was suggested as possible nephrotoxic mechanism of 3-MCPD. β-Chlorolactaldehyde, produced from 3-MCPD via alcohol dehydrogenase, has been shown to inhibit glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase, both enzymes involved in glycolysis (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 β-chlorolactic acid) in the kidney was also thought to contribute to the kidney toxicity of 3-MCPD (Jones et al., 1981).

In a recent metabolomics study in rats exposed to 3-MPCD (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 lysosomal β-galactosidase in kidney and epididymis, leading to decreased hydrolysis of galactosylglycerol to galactose and glycerol and elevation of galactosylglycerol in the urine (Li et al., 2010).

To study the molecular mechanisms of 3-MCPD fatty acid ester toxicity, a comparative proteomic analysis was performed based on a 28-day oral toxicity study in male Wistar rats which were treated with equimolar doses of 3-MCPD (10 mg/kg bw) or 3-MCPD dipalmitate (53 mg/kg bw) and a lower dose of 3-MCPD dipalmitate (13.3 mg/kg bw). The snap-frozen kidney samples were analysed by two-dimensional gel electrophoresis-mass spectrometry, and the Ingenuity Pathway Analysis was used for data evaluation. Both 3-MCPD and 3-MCPD dipalmitate treatments caused an increased expression of alcohol dehydrogenase in the rat kidney – the enzyme which was previously postulated to be involved in the metabolic pathway of 3-MCPD to β-chlorolactaldehyde. Moreover, several glycolytic enzymes (e. g. triosephosphate isomerise, which has been previously been shown to be inhibited by β-chlorolactaldehyde) were downregulated in the kidney of 3-MCPD and 3-MCPD dipalmitate treated animals. Among others, isoforms of protein DJ-1 (also known as Parkinson protein 7) and glutathione *S*-transferase P (GSTP) were strongly upregulated, indicating responses to oxidative stress and perturbations of multiple cellular pathways. Patterns of protein deregulation pointed to metabolic shifts in glucose, amino acid and fatty acid metabolism, which may generally affect energy metabolism. Further network analysis revealed that in all treatment groups several proteins controlling cell survival and cell death were deregulated. The results of this study indicate similar toxicity mechanisms (mode of action) for 3-MCPD and its esters (3-MCPD dipalmitate) and confirmed the previously hypothesised (Jones et al., 1981) mechanisms of 3-MCPD-induced nephrotoxicity (Sawada et al., 2013).

Mode of action for male fertility inhibition

3-MCPD was shown to inhibit male fertility in several reproductive toxicity studies (Jones, 1983). When given to rats, 3-MCPD decreased sperm motility and impaired male fertility; alterations in sperm morphology and epididymal lesions were found. 3-MCPD also reduced fertility in males of several other

mammalian species including primates. Although the exact molecular mechanisms are unknown, the inhibition of sperm motility was suggested to be partly due to the inhibition of spermatozoa glycolysis enzymes by the 3-MPCD metabolites (Jones, 1983).

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, 1981a). The inhibition of glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase by the 3-MCPD metabolite β-chlorolactaldehyde, was suggested as possible mechanism (Jones and Porter, 1995; Lynch et al., 1998).

Significantly decreased levels of RNA and protein 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, 1981b). 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 (Sun et al., 2013).

A comparative proteomic analysis, performed based on a 28-day oral toxicity study in male Wistar rats treated with equimolar doses of 3-MCPD or 3-MCPD dipalmitate (described above in 'Mode of action of nephrotoxicity') revealed a deregulation of several proteins controlling lipid metabolism, reproductive system disease and cancer in all treatment groups. Also in testis, one isoform of protein DJ-1 was among the most upregulated proteins in all treatment groups, suggesting a pivotal role of protein DJ-1 in 3-MCPD-mediated toxicity. Network analysis verified close relationships between molecular effects induced by 3-MCPD and its dipalmitate ester. Altogether, the results indicate similar mode of action for 3-MCPD and its esters (3-MCPD dipalmitate) (Sawada et al., 2015).

Mode of action for neurotoxicity

3-MCPD is considered to cause toxicity in several tissues and spermatozoa through inhibition of GAPDH and therefore glycolysis with energy depletion as result (Ford and Waites, 1982; Kwack et al., 2004; Skamarauskas et al., 2007). This mechanism may be involved also in neurons because cytotoxicity of 3-MCPD in primary mouse neocortical cells was associated with a drop in cellular ATP, and both decrease in ATP and cell death were ameliorated by addition of pyruvate to the culture medium (Sheline and Choi, 1998). However, in brains of both rats and mice, toxicity of 3-MPCD is selective towards glial cells with loss of neurons occurring subsequently to death of astrocytes (Cavanagh and Nolan, 1993). In brain slices from rats injected i.p. (up to 160 mg/kg bw) there was only a modest (46%) reduction in GAPDH activity and no drop in pyruvate or lactate concentrations, indicating limited effect on energy metabolism. Furthermore, brain lesions following 3-MCPD treatment do not fully map onto areas of glucose utilisation (rats) or cerebral blood flow (mice) with the inferior colliculi identified as a particularly susceptible region in rats, suggesting additional effects on other pathways (Cavanagh et al., 1993). A possible alternative mechanism was identified in brains of rat where an i.p. injection caused regionally selective decrease of glutathione reductase and glutathione in the inferior colliculi (Skamarauskas et al., 2007).

Kim (2008) studied the effects of 3-MCPD on the expression of neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) in male Sprague-Dawley rats by oral administration on a daily basis for 13 weeks (each group n = 5; 10 or 30 mg/kg bw, control groups received saline). The authors concluded that the observed effects in the nNOS and iNOS expression were mediated, at least in part, by disturbances of the nitric oxide signalling pathway and that the caudal area may be more vulnerable to 3-MCPD than the rostral area of the neocortex and striatum. It can be speculated that the observed reduction in expression of nNOS and iNOS could be a compensation for the decrease of glutathione reductase and glutathione observed by others (Skamarauskas et al., 2007) and, therefore, a reduced capacity to sequester reactive oxygen and nitrogen species.

Mode of action for carcinogenicity

The finding of Leydig cell tumours was discussed from a mechanistic point of view by Lynch et al. (1998). The authors claim that this tumour type has a relatively high spontaneous incidence in F344 rats (Bar, 1992; Gilliland and Key, 1995) resulting in an almost 100% incidence in aging male F344

rats. This finding was related to a high density of luteinizing hormone (LH) receptors in F344 Leydig cells (Prentice and Meikler, 1995). Furthermore, rat Leydig cells bear receptors for the luteinizing hormone releasing hormone (LHRH) which are not found in human or murine Leydig cells (Clayton and Huhtaniemi, 1982; Wang et al., 1983). Testicular damage resulting in a loss of testosterone production, perturbation of metabolic activation of testosterone and/or of androgen receptor binding can all result in an increased level of LH. In rats LH is a potent activator of Leydig cell proliferation which can eventually lead to Leydig cell tumours (Prentice and Meikler, 1995). 3-MCPD affects testicular lipid (Gill and Guraya, 1993) and carbohydrate metabolism in rats and leads to a decline in testosterone production (Paz et al., 1985), followed by an increase in luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin release (Morris and Jackson, 1978). Thus, it appears that 3-MCPD can induce a compensatory pituitary response which causes Leydig cell tumours.

The human relevance of the rat findings was also questionable since Leydig cell tumours are rare in humans and drugs which increase LH levels in human and rodents are known to induce Leydig cell proliferation and Leydig cell tumours in rats but not in humans (Roberts et al., 1989; Bär, 1992; Crisp et al., 1998).

The finding of an enhanced incidence of mammary tumours in male rats is a rare event, it is not reported for most carcinogens (Boorman et al., 1990b). In F344 rats bearing Leydig cell tumours, mammary tumour rates were significantly increased. It has been shown that hormones such as estrogens, progesterone and prolactin which are produced by Leydig cell tumours (Jacobs and Huseby, 1968; Turek and Desjardins, 1979; Amador et al., 1985; Reddy and Rao, 1987; Chatani et al., 1990; Konishi et al., 1991) act as strong proliferative stimuli in the mammary gland (Neumann, 1991). Thus, it has been surmised that the finding of mammary gland tumours in male rats is due to testicular damage, formation of Leydig cell tumours and an abnormal release of proliferative hormones which act on the mammary gland (Lynch et al.,1998).

Leydig cell tumours of the testis are rare in both humans and laboratory animals. However, in certain strains of rat their incidence increases with age so that in animals at the end of chronic toxicological studies over 1–2 years their prevalence is significant reaching 70% in some studies. (Prentice and Meikler, 1995, Cook, Klinefelter, Hardisty, Sharpe, Foster 1999). This observation has been well documented in Fischer F355 rats. Leydig cell adenomas are more frequently observed than Leydig cell carcinomas. The testicular steroid secretion is changed in tumour bearing animals such that serum testosterone concentrations are reduced while oestradiol concentrations are raised. (Cook, Murray, Frame, Hurt 1992, Turek, Desjardins, 1979). It is likely that most Leydig cell tumours arise because of a hormonal disturbance especially involving elevated secretion of pituitary prolactin. The consensus is that the Leydig cell tumours are rat strain-specific and may not be relevant to man. In particular, occurrence of Leydig cell hyperplasia in test species after lifetime exposure to a chemical does not constitute a cause for concern in a risk assessment for carcinogenic potential for humans (Clegg et al., 1997).

In the present study 3-MCPD was associated with Leydig cell tumours as well as an increased incidence of mammary gland hyperplasia in male rats. Mammary gland hyperplasia was not documented in any other studies of 3-MCPD in male animals. The mammary gland in male rodents does not develop if testicular steroidogenesis is normal. However, exogenous oestrogen is capable of promoting extensive growth and proliferation of the breast tissue in male animals (Lucas et al., 2008). It is likely that in the present 3-MCPD study that male rat mammary hyperplasia is a consequence of the pathologically elevated oestrogen secretion arising from the testis with the Leydig cell adenomas.

The finding of benign kidney tumours in rats is strongly associated with a progressive tubular damage and subsequent nephropathy in those animals; kidney tumours can arise on the grounds of a prolonged nephropathy with local regenerative tissue responses as has been described for the rat (Montgomery and Seely, 1990; Hard, 1998; Alden and Frith, 1991). Considering the absence of *in vivo* genotoxicity of 3-MCPD and the close link with and sequential occurrence of nephropathy and kidney tumour formation in rats, a non-genotoxic mode of action at nephrotoxic doses appears likely.

2-MCPD

In a rat study 2-MCPD failed to produce kidney damage or diuresis at a dose of 200 mg/kg bw, whereas its isomer 3-MCPD produced renal failure, enlarged kidneys, and long lasting diuresis after single dose of 75 mg/kg bw (Jones and Fakhouri, 1979). These differences were explained by the fact that metabolism of 2-MCPD to β-chlorolactaldehyde and β-chlorolactate cannot occur, which is believed to play an important role in nephrotoxicity of 3-MCPD (Lynch et al., 1998).

The subchronic toxicity of 2-MCPD (see Section 3.3.2.) has been described in a repeated dose 28-day oral protocol in young adult male and female Sprague-Dawley rats (Perrin et al., 1994). The authors suggest that the adverse effects observed are due to oxidative stress.

The underlying mechanisms for renal toxicity and the destruction of striated muscles, including the heart, are unknown.

Glycidol

Glycidol reacts readily with cellular glutathione and conjugation with glutathione seems to be the major detoxification route of glycidol) as shown *in vivo* (NTP, 1990; refer to section 'Metabolism'). In rat, a significant decrease in hepatic glutathione content (glutathione depletion) was observed following exposure to glycidol by gavage (single dose, 168 mg/kg bw) (Montaldo et al., 1984; cited by IARC, 2000).

Onami et al. (2015) detected higher levels of 3-MCPD than glycidol in serum after dosing rats with glycidol, suggesting that some of the toxicity of glycidol may be due to its partial conversion to 3-MCPD *in vivo*.

Glycidol and its esters, from which the free compound can be derived, possess a reactive epoxide moiety, which is likely to be responsible for the genotoxic activity of the compound without a requirement for metabolic activation (IARC, 2000). (see the respective section 'Genotoxicity' under 3.3.2.5.). Glycidol induces tumours in numerous organs in both sexes of F344 rats and B6C3F$_1$ mice. Furthermore, the tumours produced by glycidol in rats and mice (e.g. peri-testicular mesothelioma, mammary gland, brain, thyroid in rats; Harderian gland, lung, mammary gland in mice) are similar to those produced by other low molecular weight epoxide (or epoxide-forming) carcinogens, including ethylene oxide, glycidamide, and acrylamide (Melnick, 2002; EFSA, 2015).

3.4. Identification of critical effect and dose–response assessment

3-MCPD

In long-term studies, 3-MCPD causes progressive nephrotoxicity (characterised by tubular hyperplasia, adenoma and carcinoma), testicular toxicity (atrophy and arteritis), mammary glandular hyperplasia in male rats and nephrotoxicity in female rats. Related to these effects, benign tumours of the testes (Leydig cells tumours), mammary gland (fibroadenoma) and kidney (tubular adenoma) were found to develop.

Leydig cell tumours are a rat strain specific consequence of testis toxicity and probably not relevant to man (see Section 3.3.5 Mode of action). The Leydig cell tumours observed in the two above mentioned studies were therefore not considered further. The CONTAM Panel concluded that it is likely that in the study on 3-MCPD by Sunahara et al. (1993) the male rat mammary hyperplasia is a consequence of the pathologically elevated oestrogen secretion arising from the testis with the Leydig cell adenomas. Consequently it was seen as inappropriate to use mammary gland tumours in the study by Sunahara et al. (1993) for human risk assessment.

The CONTAM Panel applied the BMD analysis to the results obtained in two long-term exposure studies where rats received 3-MCPD via drinking water. In the first study (Sunahara et al., 1993; described in Section 3.3.2.), Fisher 344 rats were exposed to nominal concentrations of 0, 2, 50 or 100 mg/L. However, in order to derive the dose levels, the CONTAM Panel corrected the nominal concentrations considering the background concentration of 2.7 mg 3-MCPD/L reported in drinking water used as vehicle. The corrected doses, applying the same dose-specific conversion factors applied by the authors of the study resulted in the following estimated average daily doses (for nominal concentrations of 0, 2, 50 or 100 mg/L, respectively):

Males: 0.15, 1.3, 5.5 or 29 mg/kg bw per day
Females: 0.19, 1.6, 7.4 or 36 mg/kg bw per day

Cho et al. (2008a) exposed Sprague-Dawley rats to concentrations of 0, 25, 100 or 400 mg/L, converted by the authors to average daily doses of 0, 2.0, 8.3 or 29.5 mg/kg bw per day, and 0, 2.7, 10.3 or 37.0 mg/kg bw per day for male and female rats, respectively.

In both studies kidney and testes resulted as the key target organs for 3-MCPD. Pre-neoplastic and neoplastic effects on male mammary glands were also observed in the Sunahara et al. (1993) study.

The results related to kidney and testis toxicity of both studies were initially screened by visual analysis for the presence of monotonic dose–response trends; the presence of a dose-response trend in the subset of results selected in the screening phase was subsequently confirmed by applying the Cochran-Armitage trend test (Haseman, 1984). The effects showing a monotonic dose-response trend were selected for BMD analysis. The full details of the BMD analyses are reported in Appendix C and D for the Sunahara et al. (1993) and Cho et al. (2008a) studies, respectively.

In the analysis of the Sunahara et al. (1993) study results, a lowest $BMDL_{10}$ of 0.10 mg/kg bw per day was calculated for the increased incidence of nephropathy in male rats (see Appendix C). In the Cho et al. (2008a) study, a lowest $BMDL_{10}$ of 0.077 mg/kg bw per day resulted from the analysis of the incidence of tubular hyperplasia in male rats (see Appendix D).

3-MCPD fatty acid esters

In a subchronic gavage study with 3-MCPD vs 3-MCPD dipalmitate (Barocelli et al., 2011), it was found that certain toxic endpoints were more sensitive towards the parent compound than towards the diester (red blood cell loss, mortality), whereas others (kidney, testis, protein urea) exhibited similar sensitivities on a molar basis. Furthermore, a toxicokinetic comparison of 3-MCPD and its dipalmitate ester showed similar oral bioavailability, based on AUCs. Taken together, these findings led the CONTAM Panel to conclude that the toxicity of 3-MCPD fatty acid esters should be considered equivalent (on a molar basis) to that of the parent compound and no dose-response modelling for the 3-MCPD fatty acid esters was needed.

Glycidol

Glycidol is genotoxic and carcinogenic and carcinogenicity was seen as the critical effect of glycidol. The results of the 2 year NTP (1990) study on glycidol were considered for the dose–response assessment. In this study, rats and mice were exposed by gavage to 0, 37.5 or 75 mg glycidol/kg bw per day, and to 0, 25 or 50 mg/kg bw per day, respectively. Both in rats and mice glycidol dose dependently increased the incidence of tumours in different tissues.

As only two dose levels were administered in the study, the CONTAM Panel did not consider the data suitable for BMD modelling. As such, the EFSA Guidance (2009) was followed and a T25 approach (25% increase in incidence of a specific tumour above background incidence in the lifespan of the species) was used. The CONTAM Panel noted that the lowest T25 was 14.2 mg/kg bw per day for the incidence of peritoneal mesothelioma in male rats (Appendix F).

As the animals in these studies were dosed on 5 out of 7 days per week, the Contam Panel considered that with dosing 7 days per week the tumour incidence would have been greater. The T25 was therefore adjusted by multiplying by 5/7 (0.71) to compensate for the lower cumulative administered dose (Benford et al., 2010), giving an estimated T25 of 10.2 mg/kg bw per day for peritoneal mesothelioma. Table 36 gives the T25 values adjusted for dosing duration for the various tumour incidences in rats and mice.

Table 36: T25 values for tumour incidences (calculated from 2 year NTP study on glycidol, 1990) in rats and mice. The T25 values are adjusted for dosing duration

	T25 mg/kg bw per day
Male rats	
Peritoneal mesothelioma	10.2
Female rats	
Mammary gland fibroadenoma	11.7
Male mice	
Harderian gland adenoma	22.6
Female mice	
Harderian gland adenoma	24.1

3.4.1. Derivation of health-based guidance values

3-MCPD and 3-MCPD fatty acid esters

The CONTAM Panel considered that the intact 3-MCPD fatty acid esters are not responsible for the relevant adverse effects, which are due to the release of free 3-MCPD upon ingestion. There is only one long-term study with a 3-MCPD fatty acid ester (dipalmitate) in rodents. In this subchronic gavage study with 3-MCPD vs 3-MCPD dipalmitate (Barocelli et al., 2011), it was found that certain toxic endpoints were more sensitive towards the parent compound than towards the diester (red blood cell loss, mortality), whereas others (kidney, testis, protein urea) exhibited similar sensitivities on a molar basis (Table 37 and Appendix E). Furthermore, a toxicokinetic comparison of 3-MCPD and its dipalmitate ester showed similar oral bioavailability, based on AUCs. Taken together, these findings led the CONTAM Panel to conclude that the toxicity of 3-MCPD fatty acid esters should be considered equivalent (on a molar basis) to that of the parent compound.

Table 37: $BMDL_{10}$ and BMD_{10} (μmol/kg bw per day) calculated for various non-carcinogenic toxicological endpoints (adapted from Barocelli et al., 2011)

	3-MCPD μmol/kg bw per day		3-MCPD dipalmitate μmol/kg bw per day	
	$BMDL_{10}$	**BMD_{10}**	**$BMDL_{10}$**	**BMD_{10}**
Kidney female (degenerative tubule changes)			6.1	13.3
Kidney male (degenerative tubule changes)	22.6	50.7	29.4	69.4
Testis	54.3	76.0	74.8	108.7
Proteinuria male	24.4	57.9	31.6	79.4
RBC 5% loss male	31.7	65.1	41.9	90.3
RBC 5% loss female	23.5	40.7	152.6	315.7
Mortality female	20.8	66.9	< 270	< 270

Non-neoplastic and neoplastic effects

The CONTAM Panel evaluated the risks of non-neoplastic and neoplastic effects. These were combined since the analysis of mode of action of 3-MCPD in rats revealed that it acts as a nephrotoxic agent but lacks convincing evidence for a genotoxic mode of action *in vivo*. Thus, renal hyperplasia was identified as the most sensitive non-neoplastic effect that might also lead to cancer by a non-genotoxic mode of action. Sustained hyperplasia, is recognised as such a mode of action (Wilkinson and Killeen, 1996). A $BMDL_{10}$ value of 0.077 mg/kg bw per day derived from the 3-MCPD-induced renal tubular hyperplasia in male rats was selected as the reference point. This lesion is considered as both the most sensitive and highly indicative since renal toxicity has been observed in rats of both sexes in several independent studies.

For the protection of human health from neoplastic and adverse non-neoplastic effects of 3-MCPD, the CONTAM Panel applied an overall uncertainty factor of 100 to the selected reference point (77 μg/kg bw per day) to account for intraspecies and interspecies differences and derived a rounded group TDI of 0.8 μg/kg bw per day. The CONTAM Panel concluded that the TDI of 0.8 μg/kg bw per day constitutes a group TDI for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).

2-MCPD and 2-MCPD fatty acid esters

The data on 2-MCPD toxicity are limited to a single study, a 24 h dose ranging protocol informing a 28-day repeated dose study. Cardiac and renal toxicity were observed. In the absence of a long-term study from which the toxicological profile and the mode of action could be firmly established, the CONTAM Panel concluded that the available data were insufficient to derive a HBGV for 2-MCPD and 2-MCPD fatty acid esters.

Glycidol and glycidyl esters

The CONTAM Panel only considered toxicity studies in animals with glycidol since no relevant *in vivo* data were identified for glycidyl esters. The dose-response considerations were made for glycidol based on the likely complete release of the parent compound after ingestion.

There is evidence that the mode of action of glycidol as a carcinogen is mainly due to its electrophilic properties which enable the compound to bind covalently to DNA and cause genotoxic effects. The CONTAM Panel considered the dose-response data inadequate for BMD modelling. In agreement with the EFSA Guidance with respect to substances that are genotoxic and carcinogenic, T25 values were calculated for the incidence of tumours observed in rats and mice following long-term exposure to glycidol. The lowest T25 of 10 mg/kg bw per day for peritoneal mesothelioma in male rats was used as the reference point (EFSA, 2005).

3.5. Risk characterisation

3-MCPD and 3-MCPD fatty acid esters

The exposure to 3-MCPD including 3-MCPD from fatty acid esters (described in Section 3.2.4, Tables 21 and 22) showed little difference between LB and UB estimates, and the risk characterisation is therefore based on MB estimates of exposure.

In 'Infants', 'Toddlers' and 'Other children' the median across dietary surveys of the mean MB exposure was 0.7 to 0.9 μg/kg bw and the maximum MB ranged up to 1.5 μg/kg bw, indicating that in more than half of the dietary surveys for these age groups the exposure was at or above the group TDI of 0.8 μg/kg bw. The mean exposure to 3-MCPD including 3-MCPD from fatty acid esters (described in Section 3.2.4, Table 21) was below the group TDI in 'Adolescents', 'Adults' and older population groups.

The median across dietary surveys of MB high exposure estimate (P95) for 'Infants', 'Toddlers' 'Other children' ranged from 1.4 to 1.7 μg/kg bw per day (shown in Table 22). All these levels were above the TDI. For 'Adolescents', the median across dietary surveys was 0.9, ranging from 0.5 to 1.3 μg/kg bw per day. This indicates that in more than half of the dietary surveys 5% of the 'Adolescents' had an exposure to 3-MCPD above the TDI. For 'Adults', 'Elderly' and 'Very elderly' the high exposure estimate across all dietary surveys ranges from 0.3 to 0.9 μg/kg bw. These values are below or only slightly above the group TDI.

The estimated exposure to 3-MCPD of infants receiving formula only (described in Table 27) was 2.4 μg/kg bw per day using mean occurrence level and 3.2 μg/kg bw per day using P95 of occurrence; both values are above the group TDI, which is exceeded up to fourfold.

Estimated exposure substantially exceeding the group TDI is of concern, this is particularly seen in the younger age groups.

2-MCPD and 2-MCPD fatty acid esters

The exposure data in Tables 23 and 24 indicate that 2-MCPD exposure is highest in the younger age groups ('Infants', 'Toddlers' and 'Other children'). Although the exposure data are available, the CONTAM Panel considered that the toxicological information on 2-MCPD and 2-MCPD fatty acid esters is insufficient for risk characterisation.

Glycidol and glycidyl esters

The CONTAM Panel concluded that it is not appropriate to establish a TDI for glycidol since it has genotoxic and carcinogenic potential. Therefore the MoE approach was chosen to characterise the risk. The CONTAM Panel followed the EFSA Guidance on risk assessment approach for substances that are genotoxic and carcinogenic (EFSA, 2005) stating that where the data are unsuitable for deriving a BMD, the T25, representing the dose corresponding to a 25% incidence of tumours, may be used. As such the reference point (T25) derived for glycidol (in Section 3.4.1) was 10.2 mg/kg bw per day. From this, MoE estimates are calculated by dividing the reference point T25 (converted to the same unit as the exposure i.e. μg/kg bw per day) by the exposure levels.

According to the aforementioned EFSA Guidance (EFSA, 2005) 'an MoE of an order of magnitude of 10,000 or higher would not be considered of low health concern under circumstances where there were greater uncertainties, for example if the MoE was calculated using a T25, or if the reference point were based on a poor animal database'. The T25 approach is inherently less conservative than $BMDL_{10}$ modelling, in that the former considers a level of tumour incidence of 25% and the latter 10%. When the reference point is based upon T25 data it is considered that the MoE should be 2.5 times higher than an MoE based upon $BMDL_{10}$ data, i.e. 25,000 (Dybing et al., 2008). Based on this consideration, the CONTAM Panel concluded that an MoE of 25,000 or larger would be of low health concern.

The MoEs derived from the exposure estimates across dietary surveys for the mean and high exposure (P95) to glycidol from esters across different population groups (Tables 25 and 26) are shown in Table 38. Considering the low range between exposure estimates based on LB and UB occurrence, the Panel considered the MOEs corresponding to MB occurrence. The complete table of MoEs across dietary surveys, based on LB, MB and UB occurrence data is shown in the Appendix (Table B.8).

Table 38: Margins of exposure (MOEs) calculated for glycidol; the table presents the MoEs for exposure across dietary surveys based on middle bound occurrence for both, mean and P95 of exposure

	MoE range across dietary surveys (using middle bound occurrence)[a]		
	Min	**Median**	**Max**
	Mean exposure		
Infants	25,500	14,600	12,800
Toddlers	25,500	17,000	11,300
Other children	34,000	17,000	11,300
Adolescents	51,000	34,000	20,400
Adults	51,000	51,000	34,000
Elderly	102,000	51,000	34,000
Very elderly	102,000	51,000	34,000
	P95 of exposure		
Infants	8,500	7,800	4,900
Toddlers	10,200	9,300	5,100
Other children	12,800	9,300	6,000
Adolescents	25,500	17,000	9,300
Adults	34,000	20,400	17,000
Elderly	51,000	20,400	17,000
Very elderly	51,000	20,400	14,600

bw: body weight; min: minimum across dietary surveys using middle-bound occurrence; median: median across dietary surveys using middle-bound occurrence; max: maximum across dietary surveys using middle-bound occurrence.
(a): The minimum, median and maximum exposures are shown.

For all age groups the MoE estimates revealed that the 95th percentile of exposure was below 25,000 in at least half of the dietary surveys; moreover, the MoEs for the age classes of 'Infants', 'Toddlers' and 'Other children' were below 25,000 in all dietary surveys. For 'Infants', 'Toddlers and 'Other childrens' also the MoEs for the mean exposure were below 25,000 in more than half of the dietary surveys.

In the case of 'Infants', a scenario was calculated for infants receiving only formula (Table 29), giving a MoE of 5,400 for the mean exposure based on MB occurrence and of 2,100 for the P95 of occurrence (Table 39). Although the period of exclusive formula consumption is relatively short (compared to lifetime exposure), it is during a critical developmental phase assumed to be particularly sensitive to carcinogens that are genotoxic (see e.g. US EPA, 2005a,b).

Table 39: Margins of exposure (MoEs) calculated for glycidol exposure in the scenarios on infants receiving only formula

	MoE based on exposure scenario MB (LB–UB)[a]
Infants (receiving only formula) – scenario based on mean occurrence	5,400 (5,700–4,900)
Infants (receiving only formula) – scenario based on P95 of occurrence	2,100

(a): The table presents the values corresponding to LB, MB and UB occurrence levels in the form MB (LB–UB). When LB and UB values were coincident, only one value was reported. The T25 of 10.2 mg/kg bw per day for peritoneal mesothelioma was used as point of departure

Although there is a high uncertainty in the reference point used as a basis for the calculation of the MoEs for glycidol, the MoEs lower than 25,000 indicate a health concern.

3.6. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of dietary exposure to 3- and 2-MCPD and glycidol has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006; EFSA, 2009b). In addition, the report on 'Characterising and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

3.6.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

3.6.2. Exposure scenario/Exposure model

A total of 7,175 occurrence values were available to estimate dietary exposure to 3- and 2-MCPD and glycidol, of which 702 were on free 3-MCPD in soy sauce, HVP and related products, 4,754 were on 3- and 2-MCPD or glycidol from esters in fats and oils and 1,719 were on 3- and 2-MCPD or glycidol from esters in other food. The data on fats and oils were mostly submitted by associations of food business operators while those on food other than fats and oils were produced in specific studies with a limited number of samples.

The food surveys used to provide the occurrence data were mostly targeted at particular food groups that were expected to potentially contain free MCPD or MCPD and glycidol fatty acid esters. Although most of the data were randomly sampled inside the respective food group and none of them were data from targeted samples, the representativeness of the entire EU market is not clearly known and might be weak in the case of food groups with small numbers of samples. The levels of free glycidol in the food samples analysed are unknown; this may lead to underestimation of exposure.

The data set on free 3-MCPD in soy sauce, HVP and related products does not include the potential contribution of 3-MCPD released from the esterified form; this may lead to underestimation of 3-MCPD occurrence in these food groups. The data set on fats and oils does not include the potential contribution from 3- and 2-MCPD in free form; however, the proportion of 3- and 2-MCPD in free form in fats and oils is expected to be minimal with respect to the esterified forms, therefore the potential underestimation of exposure would also be minimal.

The occurrence data on infant formulae and those on 26 out of 28 samples of 'fried or baked fish' do not include the potential contribution from free 3- and 2-MCPD; this may lead to underestimation of the occurrence of 3- and 2-MCPD in these food groups.

The use of occurrence data on fried or baked fish to calculate the exposure related to the consumption of all fish may overestimate the exposure contribution of this food group. Similarly, overestimation of the exposure contribution is possible for meat (where occurrence values for fried or roast meat were used) and charcuterie products (where occurrence values for smoked meat were used). Data were missing for different food groups where some detectable content of 3- and 2-MCPD and glycidol would be expected. In some cases (e.g. oil-based sauces and condiments) the information available allowed an exposure to be modelled based on the occurrence in fats and oils; in other cases (e.g. ice cream or chocolate) it was not possible to model the exposure; only the food groups where an occurrence value was available or could be modelled were included in the exposure scenarios. Using available data to model the occurrence in food groups where measured data were not available is a source of uncertainty.

While occurrence data from a relatively recent period were used, current industry action to mitigate the formation of 3- and 2-MCPD fatty acid esters and glycidol fatty acid esters during oil refining might have led to a recent reduction in their levels in certain oils, leading to an overestimate of the exposure.

Consumption data for 'Infants' were available only from a limited number of dietary surveys; the model used for infants receiving only formula was based on assumptions. In both cases, over- or underestimation of exposure is possible.

3.6.3. Other uncertainties

The experimental data for the toxicology and toxicokinetics of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and GE in food has been generated in experimental animals and its relevance to humans remains uncertain.

The optical isomers of 3-MCPD differ in their toxicity. In the occurrence data provided there is no information on the isomeric composition of the free 3-MCPD or the 3-MCPD fatty acid esters.

Absorption rates and rates of hydrolysis of 3- and 2-MCPD fatty acid esters and glycidol fatty acid esters might vary with different fatty acids or between 3- and 2-MCPD mono- and diesters. There is a lack on toxicity data on 2-MCPD. The mode and mechanism of action of 2-MCPD is unknown.

Based on lower bioavailability of glycidol in monkeys, relative to rats, following controlled oral dosing with either glycidol or an esterified form, the rate and extent of hydrolysis of esterified forms of MCPD and glycidol by humans could be less than quantitative, as conservatively assumed in this Opinion.

Conversion of glycidol to 3-MCPD *in vivo* has been observed; however, the extent of conversion is unknown.

The data set used to derive a point of departure for glycidol carcinogenicity was deemed unsuitable for $BMDL_{10}$ modelling (control and two glycidol doses only). The T25 approach implemented as an alternative does not include an estimate of variability, which introduces an uncertainty because differences in sensitivity between individuals were not considered. Also, the T25 was extrapolated from the lowest dose (37.5 mg/kg body weight), which resulted in a 62% tumour incidence. Thus, it can only be stated with reasonable certainty that the dose resulting in a mean tumour incidence of 25% is < 37.5 mg/kg body weight, a dose fourfold greater than the calculated point of departure of 10 mg/kg body weight.

In addition, the rats in this study were dosed with glycidol on 5 out of the 7 days per week. To reflect this the resulting T25 (14.2 mg/kg body weight) was multiplied by 5/7, giving a point of departure of 10.2 mg/kg body weight. It is uncertain if this calculation reflects the toxicokinetics of dosing 7 days per week.

The possible contribution to toxicities associated with 3-MCPD and its esters from co-exposure to glycidyl esters in the diet, based on *in vivo* conversion of glycidol to 3-MCPD, cannot be quantified at this time.

3.6.4. Summary of uncertainties

In Table 40, a summary of the uncertainty evaluation for 3- and 2-MCPD and glycidol is presented which 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.

Table 40: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to 3- and 2-MCPD and glycidol

Sources of uncertainty	Direction[(a)]
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- or 2-MCPD in samples that may contain both forms	–
Unknown levels of free glycidol in food	–
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 MCPD and glycidol in human GI tract	+
Extrapolation from animal data for human risk assessment	+/–
Conversion of glycidol to 3-MCPD may occur	+/–
Lack of long-term toxicity data on 2-MCPD	–
T25 approach has intrinsic uncertainties	+/–
Adjusting data to reflect dosing regime (5/7)	+/–

(a): +: uncertainty with potential to cause over-estimation of exposure/risk; –: uncertainty with potential to cause under-estimation of exposure/risk.

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

4. Conclusions

4.1. Background

- 3-monochloropropane-1,2-diol (MCPD)- and 2-monochloropropane-1,3-diol (2-MCPD) and their esters and glycidyl esters are food contaminants found at highest levels in refined vegetable oils.
- 3- and 2-MCPD esters and glycidol esters are hydrolysed to their respective free forms in the GASTROINTESTINAL (GI) tract.

4.2. Formation

- 3- and 2-MCPD and their esters are formed during the hydrochloric acid hydrolysis of cereal materials, by reaction of the acid with lipids. They are also formed during high temperature food processing operations such as the baking of low-moisture cereal based foods.
- Glycidyl esters, along with 3- and 2-MCPD esters are formed during the deodorisation step of edible oil refining, from the reaction of chloride present naturally in the oil.
- Glycidyl esters and 3- and 2-MCPD esters are mainly formed from the reaction of diacylglycerol (DAG) with chloride.

4.3. Analysis

- Free 3- and 2-MCPD are determined by validated methods based on extraction, derivatisation, and gas chromatography-mass spectrometry.
- 3- and 2-MCPD and glycidol bound as fatty acid esters are determined by the methods used for the free compounds after chemical cleavage of the ester bonds. The methods have been validated for oils and food samples.

4.4. Occurrence data

- Three categories of analytical data were considered, one on 3-MCPD (in free form) in soy sauce, hydrolysed vegetable protein (HVP) and related products; another on 3- and 2-MCPD from esters and glycidol from esters in oils/fats; and a third one on 3- and 2-MCPD (free and from esters) and glycidol (from esters) in food groups other than those mentioned above. In the third category, in most cases the contribution to the total 3- and 2-MCPD from the free form was included, while the results on glycidol were only from esters.
- Occurrence was estimated using 7,175 analytical results, more than half of which were derived from oils and fats.
- Palm oils and fats had the highest mean middle bound (MB) levels of 3-MCPD bound as esters (2,912 μg/kg), 2-MCPD bound as esters (1,565 μg/kg) and glycidol bound as esters (3,955 μg/kg).
- Levels in other oils had lower mean MB levels, ranging from 48 to 608 μg/kg for 3-MCPD from esters, from 86 to 270 μg/kg for 2-MCPD from esters and from 15 to 650 μg/kg for glycidol from esters. Levels of ester-bound 3- and 2-MCPD and glycidol in margarines and related fats were in a similar range.
- In other food groups the highest mean MB levels were in 'Potato crisps', 'Hot surface cooked pastries', 'Shortcrusts' and 'Cookies'. In these products total 3-MCPD ranged from 154 to 247 μg/kg, total 2-MCPD ranged from 79 to 135 μg/kg, whereas glycidol from esters ranged from 110 to 149 μg/kg.

4.5. Chronic exposure assessment

- The exposure to 3- and 2-MCPD was based upon the level of exposure to the parent compounds regardless of their original form (i.e. as free or as ester of fatty acids), and referred to as 3-MCPD and 2-MCPD. Likewise, exposure to glycidol refers to the parent compound, but in this case the original form was exclusively as fatty acid esters.
- The mean exposure to 3-MCPD was 0.5 to 1.5 μg/kg bw per day (MB) across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to 3-MCPD was 1.1 to 2.6 μg/kg bw per day (MB) across dietary surveys in these age groups.

- In adolescents and adult population groups (adults, elderly, very elderly) the mean exposure to 3-MCPD ranged from 0.2 to 0.7 μg/kg bw per day (MB) and the high exposure (P95) ranged from 0.3 to 1.3 μg/kg bw per day (MB).
- The mean 2-MCPD exposure across dietary surveys ranged from 0.2 to 0.7 μg/kg bw per day (MB), for 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to 2-MCPD was 0.5 to 1.2 μg/kg bw per day (MB) across dietary surveys in these age groups.
- In adolescents and adult population groups (adults, elderly, very elderly) the mean exposure to 2-MCPD ranged from 0.1 to 0.3 μg/kg bw per day (MB) and the high exposure (P95) ranged from 0.2 to 0.6 μg/kg bw per day (MB).
- The mean exposure to glycidol was 0.3 to 0.9 μg/kg bw per day (MB) across the dietary surveys for the age groups 'Infants', 'Toddlers' and 'Other children'. The high exposure (P95) to glycidol was 0.8 to 2.1 μg/kg bw per day (MB) across dietary surveys in these age groups.
- In adolescents and adult population groups (adults, elderly, very elderly) the mean exposure to glycidol ranged from 0.1 to 0.5 μg/kg bw per day (MB). The high exposure (P95) in 'Adolescents' ranged from 0.4 to 1.1 μg/kg bw per day (MB) and in adults and older population groups ranged from 0.2 to 0.7 μg/kg bw per day (MB).
- Scenarios of exposure in infants receiving formula only, based on mean consumption and mean occurrence in the formula, resulted in daily intake of 2.4 μg/kg bw for 3-MCPD, 1.0 μg/kg bw for 2-MCPD and 1.9 μg/kg bw for glycidol. Using P95 occurrence data resulted in daily intake of 3.2 μg/kg bw for 3-MCPD, 1.6 μg/kg bw for 2-MCPD and 4.9 μg/kg bw for glycidol.
- For 'Infants' the food groups 'Infant and follow-on formulae', 'Vegetable fats and oils' and 'Cookies' were the major contributors to 3- and 2-MCPD and glycidol exposure.
- For 'Toddlers' the food groups 'Vegetable fats and oils', 'Cookies' and 'Pastries and cakes' were the major contributors to 3- and 2-MCPD and glycidol exposure. 'Infant formula' and follow-on formula' were also important contributors to 3- and 2-MCPD exposure.
- For 'Other children' the food groups with highest contribution to exposure to 3- and 2-MCPD and glycidol were 'Pastries and cakes', 'Margarine and similar' and 'Cookies'. For glycidol, an additional relevant contributor was 'Fried or roast meat'. 'Vegetable fats and oils' also contributed to 3- and 2-MCPD, and glycidol exposure.
- For 'Adolescents', 'Adults', 'Elderly' and 'Very elderly' the major sources of 3- and 2-MCPD and glycidol were 'Margarine and similar' and 'Pastries and cakes'. Additionally, 'Fried or baked potato products' were important contributors to 3- and 2-MCPD exposure while 'Fried or roast meat' and in some cases 'Chocolate spreads and similar' were important contributors to glycidol exposure.

4.6. Hazard identification and characterisation

4.6.1. Toxicokinetics

- There is no available information on toxicokinetics in humans, the information below is from studies in experimental animals.

3-MCPD and 3-MCPD fatty acid esters

- 3-MCPD and its dipalmitate fatty acid esters are rapidly and efficiently absorbed into the systemic circulation following ingestion, with extensive pre-systemic de-esterification occurring in the GI tract.
- 3-MCPD is extensively metabolised with less than 5% appearing in the urine and faeces as the parent compound. The majority of 3-MCPD is eliminated from serum within a few hours of dosing with either the parent compound or its dipalmitate ester.
- The formation of 3-MCPD metabolites and their role in toxicity is not completely characterised. Metabolism includes conjugation with glutathione with subsequent formation of 2,3-dihydroxypropyl mercapturic acid and oxidation to β-chlorolactaldehyde and oxalic acid. Conjugation with glutathione is one well-characterised metabolic pathway but its extent is limited.
- Urinary excretion of 3-MCPD and its metabolites appears to predominate.

2-MCPD and 2-MCPD fatty acid esters

- No toxicokinetic data were identified. However the difference in the structural localisation of the chorine within the molecule makes it unlikely that 2-MCPD exhibits the same metabolic pattern as 3-MCPD.

Glycidol and glycidyl fatty acid esters

- Glycidol and its fatty acid esters are efficiently absorbed following ingestion. Gastro-intestinal hydrolysis of glycidyl fatty acid esters (GE) occurs and appears to be more extensive in rats than in monkeys.
- Metabolic pathways include glutathione conjugation and mercapturate formation, epoxide hydrolysis to glycerol, and conversion to 3-MCPD.
- In addition, the glycidol moiety can bind covalently to cellular nucleophiles (e.g. DNA and haemoglobin) by virtue of the electrophilic nature of the epoxide ring.

4.6.2. Toxicity in experimental animals

3-MCPD and 3-MCPD fatty acid esters

- 3-MCPD produced severe renal toxicity in rats at single i.p. doses between 100 and 120 mg/kg bw, which persists for several weeks.
- Repeated oral doses also result in renal toxicity and progressive nephropathy and renal tubule dilation can be seen after daily dose as low as 5.2 mg/kg bw in rats.
- 3-MCPD administered to rats at 30 mg/kg bw per day impaired red blood cell function by decreasing haemoglobin content and inducing volume fraction changes consistent with normocytic and normochromic anaemia.
- Neurotoxic effects such as hind limb paralysis were reported only at doses over 50 mg/kg bw per day following short-term exposure in mice.
- In long-term studies at doses as low as 2 mg/kg bw per day 3-MCPD caused progressive nephrotoxicity (characterised by tubular hyperplasia) testicular toxicity (atrophy and arteritis) and mammary glandular hyperplasia in male rats and nephrotoxicity in female rats.
- Related to these effects, benign tumours of the testes (Leydig cells tumours), mammary gland (fibroadenoma) and kidney (tubular adenoma) developed.
- The renal toxicity of 3-MCPD appears to reside with the R isomer.
- Doses between 5 and 10 mg/kg bw day 3-MCPD administered to the rat can completely impair male fertility without changing sperm production. This effect has been demonstrated in several species including primates and is reversible. The no observed adverse effect level (NOAEL) of 3-MCPD on male fertility is not clear.
- Single and multiple doses of 3-MCPD administered to the pregnant rat decreased the number of implantations and increased fetal loss but were not teratogenic. The NOAEL for multiple doses was 10 mg/kg bw per day for maternal toxicity and 30 mg/kg bw per day for fetal toxicity.
- Despite some positive genotoxicity tests *in vitro*, there is no evidence that 3-MCPD is genotoxic *in vivo* in any organ tested, including kidney, and testis.
- From the available information on 3-MCPD fatty acid esters, it can be concluded that the toxic effects for esterified 3-MCPD are the same as those seen for the free 3-MCPD, supporting the view that the esters are cleaved and toxicity primarily exerted by 3-MPCD.
- After equimolar multiple doses of 3-MCPD and 3-MCPD dipalmitate the biochemical changes associated with renal toxicity are similar in pattern and magnitude. Both compounds produce an array of renal histopathology including glomerular lesions and tubular epithelial hyperplasia.
- There is limited evidence that some esters of 3-MCPD have male antifertility effects at a similar molar dose to 3-MCPD and degenerative changes in the spermatogenic tubules have been recorded after treatment with 3-MCPD fatty acid esters.
- No studies on the *in vitro* genotoxicity of 3-MCPD fatty acid esters were identified. From the limited evidence (one study with different endpoints) available there is no indication that 3-MCPD fatty acid esters are genotoxic *in vivo*.

2-MCPD and 2-MCPD fatty acid esters

- In a 28-day study in rats, daily doses of 16 or 30 mg/kg bw per day caused severe myopathy and nephrotoxicity. From 8 days of treatment severe lesions leading to cell death developed in striated muscle particularly in cardiac myocytes that resulted in heart failure and the death of some animals. These effects were not observed at 2 mg/kg bw per day.
- No data on long-term studies for 2-MCPD or 2-MCPD fatty acid esters were identified.
- *In vitro* genotoxicity data on 2-MCPD are too limited to make any conclusion. No mammalian *in vivo* genotoxicity studies have been identified for 2-MCPD and 2-MCPD fatty acid esters.

Glycidol

- Neurotoxicity was observed after 28 days of treatment of rats with 200 mg glycidol/kg bw per day.
- Glycidol caused renal toxicity in repeated dose studies in rats and mice at doses in the range 150–400 mg/kg bw per day.
- Two-year carcinogenicity studies in mice (25 and 50 mg/kg bw/day) and rats (37.5 and 75 mg/kg bw per day) showed induction of tumours in multiple organs from both sexes. Supporting evidence for carcinogenicity of glycidol was provided by a short-term study in a transgenic mouse strain.
- Male anti-fertility effects have been noted in rats and mice. The lowest LOAEL was 25 mg/kg bw day in the rat, resulting in 36% reduction in epididymal sperm count. This may be attributed to conversion of glycidol to 3-MCPD in the stomach.
- Glycidol was maternally toxic in mice without producing any major external abnormalities in the fetus. Neurotoxicity was observed in male pups of rats exposed to a maternal dose of 49 mg glycidol/kg bw per day during pregnancy and weaning.
- There is strong evidence from *in vitro* data and some evidence from *in vivo* studies that glycidol is a genotoxic compound.

Observations in humans

- No relevant toxicity data were identified.

4.6.3. Biomarkers of exposure

- In animals urinary 3-MCPD-metabolites, including MCPD-mercaptenurate (2,3-dihydroxypropyl mercapturic acid, DHPMA) and β-chlorolactic acid, have been identified as potential biomarkers of exposure for MCPD fatty acid esters.

4.6.4 Mode of action

3-MCPD and 3-MCPD fatty acid esters

- Toxic effects to kidneys and testis were associated with oxidative metabolism of 3-MCPD to β-chlorolactaldehyde and β-chlorolactic acid.
- The inhibition of glycolysis by metabolites associated with the β-chlorolactate pathway was suggested as possible nephrotoxic mechanism of 3-MCPD and can explain the reduction in sperm motility.
- In rats regionally selective decrease of glutathione reductase activity and glutathione content were identified as a possible alternative mechanism for neurotoxicity. In brains of both rats and mice, toxicity of 3-MPCD affects glial cells with loss of neurons occurring subsequent to death of astrocytes.
- Mammary tumours in male rats were a consequence of the development of Leydig cell tumours. Leydig cell tumours were considered rat strain specific and not relevant to humans.
- Benign kidney tumours in rats is associated with tubular hyperplasia, a non-genotoxic mode of action is likely.

2-MCPD and 2-MCPD fatty acid esters

- The underlying mechanisms for renal toxicity and the destruction of striated muscles, including the heart are unknown.

Glycidol and glycidyl fatty acid esters

- Glycidol is a strong electrophilic agent which reacts readily with cellular nucleophiles.
- Detection of higher levels of 3-MCPD than glycidol in serum after dosing rats with glycidol, suggests that some of the toxicity of glycidol may be due to its partial conversion to 3-MCPD *in vivo*.
- Significant hepatic glutathione depletion was observed following exposure to glycidol.

4.6.5. Hazard characterisation

3-MCPD and 3-MCPD fatty acid esters

- The critical effect of 3-MCPD was kidney toxicity. The CONTAM Panel applied BMD analysis to the results obtained in kidney in two long-term exposure studies where rats received 3-MCPD via drinking water.
- For 3-MCPD, a tolerable daily intake (TDI) of 0.8 μg/kg bw per day was established. This was based on a chronic study in rats in which the lowest $BMDL_{10}$ of 0.077 mg/kg bw per day for renal tubular hyperplasia in males was derived and application of an overall uncertainty factor of 100.
- Noting the lack of specific data on 3-MCPD fatty acid esters and their hydrolysis, the CONTAM Panel confirmed that the toxicity of 3-MCPD fatty acid esters should be considered equivalent (on a molar basis) to that of the parent compound. Therefore the CONTAM Panel concluded that the TDI of 0.8 μg/kg bw per day constitutes a group TDI for 3-MCPD and its fatty acid esters (expressed as MCPD equivalents).

2-MCPD and 2-MCPD fatty acid esters

- No health-based guidance value could be established for 2-MCPD due to insufficient toxicological information, i.e. no data on metabolism and long-term toxicity, little information on mode of action and equivocal *in vitro* genotoxicity findings.

Glycidol and glycidyl fatty acid esters

- The CONTAM Panel only considered toxicity studies in animals with glycidol as no *in vivo* data was identified for glycidyl esters. Dose-response considerations were made for glycidol, assuming a complete hydrolysis of the esters to free glycidol following ingestion.
- The CONTAM Panel considered the dose-response data inadequate for benchmark dose (BMD) modelling.
- Based upon the EFSA Guidance on substances that are genotoxic and carcinogenic, T25 values were calculated for the incidence of tumours observed in rats and mice following long-term exposure to glycidol. The T25 of 10.2 mg/kg bw per day for peritoneal mesothelioma in male rats was used as the reference point.

4.7. Risk characterisation

3-MCPD and 3-MCPD fatty acid esters

- The mean exposure to 3-MCPD was below the group TDI of 0.8 μg/kg bw per day in 'Adolescents', 'Adults' and older age classes in all dietary surveys. In 'Infants', 'Toddlers' and 'Other children' half of the dietary surveys had mean exposure at or above the group TDI, up to a maximum of about 1.5 μg/kg bw per day in 'Toddlers' and 'Other children'.
- The high exposure (P95) for 'Infants', 'Toddlers' and 'Other children' was above the group TDI in all dietary surveys, ranging between a minimum of 1.1 μg/kg bw per day in 'Other children' or roughly 1.5 μg/kg bw per day in 'Infants' and 'Toddlers' up to about 2.5 μg/kg bw per day in all the three age classes.
- The high exposure (P95) for adolescents was at or above the group TDI in half of the dietary surveys, with exposure estimates up to 1.4 μg/kg bw per day. For 'Adults' and the older age classes, only the maximum P95 of dietary exposure to 3-MCPD was around the group TDI.
- The estimated exposure to 3-MCPD of infants receiving only formula was 2.4 μg/kg bw per day using mean occurrence and 3.2 μg/kg bw per day using P95 of occurrence; both values are above the group TDI, which is exceeded up to fourfold.

2-MCPD and 2-MCPD fatty acid esters

- No TDI could be established so although the exposure data were available, it was not possible to undertake risk characterisation.

Glycidol and glycidyl fatty acid esters

- In view of the genotoxic and carcinogenic potential of glycidol, a margin of exposure (MoE) approach was applied. MoEs were calculated by dividing the reference point of 10.2 mg/kg bw per day by the exposure levels. A MoE of 25,000 or higher was considered of low health concern.
- For 'Infants', 'Toddlers' and 'Other children' the MoE estimates for the mean exposure ranged from 34,000 to 11,300; the MoE for high (P95) exposure ranged from 12,800 to 4,900.
- For 'Adolescents' and 'Adults', 'Elderly' and 'Very elderly' age groups the MoE for the mean exposure ranged from 102,000 to 20,400, whereas at high (P95) exposure the range was from 51,000 to 9,300.
- Scenarios of exposure in infants receiving formula only resulted in a MoE of about 5,500 for the mean occurrence and 2,100 for the P95 of occurrence.

5. Recommendations

- For future data collection activities, the inclusion of samples from all food groups potentially contaminated by 3-, 2-MCPD and glycidol is recommended, including foods where mitigation measures have been enforced. For each food analysed, the levels deriving from both forms, free and ester bound, should be measured, when applicable.
- Analysis of the enantiomeric composition of 3-MCPD and its fatty acid esters is recommended for foods containing high levels of these compounds.
- Further studies are recommended on the rates and degree of release of the free compounds from 3- and 2-MCPD fatty acid mono- and diesters, and on the rates and degree of release of the free 3- and 2-MCPD and glycidol from esters of different fatty acids. Such studies should include studies to clarify the metabolic fate of the compounds.
- The mode and mechanism of action of 2-MCPD needs to be investigated.
- Long-term toxicity testing of 2-MCPD is required to provide a basis for quantitative risk assessment.
- More extensive testing of the dose-response for carcinogenesis from chronic lifetime oral administration of glycidol and its esters in rats (2-year carcinogenicity study utilising appropriate doses) would reduce uncertainty in the risk assessment.

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Abbreviations

2-AAF	2-acetylaminofluorene
1,3-DCP	1,3-dichloropropan-2-ol
2,3-DCP	2,3-dichloropropan-1-ol
2-MCPD	2-monochloropropane-1,3-diol
3-MCPD	3-monochloropropane-1,2-diol
ADH	alcohol dehydrogenase
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
APCI	atmospheric pressure chemical ionisation
ARfD	acute reference dose
ASE	accelerated solvent extraction
AUC	area under the curve
ALARA	as low as reasonably achievable
BfR	German Federal Institute for Risk Assessment
BIOCONTAM	Biological Hazard and Contaminants Unit
BMD	benchmark dose
BMDL	benchmark dose (lower confidence limit)
BSTFA	*N*,*O*-bis(trimethylsily)trifluoroacetamine
bw	body weight
cAMP	cyclic adenosine monophosphate
CDO	oleate diester
CDP	palmitate diester
CEN	European Committee for Standardization
CMP	palmitate monoester
COC	UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
CONTAM Panel	EFSA Panel on the Contaminants in the Food Chain
CPA	cyclophilin A
DGF	German Society for Fat Science

DHPMA	*N*-acetyl-S-(2,3-dihydroxypropyl)cysteine or 2,3-dihydroxypropyl mercapturic acid
diHOPrVal	*N*-(2,3-dihydroxy-propyl)valine
DIPP	Type I Diabetes Prediction and Prevention (DIPP) Study, Finland
DMN	dimethylnitrosamine
EC	European Commission
EH	epoxide hydrolase
EI	electron impact
EU	European Union
FAO/WHO	Food and Agriculture Organisation of the United Nations/World Health Organization
FEDIOL	Association of the EU Vegetable Oil and Proteinmeal Industry
FoodEx1	EFSA food classification and description system, version 1
FSA UK	Food Standards Agency
FSH	Follicle stimulating hormone
GC	gas chromatography
GC-ECD	gas chromatography with electron capture detection
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography-tandem mass spectrometry
GE	glycidyl fatty acid esters
GI	gastrointestinal tract
GL	Guideline
GST	glutathione *S*-transferase
Hb	haemoglobin
HCl	hydrochloric acid
HDC	highly damaged cells
HFB	heptafluorobutyrate
HFBA	heptafluorobutyric anhydride
HFBI	heptafluorobutyrylimidazole
HPLC	high performance liquid chromatography
HPV	hydrolysed vegetable protein
IARC	International Agency for Research on Cancer
IMACE	European Margarine Association
iNOS	inducible nitric oxide synthase
IOM	Institute of Medicine of the US National Academies of Science
i.p.	intraperitoneal injection
i.v.	intravenous injection
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre of the European Commission
JRC-IRMM	Joint Research Centre of the European Commission and Institute for Reference Materials and Measurements
LB	lower bound
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectromery
LD_{50}	median lethal dose
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LMBG	German Food and Feed Code
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification
LVI	large volume injection
MB	middle bound
MBPD	monobromopropanediol
MCPD	monochloropropanediol
MF	mutation frequencies
MMS	methyl methanesulfonate
MN	micronucleus

MOE	margin of exposure
MRM	multiple reaction monitoring
NaCl	sodium chloride
NCI	negative chemical ionisation
NITR	Korean National Institute of Toxicological Research
nNOS	neuronal nitric oxide synthase
NOAEL	no observed adverse effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PBA	pheylboronic acid
PCE	polychromatic erythrocytes
PCE/NCE	polychromatic erythrocytes to normochromatic erythrocytes
PLE	pressurised liquid extraction
PMTDI	provisional maximum tolerable daily intake
RBC	red blood cells
RET	retiulocytes
SAS	SAS statistical software
SCE	sister chromatide exchange
SCF	EU Scientific Committee for Food
SIM	selected ion monitoring
SLA	service level agreement
SN2	Second-order Nucleophilic Substitution
SPE	solid phase extraction
SSD	standard sampling description
TDI	tolerable daily intake
UB	upper bound
UDS	unscheduled DNA synthesis
UPLC-MS	ultra-performance liquid chromatography-mass spectrometry
WHO	World Health Organization
WHO/IPCS	World Health Organization/International Programme on Chemical Safety
w.w.	whole weight

Appendix A – EFSA guidance documents applied for the assessment

- EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a request from EFSA related to uncertainties in Dietary Exposure Assessment. EFSA Journal 2006;4(1):438, 54 pp. doi:10.2903/j.efsa.2007.438
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- EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. doi:10.2903/j.efsa.2010.1557
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- EFSA SC (EFSA Scientific Committee), 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579
- EFSA SC (EFSA Scientific Committee), 2012. Scientific Opinion on Risk Assessment Terminology. EFSA Journal 2012;10(5):2664, 43 pp. doi:10.2903/j.efsa.2012.2664

Appendix B – Support tables for the occurrence and exposure sections

Table B.1: Structure of food groups (N = 68) used to organise the analytical results in the present report. Where the groups correspond to FoodEx1 groups, the corresponding code is provided. Groups created for the purpose of this report are flagged as 'ad-hoc'

Code[a]	Food group Name[b]	Note
A.01.001580	Herbs, spices and condiments	
A.01.001625	Herb and spice mixtures	
A.01.001632	Seasoning or extracts	
A.01.001640	Stock cubes (bouillon cube)	
Ad-hoc grp	Other seasoning products	Seasoning or extracts other than stock cubes
A.01.001649	Condiment	
A.01.001660	Soy sauce	
Ad-hoc grp	Other condiment sauces	Sauces included in the condiment group, other than soy sauce
A.01.001665	Dressing	The group includes mayonnaise and similar sauces used as dressing
A.01.001684	Savoury sauces	
A.01.001757	Protein and amino acids supplements	
Ad-hoc grp	Hydrolysed vegetable proteins	Subgroup of the FoodEx1 group A.01.001757 Protein and amino acids supplements including only hydrolysed vegetable proteins
A.01.001789	Composite food	
Ad-hoc grp	Dry preparations for soups (to be reconstituted)	Subgroup of the FoodEx1 group A.01.001856 Ready-to-eat soups including only dry products to be reconstituted
A.01.001346	Animal and vegetable fats and oils	
A.01.001389	Margarine and similar products	
A.01.001390	Margarine, normal fat	
A.01.001391	Margarine, low fat	
A.01.001393	Fat emulsions	
Ad-hoc grp	Special Fats	Group not corresponding to a specific FoodEx1 group, because it includes special industrial products
Ad-hoc grp	Vegetable fats and oils	Group resulting from merging A.01.001362 Vegetable fat with A.01.001367 Vegetable oil
A.01.001378	Peanut oil	
Ad-hoc grp	Coconut oil/fat	Group resulting from merging A.01.001364 Coconut fat and A.01.001369 Coconut oil
A.01.001370	Maize oil	
A.01.001375	Olive oil	
A.01.001376	Palm kernel oil	
Ad-hoc grp	Palm oil/fat	Group resulting from merging A.01.001366 Palm fat and A.01.001377 Palm oil
A.01.001380	Rape seed oil	
A.01.001383	Soya bean oil	
A.01.001384	Sunflower seed oil	
A.01.001386	Walnut oil	
Ad-hoc grp	Cereal-based products and similar	
A.01.000184	Breakfast cereals	All the subgroups are represented excluded mixed breakfast cereals and grits
A.01.000185	Cereal flakes	
A.01.000210	Muesli	

Code[(a)]	Food group Name[(b)]	Note
A.01.000220	Cereal bars	
A.01.000225	Popped cereals	
A.01.000246	Porridge	
A.01.000252	Fine bakery wares	
A.01.000302	Cookies	
Ad-hoc grp	Fatty cake products	Ad-hoc part of A.01.000253 Pastries and cakes
Ad-hoc grp	Hot surface cooked pastries	Ad-hoc part of A.01.000253 Pastries and cakes
Ad-hoc grp	Puff pastry	Ad-hoc part of A.01.000253 Pastries and cakes
Ad-hoc grp	Shortcrusts	Ad-hoc part of A.01.000253 Pastries and cakes
Ad-hoc grp	Yeast leavened pastries	Ad-hoc part of A.01.000253 Pastries and cakes
A.01.000098	Bread and rolls	
A.01.000099	Wheat bread and rolls	
A.01.000118	Rye bread and rolls	
A.01.000129	Mixed wheat and rye bread and rolls	
A.01.000141	Multigrain bread	
A.01.000144	Unleavened bread, crispbread, rusk	
Ad-hoc grp	Fried, baked or roast meat or fish products	Ad-hoc group for meat and fish baked or fried or cooked at high temperature with fat
Ad-hoc grp	Fried or roast meat	Ad-hoc group including A.01.000728 Livestock meat, A.01.000736 Poultry, A.01.000744 Game mammals, A.01.000751 Game birds, A.01.000760 Mixed meat, A.01.000766 Edible offal, farmed animals and A.01.000791 Edible offal, game animals – when they are roast or fried or cooked at high temperature with fat
Ad-hoc grp	Fried or baked fish	Subgroup of the FoodEx1 group A.01.000877 Fish when it is baked or fried or cooked at high temperature with fat
A.01.001716	Infant formulae (powder)	
A.01.001717	Infant formula, milk-based, powder	
Ad-hoc grp	Smoked meat or fish products	
Ad-hoc grp	Smoked fish	Subgroup of the FoodEx1 group A.01.000877 Fish when it is smoked
Ad-hoc grp	Smoked meat products	Subgroup of the FoodEx1 group A.01.000795 Preserved meat or A.01.000811 Sausages when the products are smoked
Ad-hoc grp	Snacks and potato products	
Ad-hoc grp	Miscellaneous snack products	Subgroup of the FoodEx1 group A.01.001878 Snack food not including potato crisps and popcorns (listed under potato products and popped cereals, respectively)
Ad-hoc grp	Potato products	
A.01.000477	Potato croquettes	
A.01.000471	French fries	
A.01.001879	Potato crisps	
Ad-hoc grp	Oven baked and pan fried potato products (including home made products like pan-fried potato pieces or Roesti)	Group collecting A.01.000475 Potato fried and A.01.000476 Potato baked

(a): FoodEx1 code in the case that the food group corresponds to a FoodEx1 food group, 'ad-hoc' in the case of groups created for the purpose of this report.
(b): The names are provided in indented form to show the hierarchical relationships.

Table B.2: Dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes

Country	Survey acronym	Survey period	No. of days per subject	No. of subjects/No. of days						
				Infants	Toddlers	Other children	Adolescents (*mean age*)	Adults	Elderly	Very elderly
Austria	ASNS – Adults	2010–2012	2					308/726	67/181	25/85
Austria	ASNS – Children	2010–2012	3			128/384	237/706			
Belgium	Regional Flanders	2002–2002	3		36/108	625/1,875	–	–	–	–
Belgium	Diet National 2004	2004	2		–	–	576/1,187 *(16a)*	1,292/2,648	511/1,045	704/1,408
Bulgaria	NSFIN	2004	1				–/162	–/691	–/151	–/200
Bulgaria	NUTRICHILD	2007	2	861/1,720	428/856	433/867	–	–	–	–
Cyprus	Childhealth	2003	3		–	–	303/909 *(13a)*	–	–	–
Czech Republic	SISP04	2003–2004	2		–	389/778	298/596 *(13a)*	1,666/3,332	–	–
Denmark	DANSDA 2005–08	2005–2008	7		–	298/2,085	377/2,622 *(13a)*	1,739/12,127	274/1,916	12/84
Denmark	IAT 2006 07	2006–2007	7	826/5,771	917/6,388	–	–	–	–	–
Estonia	NDS 1997	1997	1					–/1,866	–	–
Finland	DIPP 2001 2009	2001–2009	3	500/1,500	500/1,500	750/2,250	–	–	–	–
Finland	NWSSP07 08	2007–2008	4		–	–	306/1,186 *(13a)*	–	–	–
Finland	FINDIET 2012	2012	2		–	–	–	1,295/2,590	413/826	–
France	INCA2	2007	7		–	482/3,315	973/6,728 *(14a)*	2,276/15,727	264/1,824	84/571
Germany	VELS	2001–2002	6	159/927	348/1,947	293/1,610	–	–	–	–
Germany	EsKiMo	2006	3		–	835/2,498	393/1,179 *(11a)*	–	–	–
Germany	National Nutrition Survey II	2007	2		–	–	1,011/2,022 *(16a)*	10,419/20,838	2,006/4,012	490/980

Country	Survey acronym	Survey period	No. of days per subject	No. of subjects/No. of days						
				Infants	Toddlers	Other children	Adolescents (*mean age*)	Adults	Elderly	Very elderly
Greece	Regional Crete	2004–2005	3			838/2,508	–	–	–	–
Greece	DIET LACTATION GR	2005–2007	3		–	–	–	65/350	–	–
Hungary	National Repr Surv	2003	3		–	–	–	1,074/3,222	206/618	80/240
Ireland	NANS 2012	2008–2010	4		–	–	–	1,274/5,096	149/596	77/308
Italy	INRAN SCAI 2005 06	2005–2006	3	16/48	36/108	193/579	247/741 *(14a)*	2,313/6,939	290/870	228/684
Latvia	EFSA TEST	2008	2			187/377	453/979 *(14a)*	1,271/2,655	–	–
Latvia	FC PREGNANT WOMEN 2011	2011	2		–	–	–	1,002/2,005	–	–
Netherlands	VCP kids	2006–2007	3		322/644	957/1,914	–	–	–	–
Netherlands	VCPBasis AVL2007 2010	2007–2010	2		–	447/894	1,142/2,284 *(14a)*	2,057/4,114	173/346	
Netherlands	VCP-Elderly	2010–2012	2		–	–	–	–	289/578	450/900
Poland	IZZ FAO 2000	2000	1		–/79	–/409	–/666 *(14a)*	–/2,527	–/329	–/124
Romania	Dieta Pilot Children	2012	1		–	–/205	–/567 *(14a)*	–	–	–
Romania	Dieta Pilot Adults	2012	7		–	–	–	1,254/8,770	83/581	45/315
Slovakia	SK MON 2008	2008	1		–	–	–	2,761	–	–
Slovenia	CRP 2008	2007–2008	1		–	–	–	407	–	–

Country	Survey acronym	Survey period	No. of days per subject	No. of subjects/No. of days						
				Infants	Toddlers	Other children	Adolescents (*mean age*)	Adults	Elderly	Very elderly
Spain	enKid	1998–2000	2		17/34	156/312	209/418 *(12a)*	–	–	–
Spain	AESAN	1999–2001	3		–	–	–	410/828	–	–
Spain	NUT INK05	2004–2005	2			399/798	651/1,302 *(14a)*	–	–	–
Spain	AESAN FIAB	2009	3		–	–	86/226 *(17a)*	981/2,748	–	–
Sweden	NFA	2003	4		–	1,473/5,875	1,018/4,047 *(12a)*	–	–	–
Sweden	Riksmaten 2010	2010–2011	4		–	–	–	1,430/5,680	295/1,167	72/288
United Kingdom	NDNS-Rolling Programme Years 1–3	2008–2011	4		185/737	651/2,595	666/2,653 *(14a)*	1,266/5,040	166/662	139/552
United Kingdom	DNSIYC 2011	2011	4	1,369/5,446	1,314/5,217	–	–	–	–	–

Table B.3: FoodEx1 food codes and corresponding food groups used in dietary exposure assessment of 3- and 2-MCPD and glycidol

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.000099	Wheat bread and rolls	Wheat bread and rolls	No additional calculation	Bread and bread rolls
A.01.000118	Rye bread and rolls	Rye bread and rolls	No additional calculation	Bread and bread rolls
A.01.000129	Mixed wheat and rye bread and rolls	Mixed wheat and rye bread and rolls	No additional calculation	Bread and bread rolls
A.01.000140	Multigrain bread and rolls	Multigrain bread and rolls	No additional calculation	Bread and bread rolls
A.01.000144	Unleavened bread, crisp bread and rusk	Unleavened bread, crispbread, rusk	No additional calculation	Bread and bread rolls
A.01.000154	Other bread	Bread and bread rolls	No additional calculation	Bread and bread rolls
A.01.000164	Bread products	Bread and bread rolls	No additional calculation	Bread and bread rolls
A.01.000225	Popped cereals	Popped cereals	No additional calculation	Breakfast cereals
A.01.000255	Buns	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.000256	Cake from batter	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000257	Cheese cream cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000258	Cheese cream sponge cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000259	Chocolate cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000260	Chocolate cake with fruits	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000261	Cream cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000262	Cream cheese cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000263	Cream custard cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000264	Cream custard sponge cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000265	Croissant	Puff pastry	No additional calculation	Pastries and cakes
A.01.000266	Croissant, filled with chocolate	Puff pastry	No additional calculation	Pastries and cakes
A.01.000267	Croissant, filled with cream	Puff pastry	No additional calculation	Pastries and cakes
A.01.000268	Croissant, filled with jam	Puff pastry	No additional calculation	Pastries and cakes
A.01.000270	Doughnuts	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.000272	Flan	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000273	Fruit cake	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000274	Fruit pie	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000275	Cheese pie	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000276	Fruit tart	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000277	Gingerbread	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000279	Kringles	Puff pastry	No additional calculation	Pastries and cakes
A.01.000280	Nut cream cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000281	Pancakes	Hot surface cooked pastries	No additional calculation	Pastries and cakes

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.000284	Rhubarb flan	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000285	Scone	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.000286	Sponge dough	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000287	Sponge cake	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000288	Sponge cake roll	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000289	Muffins	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000290	Waffles	Hot surface cooked pastries	No additional calculation	Pastries and cakes
A.01.000291	Apple strudel	Puff pastry	No additional calculation	Pastries and cakes
A.01.000292	Cream-cheese strudel	Puff pastry	No additional calculation	Pastries and cakes
A.01.000293	Cheese pastry goods from puff pastry	Puff pastry	No additional calculation	Pastries and cakes
A.01.000294	Croissant from puff pastry	Puff pastry	No additional calculation	Pastries and cakes
A.01.000295	Brioche	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.000297	Lebkuchen	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.000298	Dumpling	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000299	Cake marbled, with chocolate	Fatty cake products	No additional calculation	Pastries and cakes
A.01.000300	Marzipan pie	Shortcrusts	No additional calculation	Pastries and cakes
A.01.000301	Baklava	Puff pastry	No additional calculation	Pastries and cakes
A.01.000302	Biscuits (cookies)	Cookies	No additional calculation	Cookies
A.01.000471	French fries	French fries	No additional calculation	Fried or baked potato products
A.01.000475	Potato fried	Oven-baked potato products (include also homemade products like pan-fried potato pieces or Roesti)	No additional calculation	Fried or baked potato products
A.01.000476	Potato baked	Oven-baked potato products (include also homemade products like pan-fried potato pieces or Roesti)	No additional calculation	Fried or baked potato products
A.01.000477	Potato croquettes	Potato croquettes	No additional calculation	Fried or baked potato products
A.01.000728	Livestock meat	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000736	Poultry	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000744	Game mammals	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000751	Game birds	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000760	Mixed meat	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000766	Edible offal, farmed animals	Fried or roast meat	No additional calculation	Fried or roast meat

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.000791	Edible offal, game animals	Fried or roast meat	No additional calculation	Fried or roast meat
A.01.000795	Preserved meat	Smoked meat products	No additional calculation	Smoked meat products
A.01.000878	Herring (*Clupea*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000879	Sprat (*Sprattus sprattus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000880	Sardine and pilchard (*Sardina*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000881	Anchovy (*Engraulis*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000882	Shad (*Alosa*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000883	Salmon and trout (*Salmo* spp.)	Fried or baked fish	(a)	Fried or baked fish
A.01.000884	Char (*Salvelinus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000885	Smelt (*Osmerus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000886	Whitefish (*Coregonus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000887	Perch (*Perca*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000888	Bass (*Marone*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000889	Surgeonfish (*Acanthurus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000890	Mackeral (*Scomber*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000891	Tuna (*Thunnus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000892	Sea catfish and wolffish (*Anarhichas*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000893	Grey mullet (*Mugil*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000894	Cod and whiting (*Gadus* spp.)	Fried or baked fish	(a)	Fried or baked fish
A.01.000895	Hake (*Merluccius*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000896	Flounder (*Platichthys flesus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000897	Halibut (*Hippoglossus* spp.)	Fried or baked fish	(a)	Fried or baked fish
A.01.000898	Plaice (*Pleuronectes*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000899	Sole (*Limanda*; *Solea*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000900	Roach (*Rutilus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000901	Carp (*Cyprinus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000902	Barbel (*Barbus*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000903	Bream (*Charax*)	Fried or baked fish	(a)	Fried or baked fish
A.01.000904	Eels (Apodes)	Fried or baked fish	(a)	Fried or baked fish
A.01.000905	Zeomorphi (Zeomorphi)	Fried or baked fish	(a)	Fried or baked fish
A.01.000906	Lophiiformes (Pediculati)	Fried or baked fish	(a)	Fried or baked fish
A.01.000907	Selachoidei (Pleurotremata)	Fried or baked fish	(a)	Fried or baked fish
A.01.000908	Rays (Hypotremata)	Fried or baked fish	(a)	Fried or baked fish
A.01.000909	Acipenseriformes (sturgeons) (Chondrostei)	Fried or baked fish	(a)	Fried or baked fish

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.002100	Bonito (*Sarda sarda*)	Fried or baked fish	(a)	Fried or baked fish
A.01.002101	Bullet tuna (*Auxis* spp.)	Fried or baked fish	(a)	Fried or baked fish
A.01.002102	Sardinops (*Sardinops sagax*)	Fried or baked fish	(a)	Fried or baked fish
A.01.002103	Swordfish (Xiphiidae spp.)	Fried or baked fish	(a)	Fried or baked fish
A.01.000911	Fish balls	Fried or baked fish	(a)	Fried or baked fish
A.01.000912	Fishcakes	Fried or baked fish	(a)	Fried or baked fish
A.01.000913	Fish fingers	Fried or baked fish	(a)	Fried or baked fish
A.01.000919	Crustaceans	Fried or baked fish	(a)	Fried or baked fish
A.01.001295	Chocolate (Cocoa) products	Chocolate spreads and similar (22% palm oil)	Occurrence values used as calculated, but only in the spreads reported under this code	Chocolate spreads and similar
A.01.001299	Chocolate, cream	Chocolate spreads and similar (22% palm oil)	Occurrence values used as calculated	Chocolate spreads and similar
A.01.001364	Coconut fat	Coconut oil/fat	No additional calculation	Vegetable fats and oils
A.01.001366	Palm fat	Palm oil/fat	No additional calculation	Vegetable fats and oils
A.01.001368	Almond oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001369	Coconut oil	Coconut oil/fat	No additional calculation	Vegetable fats and oils
A.01.001370	Corn oil	Maize oil	No additional calculation	Vegetable fats and oils
A.01.001371	Cottonseed oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001372	Grape seed oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001373	Linseed oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001374	Oil, frying, blend	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001375	Olive oil	Olive oil	No additional calculation	Vegetable fats and oils
A.01.001376	Palm kernel oil	Palm kernel oil	No additional calculation	Vegetable fats and oils
A.01.001377	Palm oil	Palm oil/fat	No additional calculation	Vegetable fats and oils
A.01.001378	Peanut oil	Peanut oil	No additional calculation	Vegetable fats and oils
A.01.001379	Pumpkinseed oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.001380	Rapeseed oil	Rapeseed oil	No additional calculation	Vegetable fats and oils
A.01.001381	Safflower oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001382	Sesame oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001383	Soybean oil	Soya bean oil	No additional calculation	Vegetable fats and oils
A.01.001384	Sunflower oil	Sunflower seed oil	No additional calculation	Vegetable fats and oils
A.01.001385	Thistle oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001386	Walnut oil	Walnut oil	Occurrence value used as it is	Vegetable fats and oils
A.01.001387	Wheat germ oil	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001388	Fats of mixed origin	(b)	Occurrence values used without additional calculation	Vegetable fats and oils
A.01.001390	Margarine, normal fat	Margarine, normal fat	No additional calculation	Margarine and similar
A.01.001391	Margarine, low fat	Margarine, low fat	No additional calculation	Margarine and similar
A.01.001392	Margarine with other ingredients	Fat emulsions	No additional calculation	Margarine and similar
A.01.001393	Fat emulsions	Fat emulsions	No additional calculation	Margarine and similar
A.01.001625	Herb and spice mixtures	Herb and spice mixtures	No additional calculation	Herb and spice mixtures
A.01.001640	Stock cubes (bouillon cube)	Stock cubes (bouillon cube)	No additional calculation	Stock cubes (bouillon cube)
A.01.001641	Gravy thickener	Other seasoning products	No additional calculation	Other seasoning products
A.01.001642	Gravy browning	Other seasoning products	No additional calculation	Other seasoning products
A.01.001643	Gravy instant granules	Other seasoning products	No additional calculation	Other seasoning products
A.01.001644	Vegetable extracts	Other seasoning products	No additional calculation	Other seasoning products
A.01.001645	Meat extract	Other seasoning products	No additional calculation	Other seasoning products
A.01.001646	Malt extract	Other seasoning products	No additional calculation	Other seasoning products
A.01.001647	Yeast extract	Other seasoning products	No additional calculation	Other seasoning products

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.001666	Salad dressing, > 50% oil	(b)	Model combining occurrence in dressing with 50% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Dressing
A.01.001667	Salad dressing, 25–50% oil	(b)	Model combining occurrence in dressing with 35% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Dressing
A.01.001668	Salad dressing, < 25% oil	(b)	Model combining occurrence in dressing with 20% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Dressing
A.01.001669	Mayonnaise, > 50% oil	(b)	Model combining occurrence in dressing with 75% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Mayonnaise
A.01.001670	Mayonnaise, 25–50% oil	(b)	Model combining occurrence in dressing with 30% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Mayonnaise
A.01.001671	Mayonnaise, < 25 % oil	(b)	Model combining occurrence in dressing with 20% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Mayonnaise
A.01.001685	White sauce (Bechamel sauce, Cheese sauce)	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001686	Brown sauce (Gravy, Lyonnais sauce)	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001687	Cream sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001688	Butter sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.001689	Emulsion sauce (Hollandaise sauce)	(b)	Model combining occurrence in savoury sauces with 25% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Savoury sauces, oil-based
A.01.001690	Oil-based sauce	(b)	Model combining occurrence in savoury sauces with 25% occurrence value estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats)	Savoury sauces, oil-based
A.01.001691	Alcoholic sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001692	Meat sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001693	Fish sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001694	Vegetable sauce	Savoury sauces	No additional calculation	Savoury sauces, non-oil-based
A.01.001716	Infant formulae, powder	Infant formula, milk-based, powder	No additional calculation	Infant and follow-on formulae
A.01.002000	Infant formulae, liquid	Infant formula, milk-based, powder	Dividing by a factor of 7.7 (4.5 g of powder in 30 mL)	Infant and follow-on formulae
A.01.001722	Follow-on formulae, powder	Follow-on formula, milk-based, powder	No additional calculation	Infant and follow-on formulae
A.01.002010	Follow-on formulae, liquid	Follow-on formula, milk-based, powder	Dividing by a factor of 7.7 (4.5 g of powder in 30 mL)	Infant and follow-on formulae
A.01.001731	Biscuits, rusks and cookies for children	Cookies	No additional calculation	Cookies
A.01.001757	Protein and amino acids supplements	Protein and amino acids supplements	No additional calculation	Protein and amino acids supplements
A.01.001791	Sandwich and sandwich-like meal	Bread and bread rolls	No additional calculation	Bread and bread rolls
A.01.001800	Pizza and pizza-like pies	Bread and bread rolls	No additional calculation	Bread and bread rolls
A.01.001830	Meat burger	Fried or roasted meat	No additional calculation	Fried or roasted meat
A.01.001831	Meat balls	Fried or roasted meat	No additional calculation	Fried or roasted meat
A.01.001856	Ready-to-eat soups	Dry preparations for soups (to be reconstituted)	Dividing by a factor of 12 (18 grams of dry product in 200ml water)	Ready to eat soups
A.01.001879	Potato crisps	Potato crisps	No additional calculation	Fried or baked potato products

FoodEx1 code	Food group used for exposure	Occurrence values used	Calculation applied	Aggregated groups for presenting contribution to exposure
A.01.001880	Corn chips	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001881	Tortilla chips	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001882	Corn curls	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001883	Popcorn	Popped cereals	No additional calculation	Breakfast cereals
A.01.001884	Pretzels	Yeast leavened pastries	No additional calculation	Pastries and cakes
A.01.001885	Fish-based snacks	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001886	Seafood chips	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001887	Cheese puffs	Miscellaneous snack products	No additional calculation	Miscellaneous snack products
A.01.001660	Soy sauce	Soy sauce	No additional calculation	Soy sauce
A.01.001656	Barbecue sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001657	Tabasco sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001658	Horseradish sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001659	Mint sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001661	Curry sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001662	Salsa	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001663	Tartar sauce	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001664	Mixed condiment	Other condiment sauces	No additional calculation	Other condiment sauces
A.01.001757	Protein and amino acids supplements	Protein and amino acids supplements	No additional calculation	Protein and amino acids supplements
A.01.000185	Cereal flakes	Cereal flakes	No additional calculation	Breakfast cereals
A.01.000210	Muesli	Muesli	No additional calculation	Breakfast cereals
A.01.000220	Cereal bars	Cereal bars	No additional calculation	Breakfast cereals
A.01.000233	Mixed breakfast cereals	Muesli	No additional calculation	Breakfast cereals
A.01.000246	Porridge	Porridge	No additional calculation	Porridge
A.01.000811	Sausages	Smoked meat products	No additional calculation	Smoked meat products

(a): In fish and fish products, the FoodEx code does not record the treatment, therefore the occurrence value for fried fish was applied, because the occurrence levels in fried fish are either higher (more conservative choice) or similar to those in smoked fish. It is here assumed that fish are either fried or smoked. No occurrence data are available for potential combination of the two treatments.

(b): Occurrence estimated based on the available occurrence in oils (excluding olive oil and palm/coconut fats) and the 2011 oil consumption in the EU (27 Members States aggregated).

Table B.4: Occurrence values used for the FoodEx1 food groups used for the exposure assessment of 3-MCPD, 2-MCPD and glycidol, following the approach described in Table B.3

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.000099	Wheat bread and rolls	24	31	37	10	15	19	11	12	12
A.01.000118	Rye bread and rolls	1.91	8.45	14	0	4.64	9.29	0.08	0.35	0.63
A.01.000129	Mixed wheat and rye bread and rolls	5.2	11	17	1.45	6.06	10	0.55	0.79	1.04
A.01.000140	Multigrain bread and rolls	13	19	25	2.66	7.25	11	3.83	4.07	4.31
A.01.000144	Unleavened bread, crisp bread and rusk	95	101	108	44	49	54	27	28	28
A.01.000154	Other bread	23	29	36	9.81	14	19	7.77	8.03	8.3
A.01.000164	Bread products	23	29	36	9.81	14	19	7.77	8.03	8.3
A.01.000225	Popped cereals	23	29	35	12	17	22	14	15	16
A.01.000255	Buns	127	133	138	60	65	70	81	81	82
A.01.000256	Cake from batter	132	138	145	66	71	75	102	102	103
A.01.000257	Cheese-cream cake	132	138	145	66	71	75	102	102	103
A.01.000258	Cheese-cream sponge cake	132	138	145	66	71	75	102	102	103
A.01.000259	Chocolate cake	132	138	145	66	71	75	102	102	103
A.01.000260	Chocolate cake with fruits	132	138	145	66	71	75	102	102	103
A.01.000261	Cream cake	132	138	145	66	71	75	102	102	103
A.01.000262	Cream-cheese cake	132	138	145	66	71	75	102	102	103
A.01.000263	Cream-custard cake	132	138	145	66	71	75	102	102	103
A.01.000264	Cream-custard sponge cake	132	138	145	66	71	75	102	102	103
A.01.000265	Croissant	100	106	112	42	47	53	19	21	23
A.01.000266	Croissant, filled with chocolate	100	106	112	42	47	53	19	21	23
A.01.000267	Croissant, filled with cream	100	106	112	42	47	53	19	21	23
A.01.000268	Croissant, filled with jam	100	106	112	42	47	53	19	21	23
A.01.000270	Doughnuts	127	133	138	60	65	70	81	81	82
A.01.000272	Flan	148	154	160	75	79	84	148	149	149
A.01.000273	Fruit cake	148	154	160	75	79	84	148	149	149
A.01.000274	Fruit pie	148	154	160	75	79	84	148	149	149
A.01.000275	Cheese pie	148	154	160	75	79	84	148	149	149

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.000276	Fruit tart	148	154	160	75	79	84	148	149	149
A.01.000277	Gingerbread	132	138	145	66	71	75	102	102	103
A.01.000279	Kringles	100	106	112	42	47	53	19	21	23
A.01.000280	Nut cream cake	132	138	145	66	71	75	102	102	103
A.01.000281	Pancakes	242	247	253	118	123	128	136	137	138
A.01.000284	Rhubarb flan	148	154	160	75	79	84	148	149	149
A.01.000285	Scone	127	133	138	60	65	70	81	81	82
A.01.000286	Sponge dough	132	138	145	66	71	75	102	102	103
A.01.000287	Sponge cake	132	138	145	66	71	75	102	102	103
A.01.000288	Sponge cake roll	132	138	145	66	71	75	102	102	103
A.01.000289	Muffins	132	138	145	66	71	75	102	102	103
A.01.000290	Waffles	242	247	253	118	123	128	136	137	138
A.01.000291	Apple strudel	100	106	112	42	47	53	19	21	23
A.01.000292	Cream-cheese strudel	100	106	112	42	47	53	19	21	23
A.01.000293	Cheese pastry goods from puff pastry	100	106	112	42	47	53	19	21	23
A.01.000294	Croissant from puff pastry	100	106	112	42	47	53	19	21	23
A.01.000295	Brioche	127	133	138	60	65	70	81	81	82
A.01.000297	Lebkuchen	127	133	138	60	65	70	81	81	82
A.01.000298	Dumpling	132	138	145	66	71	75	102	102	103
A.01.000299	Cake marbled, with chocolate	132	138	145	66	71	75	102	102	103
A.01.000300	Marzipan pie	148	154	160	75	79	84	148	149	149
A.01.000301	Baklava	100	106	112	42	47	53	19	21	23
A.01.000302	Biscuits (cookies)	194	200	206	98	103	107	134	134	135
A.01.000471	French fries	51	57	63	19	23	28	40	41	41
A.01.000475	Potato fried	33	40	47	23	28	32	6.36	6.38	6.4
A.01.000476	Potato baked	33	40	47	23	28	32	6.36	6.38	6.4
A.01.000477	Potato croquettes	23	30	37	12	17	21	4.8	5	5.21
A.01.000728	Livestock meat	17	23	29	2.97	8.38	13	42	43	44
A.01.000736	Poultry	17	23	29	2.97	8.38	13	42	43	44

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.000744	Game mammals	17	23	29	2.97	8.38	13	42	43	44
A.01.000751	Game birds	17	23	29	2.97	8.38	13	42	43	44
A.01.000760	Mixed meat	17	23	29	2.97	8.38	13	42	43	44
A.01.000766	Edible offal, farmed animals	17	23	29	2.97	8.38	13	42	43	44
A.01.000791	Edible offal, game animals	17	23	29	2.97	8.38	13	42	43	44
A.01.000795	Preserved meat	17	24	30	0.21	6.24	12	24	27	30
A.01.000878	Herring (*Clupea*)	42	42	43	13	14	15	30	30	30
A.01.000879	Sprat (*Sprattus sprattus*)	42	42	43	13	14	15	30	30	30
A.01.000880	Sardine and pilchard (*Sardina*)	42	42	43	13	14	15	30	30	30
A.01.000881	Anchovy (*Engraulis*)	42	42	43	13	14	15	30	30	30
A.01.000882	Shad (*Alosa*)	42	42	43	13	14	15	30	30	30
A.01.000883	Salmon and trout (*Salmo* spp.)	42	42	43	13	14	15	30	30	30
A.01.000884	Char (*Salvelinus*)	42	42	43	13	14	15	30	30	30
A.01.000885	Smelt (*Osmerus*)	42	42	43	13	14	15	30	30	30
A.01.000886	Whitefish (*Coregonus*)	42	42	43	13	14	15	30	30	30
A.01.000887	Perch (*Perca*)	42	42	43	13	14	15	30	30	30
A.01.000888	Bass (*Marone*)	42	42	43	13	14	15	30	30	30
A.01.000889	Surgeonfish (*Acanthurus*)	42	42	43	13	14	15	30	30	30
A.01.000890	Mackeral (*Scomber*)	42	42	43	13	14	15	30	30	30
A.01.000891	Tuna (*Thunnus*)	42	42	43	13	14	15	30	30	30
A.01.000892	Sea catfish and wolffish (*Anarhichas*)	42	42	43	13	14	15	30	30	30
A.01.000893	Grey mullet (*Mugil*)	42	42	43	13	14	15	30	30	30
A.01.000894	Cod and whiting (*Gadus* spp.)	42	42	43	13	14	15	30	30	30
A.01.000895	Hake (*Merluccius*)	42	42	43	13	14	15	30	30	30
A.01.000896	Flounder (*Platichthys flesus*)	42	42	43	13	14	15	30	30	30
A.01.000897	Halibut (*Hippoglossus* spp.)	42	42	43	13	14	15	30	30	30
A.01.000898	Plaice (*Pleuronectes*)	42	42	43	13	14	15	30	30	30
A.01.000899	Sole (*Limanda*; *Solea*)	42	42	43	13	14	15	30	30	30
A.01.000900	Roach (*Rutilus*)	42	42	43	13	14	15	30	30	30
A.01.000901	Carp (*Cyprinus*)	42	42	43	13	14	15	30	30	30

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.000902	Babel (*Barbus*)	42	42	43	13	14	15	30	30	30
A.01.000903	Bream (*Charax*)	42	42	43	13	14	15	30	30	30
A.01.000904	Eels (Apodes)	42	42	43	13	14	15	30	30	30
A.01.000905	Zeomorphi (Zeomorphi)	42	42	43	13	14	15	30	30	30
A.01.000906	Lophiiformes (Pediculati)	42	42	43	13	14	15	30	30	30
A.01.000907	Selachoidei (Pleurotremata)	42	42	43	13	14	15	30	30	30
A.01.000908	Rays (Hypotremata)	42	42	43	13	14	15	30	30	30
A.01.000909	Acipenseriformes (sturgeons) (Chondrostei)	42	42	43	13	14	15	30	30	30
A.01.002100	Bonito (*Sarda sarda*)	42	42	43	13	14	15	30	30	30
A.01.002101	Bullet tuna (*Auxis* spp.)	42	42	43	13	14	15	30	30	30
A.01.002102	Sardinops (*Sardinops sagax*)	42	42	43	13	14	15	30	30	30
A.01.002103	Swordfish (Xiphiidae spp.)	42	42	43	13	14	15	30	30	30
A.01.000911	Fish balls	42	42	43	13	14	15	30	30	30
A.01.000912	Fishcakes	42	42	43	13	14	15	30	30	30
A.01.000913	Fish fingers	42	42	43	13	14	15	30	30	30
A.01.000919	Crustaceans	42	42	43	13	14	15	30	30	30
A.01.001295	Chocolate (Cocoa) products	641	641	641	344	344	345	870	870	870
A.01.001299	Chocolate, cream	641	641	641	344	344	345	870	870	870
A.01.001364	Coconut fat	608	608	608	143	169	194	472	476	479
A.01.001366	Palm fat	2,912	2,912	2,912	1,563	1,565	1,566	3,954	3,955	3,955
A.01.001368	Almond oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001369	Coconut oil	608	608	608	143	169	194	472	476	479
A.01.001370	Corn oil	502	503	505	233	233	233	647	650	654
A.01.001371	Cottonseed oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001372	Grape seed oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001373	Linseed oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001374	Oil, frying, blend	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001375	Olive oil	48	48	49	85	86	88	0	15	31
A.01.001376	Palm kernel oil	624	624	624	249	270	291	415	421	428
A.01.001377	Palm oil	2,912	2,912	2,912	1,563	1,565	1,566	3,954	3,955	3,955

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.001378	Peanut oil	229	229	229	90	102	115	133	148	162
A.01.001379	Pumpkinseed oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001380	Rapeseed oil	224	232	239	78	109	140	144	166	188
A.01.001381	Safflower oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001382	Sesame oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001383	Soybean oil	392	394	396	159	167	175	157	171	186
A.01.001384	Sunflower oil	517	521	524	207	218	229	259	269	279
A.01.001385	Thistle oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001386	Walnut oil	236	236	236	127	127	127	247	247	247
A.01.001387	Wheat germ oil	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001388	Fats of mixed origin	382.07	387.12	391.40	149.77	167.53	185.31	208.20	223.24	238.47
A.01.001390	Margarine, normal fat	667	668	669	224	236	248	580	582	584
A.01.001391	Margarine, low fat	215	218	220	101	104	107	204	209	213
A.01.001392	Margarine with other ingredients	181	181	181	77	80	84	114	114	114
A.01.001393	Fat emulsions	181	181	181	77	80	84	114	114	114
A.01.001625	Herb and spice mixtures	19	23	27	0	0	0	0	0	0
A.01.001640	Stock cubes (bouillon cube)	8.49	10	13	0	0	0	0	0	0
A.01.001641	Gravy thickener	11	14	17	0	0	0	0	0	0
A.01.001642	Gravy browning	11	14	17	0	0	0	0	0	0
A.01.001643	Gravy instant granules	11	14	17	0	0	0	0	0	0
A.01.001644	Vegetable extracts	11	14	17	0	0	0	0	0	0
A.01.001645	Meat extract	11	14	17	0	0	0	0	0	0
A.01.001646	Malt extract	11	14	17	0	0	0	0	0	0
A.01.001647	Yeast extract	11	14	17	0	0	0	0	0	0
A.01.001666	Salad dressing, > 50% oil	198.04	202.31	205.70	74.88	83.77	92.66	104.10	111.62	119.23
A.01.001667	Salad dressing, 25–50% oil	140.72	144.24	146.99	52.42	58.64	64.86	72.87	78.13	83.46
A.01.001668	Salad dressing, < 25% oil	83.41	86.17	88.28	29.95	33.51	37.06	41.64	44.65	47.69
A.01.001669	Mayonnaise, > 50% oil	293.55	299.09	303.55	112.33	125.65	138.98	156.15	167.43	178.85
A.01.001670	Mayonnaise, 25–50% oil	121.62	124.89	127.42	44.93	50.26	55.59	62.46	66.97	71.54
A.01.001671	Mayonnaise, < 25% oil	83.41	86.17	88.28	29.95	33.51	37.06	41.64	44.65	47.69

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.001685	White sauce (Bechamel sauce, Cheese sauce)	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001686	Brown sauce (Gravy, Lyonnais sauce)	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001687	Cream sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001688	Butter sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001689	Emulsion sauce (Hollandaise sauce)	96.78	102.13	107.29	37.44	41.88	46.33	52.05	55.81	59.62
A.01.001690	Oil-based sauce (Pesto, Aioli sauce)	96.78	102.13	107.29	37.44	41.88	46.33	52.05	55.81	59.62
A.01.001691	Alcoholic sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001692	Meat sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001693	Fish sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001694	Vegetable sauce	1.26	5.35	9.44	0	0	0	0	0	0
A.01.001716	Infant formulae, powder	108	108	109	31	44	58	80	87	94
A.01.002000	Infant formulae, liquid	14.03	14.03	14.16	4.03	5.71	7.53	10.39	11.30	12.21
A.01.001722	Follow-on formulae, powder	108	108	109	31	44	58	80	87	94
A.01.002010	Follow-on formulae, liquid	14.03	14.03	14.16	4.03	5.71	7.53	10.39	11.30	12.21
A.01.001731	Biscuits, rusks and cookies for children	194	200	206	98	103	107	134	134	135
A.01.001757	Protein and amino acids supplements	22	25	28	0	0	0	0	0	0
A.01.001791	Sandwich and sandwich-like meal	23	29	36	9.81	14	19	7.77	8.03	8.3
A.01.001800	Pizza and pizza-like pies	23	29	36	9.81	14	19	7.77	8.03	8.3
A.01.001830	Meat burger	17	23	29	2.97	8.38	13	42	43	44
A.01.001831	Meat balls	17	23	29	2.97	8.38	13	42	43	44
A.01.001856	Ready-to-eat soups	0.73	0.83	1	0	0	0	0	0	0
A.01.001879	Potato crisps	210	216	223	131	135	140	110	110	110
A.01.001880	Corn chips	112	119	126	62	67	71	12	15	17
A.01.001881	Tortilla chips	112	119	126	62	67	71	12	15	17
A.01.001882	Corn curls	112	119	126	62	67	71	12	15	17
A.01.001883	Popcorn	23	29	35	12	17	22	14	15	16
A.01.001884	Pretzels	127	133	138	60	65	70	81	81	82
A.01.001885	Fish-based snacks	112	119	126	62	67	71	12	15	17

FoodEx1 code	Food group used for exposure	3-MCPD (μg/kg)			2-MCPD (μg/kg)			Glycidol (μg/kg)		
		LB	MB	UB	LB	MB	UB	LB	MB	UB
A.01.001886	Seafood chips	112	119	126	62	67	71	12	15	17
A.01.001887	Cheese puffs	112	119	126	62	67	71	12	15	17
A.01.001660	Soy sauce	1.1	4.47	7.85	0	0	0	0	0	0
A.01.001656	Barbecue sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001657	Tabasco sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001658	Horseradish sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001659	Mint sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001661	Curry sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001662	Salsa	4.23	8.75	13	0	0	0	0	0	0
A.01.001663	Tartar sauce	4.23	8.75	13	0	0	0	0	0	0
A.01.001664	Mixed condiment	4.23	8.75	13	0	0	0	0	0	0
A.01.001757	Protein and amino acids supplements	22	25	28	0	0	0	0	0	0
A.01.000185	Cereal flakes	6.09	12	19	3.77	8.71	13	5.45	6.29	7.14
A.01.000210	Muesli	88	95	102	46	50	55	83	84	85
A.01.000220	Cereal bars	14	21	29	7.3	12	16	11	12	12
A.01.000233	Mixed breakfast cereals	88	95	102	46	50	55	83	84	85
A.01.000246	Porridge	0.6	8.8	17	0	5.94	11	0	2.98	5.97
A.01.000811	Sausages	17	24	30	0.21	6.24	12	24	27	30

3-MCPD: 3-monochloropropane-1,2-diol; 2-MCPD: 2-monochloropropane-1,3-diol; LB: lower bound; MB: middle bound; UB: upper bound.

Table B.5: Contribution of Food groups to MB mean exposure to 3-MCPD by population groups (represented as number of surveys where the % contribution of the specific food group falls into one of the predefined ranges of % contribution)

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
Infants	Bread and bread rolls	2	1	3					
	Breakfast cereals	3	2						
	Cookies	1	1	1	2	1			
	Fried or baked potato products	3	1						
	Fried or baked fish	3	3						
	Fried or roasted meat		6						
	Herb and spice mixtures	3							
	Infant and follow-on formulae							4	2
	Margarine and similar	3	1		1				
	Mayonnaise	4							
	Miscellaneous snack products	3							
	Other condiment sauces	3							
	Other seasoning products	4							
	Pastries and cakes	1	2		1				
	Porridge	1	1						
	Ready-to-eat soups	6							
	Savoury sauces, non-oil-based	2							
	Savoury sauces, oil-based	1							
	Smoked meat products	3	3						
	Soy sauce	4							
	Stock cubes (bouillon cube)	3							
	Vegetable fats and oils		1	1	2	1	1		
Toddlers	Bread and bread rolls	1		1	8				
	Breakfast cereals	3	6	1					
	Chocolate spreads and similar	1	1		1				
	Cookies		3		4	1	1	1	
	Dressing	2	1						
	Fried or baked potato products	2	3	3	2				
	Fried or baked fish	1	7		2				
	Fried or roasted meat		5	4	1				
	Herb and spice mixtures	6							
	Infant and follow-on formulae		5	2		1	1		

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Margarine and similar	2	1	1	3		1		
	Mayonnaise	8							
	Miscellaneous snack products	6	1						
	Other condiment sauces	5							
	Other seasoning products	7							
	Pastries and cakes	1		2	2	3	1		
	Porridge	4							
	Protein and amino acids supplements	1							
	Ready-to-eat soups	9							
	Savoury sauces, non-oil-based	6							
	Savoury sauces, oil-based	5							
	Smoked meat products		9	1					
	Soy sauce	7							
	Stock cubes (bouillon cube)	7							
	Vegetable fats and oils	1	2	1	3	1	2		
Other children	Bread and bread rolls	1		4	13				
	Breakfast cereals	7	11						
	Chocolate spreads and similar	1		2	2	1			
	Cookies	1	2	4	5	4	2		
	Dressing	4	3						
	Fried or baked potato products	1	7	5	4	1			
	Fried or baked fish	4	11	3					
	Fried or roasted meat		5	11	2				
	Herb and spice mixtures	10							
	Margarine and similar	3	3	3	4	2	1	1	
	Mayonnaise	12	4						
	Miscellaneous snack products	12	1						
	Other condiment sauces	11							
	Other seasoning products	9							
	Pastries and cakes	1			6	4	5	1	
	Porridge	7							
	Protein and amino acids supplements	2							
	Ready-to-eat soups	15							

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Savoury sauces, non-oil-based	13							
	Savoury sauces, oil-based	9							
	Smoked meat products	1	13	4					
	Soy sauce	13							
	Stock cubes (bouillon cube)	12							
	Vegetable fats and oils	3	4	3	4	2	1		
Adolescents	Bread and bread rolls	1		2	13	1			
	Breakfast cereals	5	12						
	Chocolate spreads and similar		1	1	2	1			
	Cookies	1	3	4	7	2			
	Dressing	6	1						
	Fried or baked potato products		3	9	3	1	1		
	Fried or baked fish	4	9	4					
	Fried or roasted meat		2	9	6				
	Herb and spice mixtures	9							
	Margarine and similar	2	3	4	3	2		2	
	Mayonnaise	7	8		1				
	Miscellaneous snack products	11	1						
	Other condiment sauces	11							
	Other seasoning products	7							
	Pastries and cakes	1			7	4	4		
	Porridge	5							
	Protein and amino acids supplements	1							
	Ready-to-eat soups	15							
	Savoury sauces, non-oil-based	13							
	Savoury sauces, oil-based	9							
	Smoked meat products		11	6					
	Soy sauce	13							
	Stock cubes (bouillon cube)	11							
	Vegetable fats and oils	2	3	5	5	1			
Adults	Bread and bread rolls			1	14	2			
	Breakfast cereals	6	11						
	Chocolate spreads and similar	5	3						

Population group	Food groups	Number of dietary surveys with % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Cookies		5	6	6				
	Dressing	5	2						
	Fried or baked potato products	2	7	4	4				
	Fried or baked fish	1	12	2	2				
	Fried or roasted meat			11	6				
	Herb and spice mixtures	11							
	Margarine and similar	2	3	2	6	2	1	1	
	Mayonnaise	7	7		1				
	Miscellaneous snack products	14							
	Other condiment sauces	14							
	Other seasoning products	7							
	Pastries and cakes	1	1	1	5	5	2	1	
	Porridge	8	2						
	Protein and amino acids supplements	5							
	Ready-to-eat soups	16							
	Savoury sauces, non-oil-based	13							
	Savoury sauces, oil-based	10	1						
	Smoked meat products		11	6					
	Soy sauce	17							
	Stock cubes (bouillon cube)	14							
	Vegetable fats and oils	1	3	4	6			2	
Elderly	Bread and bread rolls			1	12		1		
	Breakfast cereals	6	8						
	Chocolate spreads and similar	5							
	Cookies		7	5	2				
	Dressing	6		1					
	Fried or baked potato products	2	8	2	1				
	Fried or baked fish	2	8	3	1				
	Fried or roasted meat			13	1				
	Herb and spice mixtures	11							
	Margarine and similar	1	1	2	4	2	2	2	
	Mayonnaise	10	2						
	Miscellaneous snack products	7							
	Other condiment sauces	11							

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Other seasoning products	8							
	Pastries and cakes	1	1	1	3	4	2	1	
	Porridge	7	3						
	Protein and amino acids supplements	2							
	Ready-to-eat soups	13							
	Savoury sauces, non-oil-based	10							
	Savoury sauces, oil-based	10							
	Smoked meat products		13	1					
	Soy sauce	11							
	Stock cubes (bouillon cube)	8							
	Vegetable fats and oils		5	1	6			2	
Very elderly	Bread and bread rolls			1	10		1		
	Breakfast cereals	6	6						
	Chocolate spreads and similar	1							
	Cookies		4	5	3				
	Dressing	2	2						
	Fried or baked potato products	2	8	1					
	Fried or baked fish	2	6	4					
	Fried or roasted meat		1	10	1				
	Herb and spice mixtures	6							
	Margarine and similar	1		1	3	4		2	
	Mayonnaise	8	1						
	Miscellaneous snack products	2							
	Other condiment sauces	8							
	Other seasoning products	5							
	Pastries and cakes	2		1	3	2	1	2	
	Porridge	5	1						
	Protein and amino acids supplements	1							
	Ready-to-eat soups	11							
	Savoury sauces, non-oil-based	8							
	Savoury sauces, oil-based	7	1						
	Smoked meat products		11	1					
	Soy sauce	9							

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Stock cubes (bouillon cube)	6							
	Vegetable fats and oils		4	1	5			2	

3-MCPD: 3-monochloropropane-1,2-diol; MB: middle bound.

Table B.6: Contribution of food groups to MB mean exposure to 2-MCPD by population groups. The contribution is represented as number of surveys where the % contribution of the specific food group falls into one of the pre-defined ranges of % contribution

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
Infants	Bread and bread rolls	2	1	3					
	Breakfast cereals	1	4						
	Cookies	1	1		3	1			
	Fried or baked potato products	3	1						
	Fried or baked fish	3	3						
	Fried or roasted meat		6						
	Infant and follow-on formulae							5	1
	Margarine and similar	3	1		1				
	Mayonnaise	4							
	Miscellaneous snack products	2	1						
	Pastries and cakes	1	2		1				
	Porridge	1	1						
	Savoury sauces, oil-based	1							
	Smoked meat products	6							
	Vegetable fats and oils		1		2	2	1		
Toddlers	Bread and bread rolls	1		1	7	1			
	Breakfast cereals	3	5	2					
	Chocolate spreads and similar	1	1		1				
	Cookies		2	1	4	1	2		
	Dressing	2	1						
	Fried or baked potato products	2	3	3	2				
	Fried or baked fish	3	5	2					
	Fried or roasted meat		6	4					
	Infant and follow-on formulae		6	1		1	1		
	Margarine and similar	2	2	3		1			
	Mayonnaise	8							
	Miscellaneous snack products	5	2						

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Pastries and cakes	1		2	2	3		1	
	Porridge	4							
	Savoury sauces, oil-based	5							
	Smoked meat products	1	9						
	Vegetable fats and oils	1	2		2	3	2		
Other children	Bread and bread rolls	1		4	13				
	Breakfast cereals	6	8	4					
	Chocolate spreads and similar	1		1	3	1			
	Cookies	1	2	4	5	4	2		
	Dressing	5	2						
	Fried or baked potato products	1	3	8	5	1			
	Fried or baked fish	5	13						
	Fried or roasted meat		9	9					
	Margarine and similar	3	4	5	1	2	1	1	
	Mayonnaise	12	4						
	Miscellaneous snack products	12	1						
	Pastries and cakes	1			5	5	4	2	
	Porridge	7							
	Savoury sauces, oil-based	9							
	Smoked meat products	1	17						
	Vegetable fats and oils	3	3	3	2	5	1		
Adolescents	Bread and bread rolls	1		1	14	1			
	Breakfast cereals	4	12	1					
	Chocolate spreads and similar		1		3	1			
	Cookies	1	3	4	7	1	1		
	Dressing	6	1						
	Fried or baked potato products		2	9	3	2	1		
	Fried or baked fish	4	13						
	Fried or roasted meat		5	12					
	Margarine and similar	2	5	3	3	1	1	1	
	Mayonnaise	8	7	1					
	Miscellaneous snack products	10	2						
	Pastries and cakes	1			7	3	5		
	Porridge	5							
	Savoury sauces, oil-based	9							
	Smoked meat products		17						
	Vegetable fats and oils	2	3	3	3	4	1		
Adults	Bread and bread rolls				15	2			
	Breakfast cereals	5	12						

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Chocolate spreads and similar	4	4						
	Cookies		3	7	7				
	Dressing	5	2						
	Fried or baked potato products	2	5	5	5				
	Fried or baked fish	4	10	3					
	Fried or roasted meat			16	1				
	Margarine and similar	2	3	6	3	2	1		
	Mayonnaise	7	7	1					
	Miscellaneous snack products	14							
	Pastries and cakes	1	1	1	5	3	4	1	
	Porridge	8	2						
	Savoury sauces, oil-based	10	1						
	Smoked meat products		17						
	Vegetable fats and oils	1	3	1	6	2	1	2	
Elderly	Bread and bread rolls				12	2			
	Breakfast cereals	5	9						
	Chocolate spreads and similar	5							
	Cookies		6	5	3				
	Dressing	6		1					
	Fried or baked potato products	2	5	5	1				
	Fried or baked fish	2	10	2					
	Fried or roasted meat		3	11					
	Margarine and similar	2		2	4	3	2	1	
	Mayonnaise	10	2						
	Miscellaneous snack products	7							
	Pastries and cakes	1	1	1	1	6	1	2	
	Porridge	6	3	1					
	Savoury sauces, oil-based	10							
	Smoked meat products		14						
	Vegetable fats and oils		5		6		1	2	
Very elderly	Bread and bread rolls			1	9	1	1		
	Breakfast cereals	5	7						
	Chocolate spreads and similar	1							
	Cookies		4	4	4				
	Dressing	3	1						
	Fried or baked potato products	2	7	2					
	Fried or baked fish	2	9	1					
	Fried or roasted meat		3	9					

Population group	Food groups	Number of dietary surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Margarine and similar	1		1	6	1	1	1	
	Mayonnaise	8	1						
	Miscellaneous snack products	2							
	Pastries and cakes	1	1	1	1	4		3	
	Porridge	3	3						
	Savoury sauces, oil-based	7	1						
	Smoked meat products		12						
	Vegetable fats and oils		4		5		1	2	

2-MCPD: 2-monochloropropane-1,3-diol; MB: middle bound.

Table B.7: Contribution of Food groups to MB mean exposure to glycidol by population groups (represented as number of surveys where the % contribution of the specific food group falls into one of the pre-defined ranges of % contribution)

Population group	Food groups	Number of surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
Infants	Bread and bread rolls	2	4						
	Breakfast cereals	3	2						
	Cookies	1	1	1	2	1			
	Fried or baked potato products	4							
	Fried or baked fish	3	3						
	Fried or roasted meat			5	1				
	Infant and follow-on formulae							3	3
	Margarine and similar	3		1	1				
	Mayonnaise	4							
	Miscellaneous snack products	2	1						
	Pastries and cakes	1	2		1				
	Porridge	2							
	Savoury sauces, oil-based	1							
	Smoked meat products	3	3						
	Vegetable fats and oils		2	1	1	1		1	
Toddlers	Bread and bread rolls	1	1	8					
	Breakfast cereals	3	6	1					
	Chocolate spreads and similar	1		1	1				
	Cookies		3		5		2		
	Dressing	2	1						
	Fried or baked potato products	3	3	3	1				
	Fried or baked fish	1	6	1	2				
	Fried or roasted meat			2	5	2	1		
	Infant and follow-on formulae		5	2		2			
	Margarine and similar	2	1	1	3			1	
	Mayonnaise	8							
	Miscellaneous snack products	7							

Population group	Food groups	Number of surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Pastries and cakes	1		2	4	1	1		
	Porridge	4							
	Savoury sauces, oil-based	5							
	Smoked meat products		5	4	1				
	Vegetable fats and oils	1	3	1	2	2	1		
Other children	Bread and bread rolls	1	8	9					
	Breakfast cereals	8	9	1					
	Chocolate spreads and similar	1			2	2	1		
	Cookies	1	3	3	7	3	1		
	Dressing	5	2						
	Fried or baked potato products	1	10	4	3				
	Fried or baked fish	4	11	3					
	Fried or roasted meat			4	8	6			
	Margarine and similar	3	3	3	4	1	1	2	
	Mayonnaise	13	3						
	Miscellaneous snack products	13							
	Pastries and cakes	1		1	6	6	2	1	
	Porridge	7							
	Savoury sauces, oil-based	9							
	Smoked meat products		7	11					
	Vegetable fats and oils	3	7	2	4	1			
Adolescents	Bread and bread rolls	1	3	11	2				
	Breakfast cereals	5	12						
	Chocolate spreads and similar		1		1	2	1		
	Cookies	1	4	4	7	1			
	Dressing	6	1						
	Fried or baked potato products		11	1	4	1			
	Fried or baked fish	4	9	4					
	Fried or roasted meat				9	6	2		

Population group	Food groups	Number of surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Margarine and similar	2	3	2	5	1	1	2	
	Mayonnaise	10	5	1					
	Miscellaneous snack products	12							
	Pastries and cakes	1		2	5	6	2		
	Porridge	5							
	Savoury sauces, oil-based	9							
	Smoked meat products		6	11					
	Vegetable fats and oils	3	7	2	4				
Adults	Bread and bread rolls		3	12	2				
	Breakfast cereals	6	11						
	Chocolate spreads and similar	4	2	2					
	Cookies		6	8	3				
	Dressing	6	1						
	Fried or baked potato products	2	9	5	1				
	Fried or baked fish	1	12	4					
	Fried or roasted meat				4	11	2		
	Margarine and similar	2	1	3	6	2	2	1	
	Mayonnaise	7	7	1					
	Miscellaneous snack products	14							
	Pastries and cakes	1	1	1	7	4	1	1	
	Porridge	10							
	Savoury sauces, oil-based	10	1						
	Smoked meat products		4	12	1				
	Vegetable fats and oils	2	3	5	4		1	1	
Elderly	Bread and bread rolls		4	9	1				
	Breakfast cereals	5	9						
	Chocolate spreads and similar	4	1						
	Cookies	1	7	4	2				
	Dressing	6	1						

Population group	Food groups	Number of surveys With % contribution to the MB mean chronic dietary exposure in the ranges:							
		< 1%	1–5%	5–10%	10–20%	20–30%	30–40%	40–60%	> 60%
	Fried or baked potato products	5	7	1					
	Fried or baked fish	2	9	2	1				
	Fried or roasted meat				7	6	1		
	Margarine and similar	1	1		4	2	3	3	
	Mayonnaise	11	1						
	Miscellaneous snack products	7							
	Pastries and cakes	1	1	1	4	3	2	1	
	Porridge	9	1						
	Savoury sauces, oil-based	10							
	Smoked meat products		7	7					
	Vegetable fats and oils		5	3	4	1		1	
Very elderly	Bread and bread rolls		4	7	1				
	Breakfast cereals	7	5						
	Chocolate spreads and similar		1						
	Cookies	1	4	5	2				
	Dressing	3	1						
	Fried or baked potato products	6	4	1					
	Fried or baked fish	2	6	4					
	Fried or roasted meat				9	2	1		
	Margarine and similar	1			3	4	1	2	
	Mayonnaise	8	1						
	Miscellaneous snack products	2							
	Pastries and cakes	2		1	2	2	2	2	
	Porridge	5	1						
	Savoury sauces, oil-based	7	1						
	Smoked meat products		8	4					
	Vegetable fats and oils	1	3	5	1		1	1	

MB: middle bound.

Table B.8: Margins of exposure (MOEs) calculated for glycidol exposure; the table presents the MoEs for median, minimum and maximum exposure across dietary surveys for both, average and P95 of exposure

	MoE based on exposure estimates (median (min–max)) across dietary surveys[(a)]		
	LB	**MB**	**UB**
	Mean exposure		
Infants	14,600 (34,000–14,600)	14,600 (25,500–12,800)	14,600 (25,500–12,800)
Toddlers	17,000 (25,500–11,300)	17,000 (25,500–11,300)	17,000 (20,400–11,300)
Other children	20,400 (34,000–11,300)	17,000 (34,000–11,300)	17,000 (34,000–10,200)
Adolescents	34,000 (51,000–20,400)	34,000 (51,000–20,400)	34,000 (51,000–20,400)
Adults	51,000 (102,000–34,000)	51,000 (51,000–34,000)	51,000 (51,000–34,000)
Elderly	51,000 (102,000–34,000)	51,000 (102,000–34,000)	34,000 (102,000–340,00)
Very elderly	51,000 (102,000–34,000)	51,000 (102,000–34,000)	34,000 (102,000–34,000)
	P95 of exposure		
Infants	7,800 (8,500–4,900)	7,800 (8,500–4,900)	6,800 (7,800–4,600)
Toddlers	9,300 (10,200–5,100)	9,300 (10,200–5,100)	8,500 (10,200–4,900)
Other children	9,300 (12,800–6,000)	9,300 (12,800–6,000)	9,300 (12,800–6,000)
Adolescents	17,000 (25,500–9,300)	17,000 (25,500–9,300)	14,600 (25,500–9,300)
Adults	20,400 (34,000–17,000)	20,400 (34,000–17,000)	20,400 (34,000–14,600)
Elderly	20,400 (51,000–17,000)	20,400 (51,000–17,000)	20,400 (34,000–17,000)
Very elderly	20,400 (51,000–14,600)	20,400 (51,000–14,600)	20,400 (51,000–12,800)

bw: body weight; LB: lower-bound; MB: middle bound; UB: upper-bound.
(a): The values corresponding to LB, MB and UB occurrence are shown.

Appendix C – Benchmark dose modelling of data from Sunahara et al. (1993)

BMD modelling of the results of the 2-year drinking water study of Sunahara et al. (1993).

The dose–response analysis was performed on the results of the long-term toxicity study performed by Sunahara et al. (1993). In this study, Fisher 344 rats (50 animals/sex per treatment group) were exposed to 3-monochloropropane-1,2-diol (3-MCPD) at nominal concentrations of 0, 20, 50 or 100 mg/L in drinking water (see Section 3.4.2.3). Average daily doses of 0, 1.1, 5.2 or 28 mg/kg body weight (bw) per day, and 0, 1.4, 7.0 or 35 mg/kg bw per day were calculated for male and female rats, respectively, by the study authors. A background concentration of 2.7 mg/L 3-MCPD was detected in drinking water used in the study. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) calculated the intake in controls as 0.11 mg/kg bw in males and 0.14 mg/kg bw in females, and used these corrected values for the dose-response analysis.

The relevant results of the study were initially screened by visual analysis for the presence of monotonic dose–response trends; the presence of a dose–response trend in the subset of results selected in the screening phase was subsequently confirmed by applying the Cochran–Armitage trend test (Haseman, 1984). The results with $p < 0.05$ in the aforementioned trend test were selected for benchmark dose (BMD) analysis, which was performed by means of the software BMDS v2.6.0.86 (US EPA). The following effects were subjected to the BMD analysis:

- Tubular hyperplasia (both sexes)
- Nephropathy (both sexes)

For the aforementioned effects, all models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10% (95% confidence level) advised by the EFSA guidance on the use of BMD (EFSA, 2009a). The models allowing for restrictions (Gamma, Log Logistic, Logprobit, Multistage and Weibull) were run both with and without the selected default restrictions. $BMDL_{10}$ were calculated separately for male and female rats. Acceptability of a model was assessed using the log-likelihood value associated with the fitted model (when tested vs the full model). In accordance with the Scientific Opinion of the EFSA (EFSA, 2009a) a goodness-of-fit was judged as sufficient if the fit showed a p-value not smaller than 0.05 (i.e. $p \geq 0.05$), using the likelihood ratio test. The lowest $BMDL_{10}$ calculated for each effect are reported in Table B.1.

Table C.1: Summary table of the lowest BMD and $BMDL_{10}$ calculated for different effects observed in the Sunahara et al. (1993) study

Effect	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day	Model	Calculation details
Tubular hyperplasia (male)	1.2	0.22	Gamma (unrestricted)	B1
Tubular hyperplasia (female)	0.83	0.29	Gamma (unrestricted)	B2
Nephropathy (male)	0.33	0.10	Loglogistic (restricted)	B3
Nephropathy (female)	1.1	0.30	Loglogistic (unrestricted)	B4

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight.

Table C.2: Male kidney hyperplasia

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0.11	50	3
1.1	50	6
5.2	50	15
28.3	50	34

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	120.43	1	< 0.001	NA		
Gamma	No	91.62	3	0.79	Yes	1.2	0.22
Logistic	NA	94.54	2	0.052	Yes	6.4	5.2
LogLogistic	No	91.58	3	0.97	Yes	1.6	0.42
LogProbit	No	91.60	3	0.85	Yes	1.8	0.58
Multistage	No	91.58	3	0.93	Yes	1.7	1.0
Multistage-Cancer	NA	92.11	2	0.59	Yes	2.6	1.9
Probit	NA	94.32	2	0.064	Yes	6.0	5.0
Weibull	No	92.11	2	0.59	Yes	2.6	1.9
Quantal-Linear	NA	92.11	2	0.59	Yes	2.6	1.9
Gamma	Yes	92.11	2	0.59	Yes	2.6	1.9
LogLogistic	Yes	91.58	3	0.97	Yes	1.6	1.1
LogProbit	Yes	93.59	2	0.13	No	4.6	3.4
Weibull	Yes	92.11	2	0.59	Yes	2.6	1.9
Multistage	Yes	92.11	2	0.59	Yes	2.6	1.9
Full	NA	91.58	4	NA	NA		

bw: body weight; BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

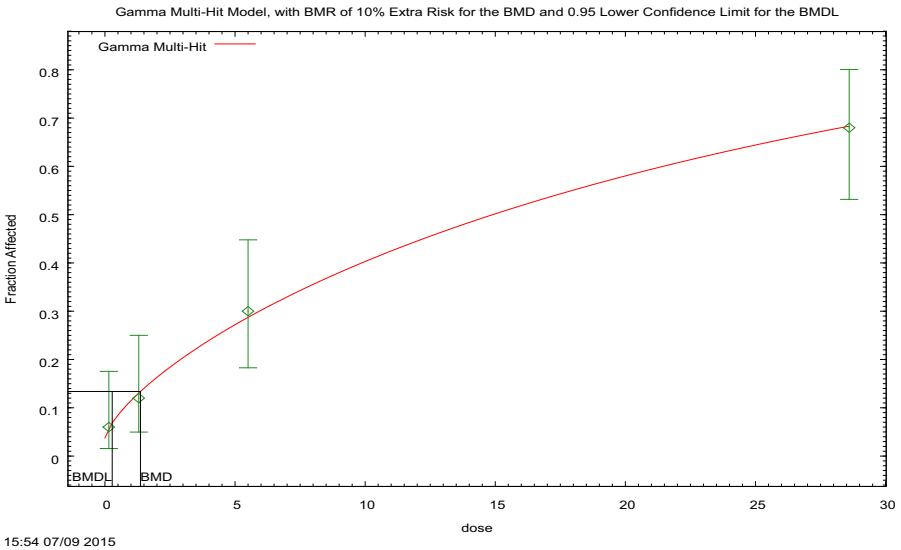


Table C.3: Female kidney hyperplasia

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0.14	50	2
1.4	50	4
7.0	50	20
35.3	50	31

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	119.52	1	< 0.001	NA		
Gamma	No	90.92	2	0.17	Yes	0.83	0.29
Logistic	NA	97.97	2	< 0.001	No	8.5	6.9
LogLogistic	No	90.34	3	0.13	Yes	1.2	0.41
LogProbit	No	90.19	3	0.16	Yes	1.6	0.47
Multistage	No	89.64	3	0.34	Yes	1.5	1.0
Multistage-Cancer	NA	93.01	2	0.022	No	3.2	2.5
Probit	NA	97.61	2	< 0.001	No	7.9	6.5
Weibull	No	93.01	2	0.022	No	3.2	2.5
Quantal-Linear	NA	93.01	2	0.022	No	3.2	2.5
Gamma	Yes	93.01	2	0.022	No	3.2	2.5
LogLogistic	Yes	90.67	2	0.23	Yes	1.9	1.3
LogProbit	Yes	95.80	2	0.014	No	5.3	3.9
Weibull	Yes	93.01	2	0.022	No	3.2	2.5
Multistage	Yes	93.01	2	0.022	No	3.2	2.5
Full	NA	89.19	4	NA	NA		

bw: body weight; BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

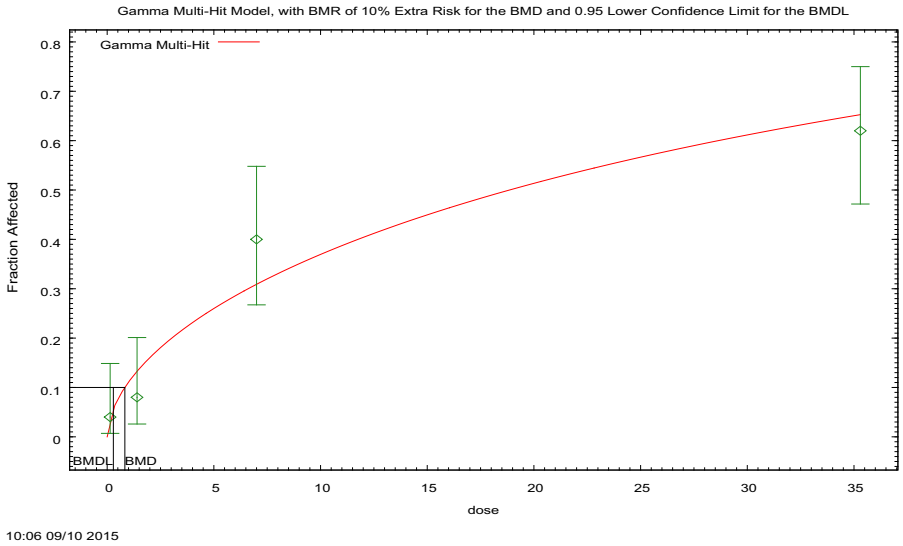


Table C.4: Male nephropathy

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0.11	50	36
1.1	50	40
5.2	50	45
28.3	50	49

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	84.54	1	< 0.001	NA		
Gamma	No	NC	NA	NC	NA	NC	NC
Logistic	NA	76.71	2	0.41	Yes	1.2	0.67
LogLogistic	No	75.84	3	0.85	Yes	0.34	< 0.001[(a)]
LogProbit	No	75.83	3	0.93	Yes	0.34	< 0.001[(a)]
Multistage	No	75.88	3	0.73	Yes	0.47	0.24
Multistage-Cancer	NA	76.54	2	0.49	Yes	0.98	0.54
Probit	NA	76.95	2	0.32	Yes	1.5	0.94
Weibull	No	NC	NA	NC	NA	NC	NC
Quantal-Linear	NA	76.54	2	0.49	Yes	0.98	0.54
Gamma	Yes	NC	NA	NC	NA	NC	NC
LogLogistic	Yes	75.84	3	0.85	Yes	0.33	0.10
LogProbit	Yes	76.38	2	0.57	Yes	1.4	0.68
Weibull	Yes	NC	NA	NC	NA	NC	NC
Multistage	Yes	76.54	2	0.49	Yes	0.98	0.54
Full	NA	75.82	4	NA	NA		

bw: body weight; BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); NA: not applicable; NC: not calculated
(a): Ratio BMD/BMDL higher than one order of magnitude.

Plot (model resulting in the lowest $BMDL_{10}$)

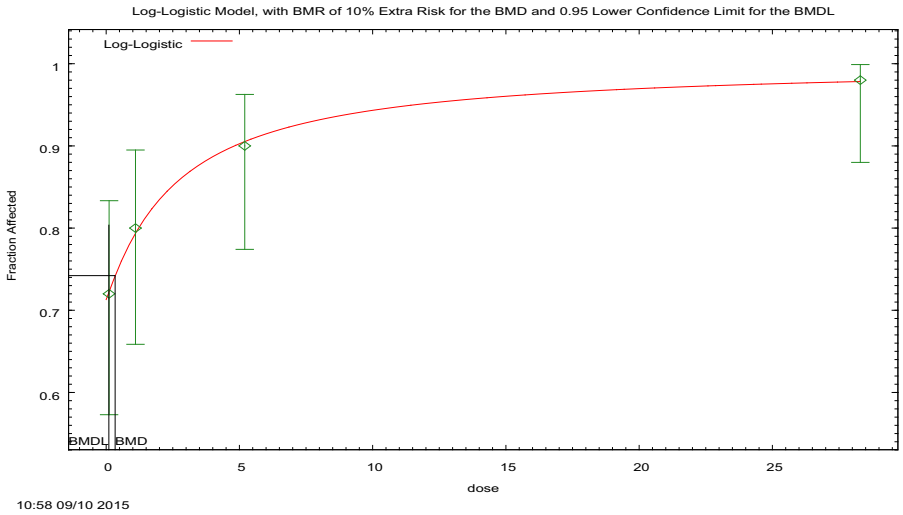


Table C.5: Female nephropathy

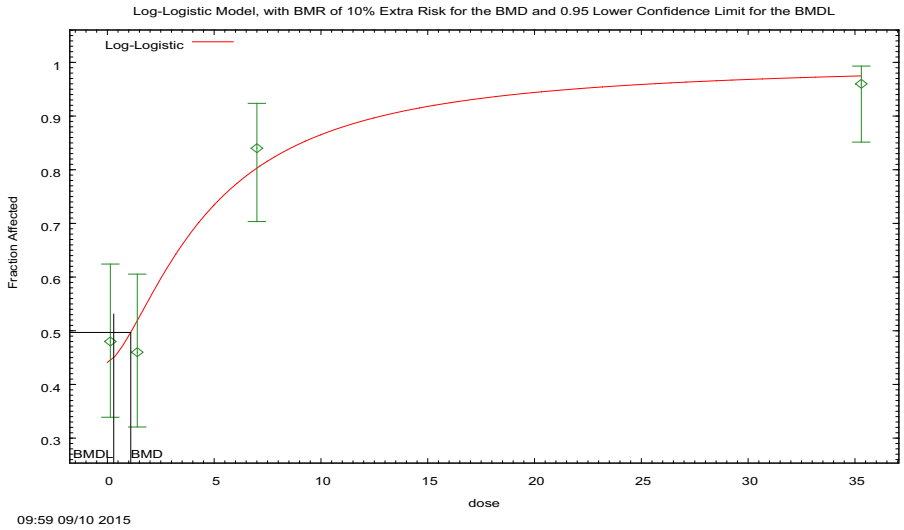
Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0.14	50	24
1.4	50	23
7.0	50	42
35.3	50	48

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	124.61	1	< 0.001	NA		
Gamma	No	NC	NA	NC	NA	NC	NC
Logistic	NA	103.69	2	0.015	No	1.7	1.2
LogLogistic	No	100.39	3	0.18	Yes	1.1	0.30
LogProbit	No	100.62	3	0.13	Yes	1.1	0.30
Multistage	No	100.60	3	0.14	Yes	0.58	0.37
Multistage-Cancer	NA	102.32	2	0.059	Yes	1.1	0.73
Probit	NA	104.52	2	0.0066	No	2.1	1.6
Weibull	No	102.32	2	0.059	Yes	1.1	0.73
Quantal-Linear	NA	102.32	2	0.059	Yes	1.1	0.73
Gamma	Yes	102.32	2	0.059	Yes	1.1	0.73
LogLogistic	Yes	100.39	3	0.18	Yes	1.1	0.30
LogProbit	Yes	100.86	2	0.25	Yes	1.5	1.0
Weibull	Yes	102.32	2	0.060	Yes	1.1	0.73
Multistage	Yes	102.32	2	0.060	Yes	1.1	0.73
Full	NA	99.50	4	NA	NA		

bw: body weight; BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); NA: not applicable; NC: not calculated.

Plot (model resulting in the lowest $BMDL_{10}$)



Appendix D – Benchmark dose modelling of data from Cho et al. (2008)

BMD modelling of the results of the 2-year drinking water study of Cho et al. (2008).

The dose–response analysis was performed on the results of the long-term toxicity study performed by Cho et al. (2008). In this study, Sprague-Dawley (SD) rats (50 animals/sex per treatment group) were exposed to 3-monochloropropane-1,2-diol (3-MCPD) at nominal concentrations of 0, 25, 100 or 400 mg/L in drinking water (see Section 3.4.2.3). Average daily doses of 0, 2.0, 8.3 or 29.5 mg/kg body weight (bw) per day, and 0, 2.7, 10.3 or 37.0 mg/kg bw per day were calculated for male and female rats, respectively. The relevant results of the study were initially screened by visual analysis for the presence of monotonic dose–response trends; the presence of a dose–response trend in the subset of results selected in the screening phase was subsequently confirmed by applying the Cochran–Armitage trend test (Haseman, 1984). The results with $p < 0.05$ in the aforementioned trend test were selected for benchmark dose (BMD) analysis, performed by means of the software BMDS v2.6.0.86 (US EPA). The following effects were subjected to the BMD analysis:

- Tubular hyperplasia (both sexes)
- Nephropathy (both sexes)
- Tubular adenoma or carcinoma (both sexes)
- Testicular atrophy

For all the aforementioned effects, all models for dichotomous (quantal) data were selected for the analysis at the default benchmark response (BMR) of 10% (95% confidence level) advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009a). The models allowing for restrictions (Gamma, Log Logistic, Logprobit and Weibull) were run both with and without the selected default restrictions. $BMDL_{10}$ were calculated separately for male and female rats. The existence of a monotonic trend was confirmed comparing the log likelihood of the null model versus the full model ($p \leq 0.05$, using the likelihood ratio test). In accordance with the Scientific Opinion of the EFSA (EFSA, 2009a) a goodness-of-fit was judged as sufficient if the fit showed a p-value not smaller than 0.05 (i.e. $p \geq 0.05$), using the likelihood ratio test. The lowest $BMDL_{10}$ calculated for each effect are reported in Table D.1.

Table D.1: Summary table of the lowest BMD and $BMDL_{10}$ calculated for different effects observed in the Cho et al. (2008) study

Effect	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day	Model	Calculation details
Tubular hyperplasia (male)	0.54	0.077	Gamma (unrestricted)	A1
Tubular hyperplasia (female)	27	14	Quantal-linear	A2
Nephropathy (male)	0.64	0.37	Loglogistic (restricted)	A3
Nephropathy (female)	3.0	1.1	Gamma (unrestricted)	A4
Testes atrophy	3.5	2.5	Quantal-linear	A5
Tubular adenoma or carcinoma (combined) (male)	25	15	Quantal-linear	A6
Tubular adenoma or carcinoma (combined) (female)	24	15	Logprobit (unrestricted)	A7

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight.

Table D.2: Male kidney hyperplasia

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0	50	1
2	50	11
8.3	50	21
29.5	50	36

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	128.86	3	< 0.001	NA		
Gamma	No	94.91	2	0.92	Yes	0.54	0.077
Logistic	NA	102.47	3	0.0005	No	5.6	4.6
LogLogistic	No	95.07	3	0.57	Yes	0.85	0.23
LogProbit	No	95.10	3	0.54	Yes	0.93	0.28
Multistage	No	95.53	3	0.27	Yes	1.3	0.91
Multistage-Cancer	NA	97.44	2	0.079	Yes	2.1	1.7
Probit	NA	102.18	2	0.0007	No	5.4	4.5
Weibull	No	94.94	3	0.81	Yes	0.64	0.14
Quantal-Linear	NA	97.44	2	0.079	Yes	2.1	1.7
Gamma	Yes	97.44	2	0.079	Yes	2.1	1.7
LogLogistic	Yes	95.36	2	0.64	Yes	1.2	0.87
LogProbit	Yes	100.33	2	0.004	No	4.0	3.0
Weibull	Yes	97.44	2	0.079	Yes	2.1	1.7
Multistage	Yes	97.44	2	0.079	Yes	2.1	1.7
Full	NA	94.91	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

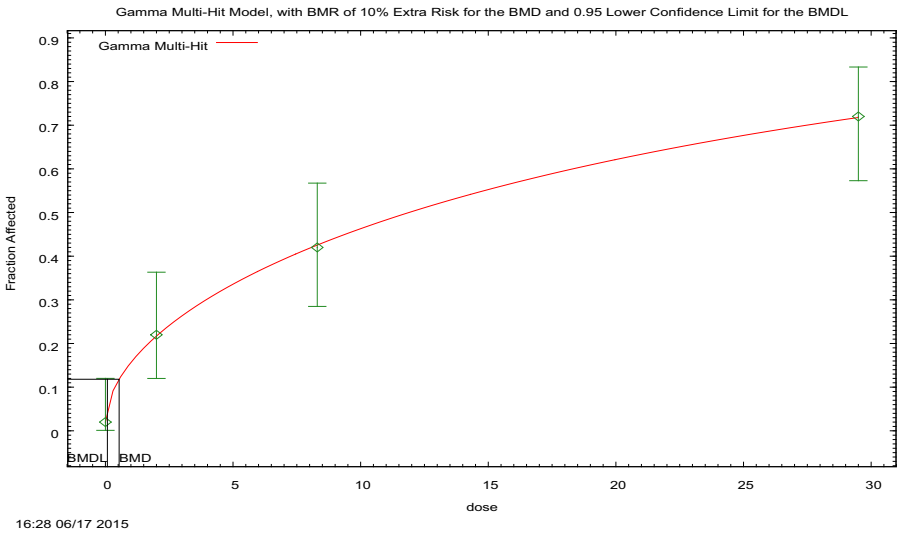


Table D.3: Female kidney hyperplasia

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0	50	1
2.7	50	0
10.3	50	1
37.0	50	10

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	45.39	1	< 0.001	NA		
Gamma	No	35.53	3	0.23	Yes	27	18
Logistic	NA	35.66	2	0.44	Yes	29	24
LogLogistic	No	35.54	3	0.23	Yes	28	18
LogProbit	No	35.53	3	0.24	Yes	26	17
Multistage	No	35.41	3	0.28	Yes	27	20
Multistage-Cancer	NA	35.61	2	0.45	Yes	28	19
Probit	NA	35.71	2	0.41	Yes	28	22
Weibull	No	35.54	3	0.23	Yes	23	19
Quantal-Linear	NA	37.13	2	0.10	Yes	27	14
Gamma	Yes	35.53	3	0.23	Yes	28	18
LogLogistic	Yes	35.54	3	0.23	Yes	28	18
LogProbit	Yes	35.53	3	0.24	Yes	26	18
Weibull	Yes	35.54	3	0.23	Yes	28	19
Multistage	Yes	35.61	2	0.45	Yes	26	19
Full	NA	34.82	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

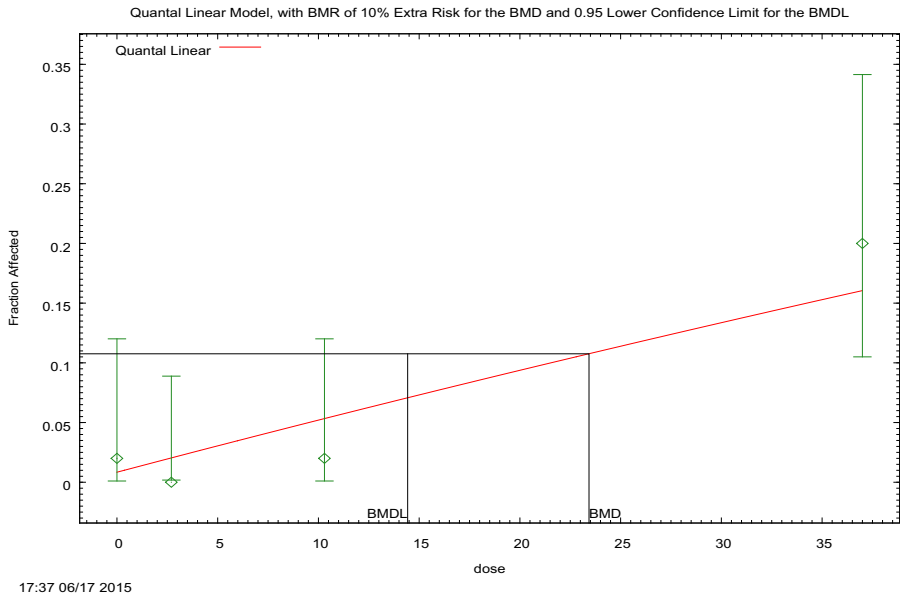


Table D.4: Male nephropathy

Incidence data		
Dose (mg/kg bw per day)	**N**	**Effect**
0	50	15
2	50	27
8.3	50	39
29.5	50	41

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	133.75	1	< 0.001	NA		
Gamma	No	115.92	3	0.16	YES	0.0076	< 0.0001[(a)]
Logistic	NA	121.86	2	0.002	NO	3.0	2.3
LogLogistic	No	115.53	3	0.28	YES	0.13	0.001[(a)]
LogProbit	No	115.57	3	0.26	YES	0.15	0.0013[(a)]
Multistage	No	115.06	3	0.64	YES	0.58	0.40
Multistage-Cancer	NA	120.23	2	0.0050	NO	1.9	1.3
Probit	NA	122.12	2	0.0008	NO	3.3	2.6
Weibull	No	115.75	3	0.21	YES	0.035	< 0.0001[(a)]
Quantal-Linear	NA	120.30	2	0.0050	NO	1.9	1.3
Gamma	Yes	120.30	2	0.0050	NO	1.9	1.3
LogLogistic	Yes	116.68	2	0.18	YES	0.64	0.37
LogProbit	Yes	121.26	2	0.0018	NO	3.1	2.0
Weibull	Yes	120.30	2	0.0050	NO	1.9	1.3
Multistage	Yes	120.30	2	0.0050	NO	1.9	1.3
Full	NA	115.00	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.
(a): Ratio BMD/BMDL higher than one order of magnitude.

Plot (model resulting in the lowest $BMDL_{10}$)

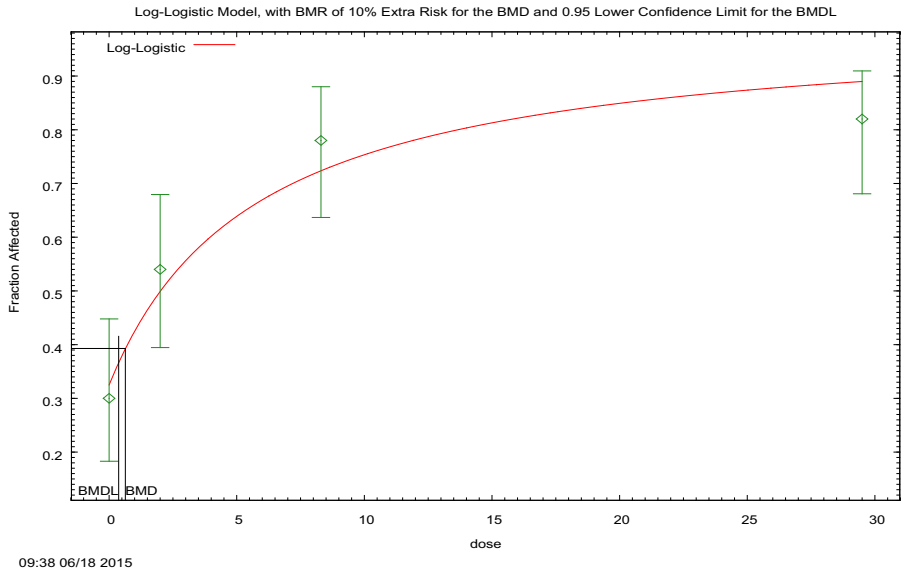


Table D.5: Female nephropathy

Incidence data		
Dose(mg/kg bw per day)	**N**	**Effect**
0	50	6
2.7	50	8
10.3	50	23
37.0	50	42

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	134.19	1	< 0.001	NA		
Gamma	No	97.14	3	0.42	YES	3.0	1.1
Logistic	NA	99.11	2	0.10	YES	5.4	4.4
LogLogistic	No	96.87	3	0.74	YES	3.6	1.8
LogProbit	No	96.83	3	0.86	YES	3.7	1.9
Multistage	No	97.26	3	0.34	YES	2.5	1.5
Multistage-Cancer	NA	97.26	3	0.34	YES	2.5	1.8
Probit	NA	99.04	2	0.11	YES	5.3	4.4
Weibull	No	97.18	3	0.39	YES	2.8	1.2
Quantal-Linear	NA	97.29	2	0.62	YES	2.3	1.8
Gamma	Yes	97.14	3	0.42	YES	3.0	1.8
LogLogistic	Yes	96.87	3	0.74	YES	3.6	1.8
LogProbit	Yes	96.85	2	0.97	YES	4.0	3.1
Weibull	Yes	97.18	3	0.39	YES	2.8	1.8
Multistage	Yes	97.26	3	0.34	YES	2.5	1.8
Full	NA	96.81	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

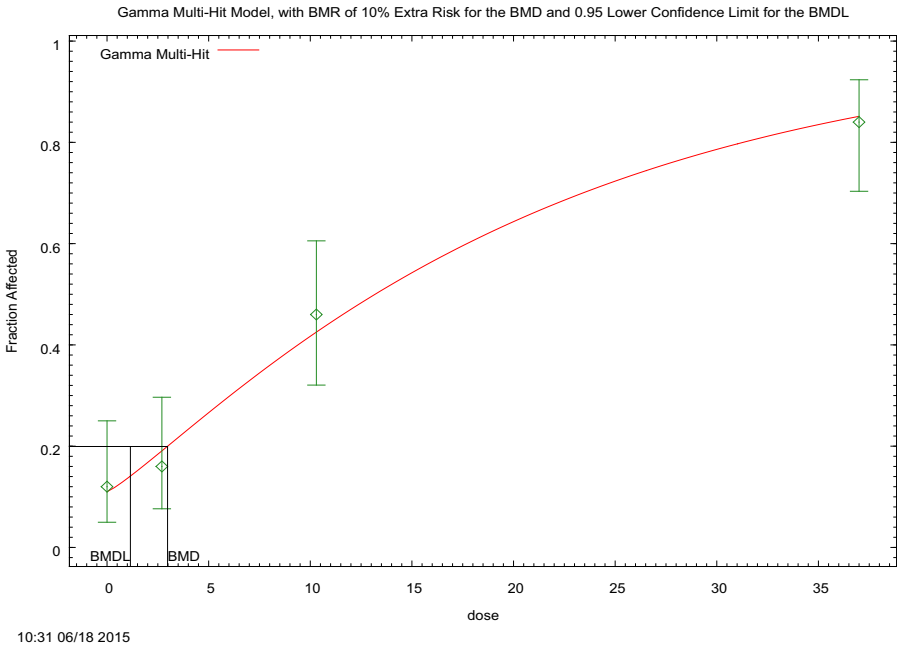


Table D.6: Testicular atrophy

Incidence data

Dose(mg/kg bw per day)	N	Effect
0	50	6
2	50	16
8.3	50	13
29.5	50	34

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	128.86	1	< 0.001	NA		
Gamma	No	112.53	3	0.017	NO	2.0	0.14
Logistic	NA	112.32	2	0.072	YES	6.2	5.0
LogLogistic	No	112.64	3	0.015	NO	9.7	0.41
LogProbit	No	113.40	3	0.0063	NO	2.2	0.22
Multistage	No	112.35	3	0.021	NO	5.8	2.1
Multistage-Cancer	NA	112.35	3	0.021	NO	5.8	2.6
Probit	NA	112.31	2	0.072	YES	6.0	4.9
Weibull	No	112.57	3	0.016	NO	7.2	0.24
Quantal-Linear	NA	112.59	2	0.055	YES	3.5	2.5
Gamma	Yes	112.65	3	0.015	NO	9.3	2.5
LogLogistic	Yes	112.64	3	0.015	NO	9.7	1.7
LogProbit	Yes	112.69	3	0.014	NO	10	5.2
Weibull	Yes	112.57	3	0.016	NO	7.2	2.5
Multistage	Yes	112.35	3	0.021	NO	5.8	2.6
Full	NA	109.69	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

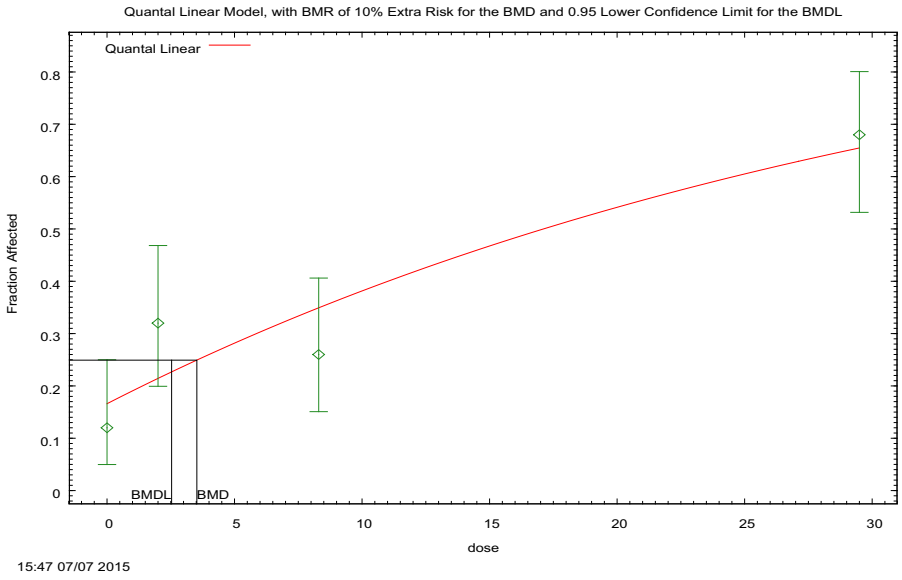


Table D.7: Male tubule adenoma or carcinoma (combined)

Incidence data

Dose(mg/kg bw per day)	N	Effect
0	50	0
2	50	0
8.3	50	1
29.5	50	7

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	33.59	1	< 0.001	NA		
Gamma	No	25.23	2	0.92	YES	24	16
Logistic	NA	25.73	2	0.56	YES	27	22
LogLogistic	No	25.23	2	0.92	YES	24	16
LogProbit	No	25.19	2	0.97	YES	23	15
Multistage	No	25.27	2	0.89	YES	24	17
Multistage-Cancer	NA	25.27	2	0.89	YES	24	17
Probit	NA	25.64	2	0.61	YES	26	21
Weibull	No	25.24	2	0.92	YES	24	16
Quantal-Linear	NA	25.88	1	0.69	YES	25	15
Gamma	Yes	25.23	2	0.93	YES	24	16
LogLogistic	Yes	25.23	2	0.92	YES	24	16
LogProbit	Yes	25.34	1	0.94	YES	23	17
Weibull	Yes	25.24	2	0.92	YES	24	16
Multistage	Yes	25.27	2	0.89	YES	24	17
Full	NA	25.15	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)

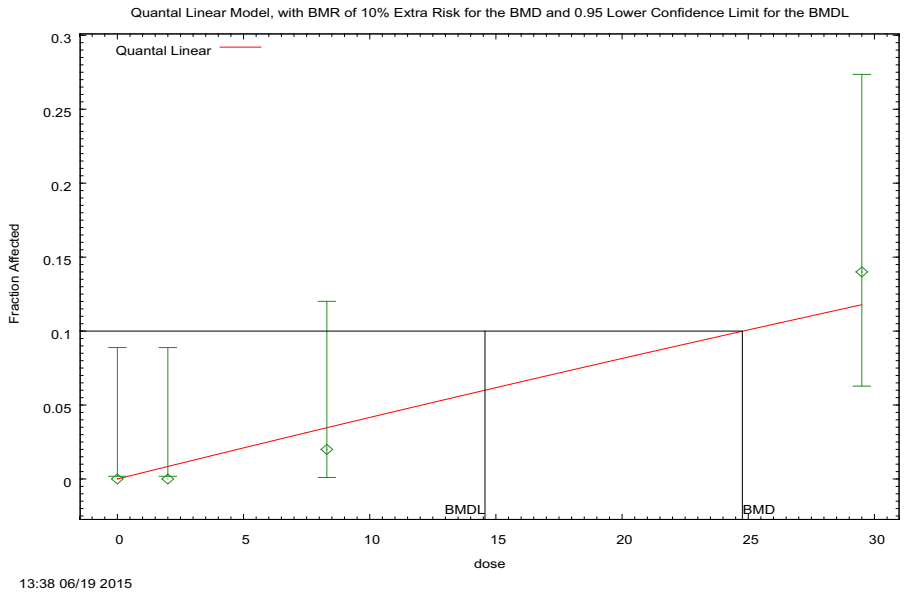


Table D.8: Female tubule adenoma or carcinoma (combined)

Incidence data

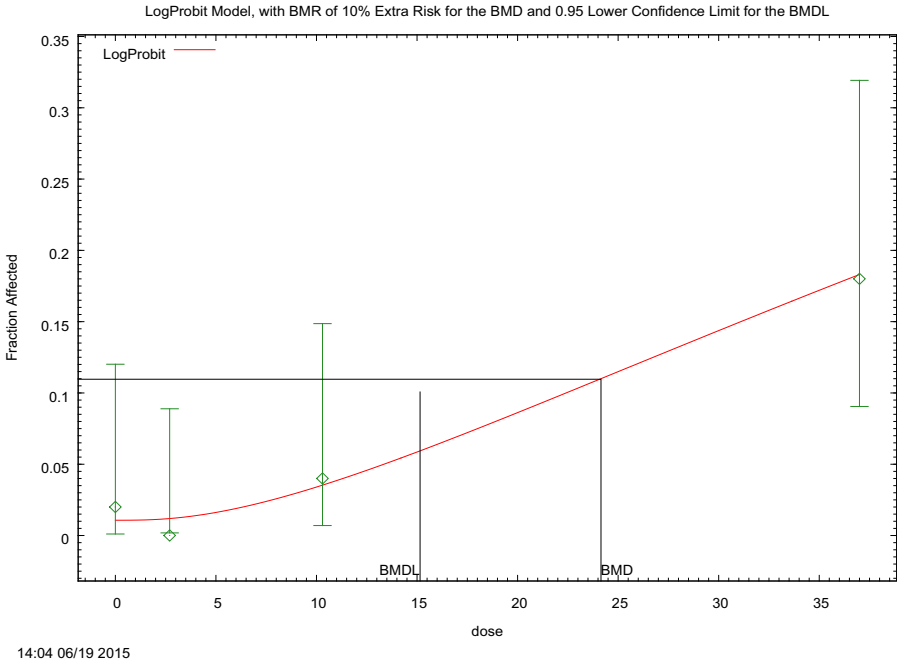
Dose(mg/kg bw per day)	N	Effect
0	50	0
2	50	0
8.3	50	1
29.5	50	7

BMD analysis results

Model	Restriction	−Log-likelihood	Number of parameters	p	Accepted	BMD_{10} mg/kg per day	$BMDL_{10}$ mg/kg per day
Null	NA	45.39	1	< 0.001	NA		
Gamma	No	37.71	3	0.19	YES	26	16
Logistic	NA	37.87	2	0.37	YES	30	24
LogLogistic	No	37.72	3	0.19	YES	26	16
LogProbit	No	37.65	3	0.21	YES	24	15
Multistage	No	37.78	3	0.18	YES	27	16
Multistage-Cancer	NA	37.78	3	0.18	YES	27	16
Probit	NA	37.84	2	0.38	YES	28	23
Weibull	No	37.73	3	0.19	YES	26	16
Quantal-Linear	NA	38.34	2	0.23	YES	24	15
Gamma	Yes	37.71	3	0.19	YES	26	16
LogLogistic	Yes	37.72	3	0.19	YES	26	16
LogProbit	Yes	37.78	2	0.40	YES	26	18
Weibull	Yes	37.73	3	0.19	YES	26	16
Multistage	Yes	37.78	3	0.18	YES	27	16
Full	NA	36.87	4	NA	NA		

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; NA: not applicable.

Plot (model resulting in the lowest $BMDL_{10}$)



Appendix E – $BMDL_{10}$ and BMD_{10} (mg/kg bw per day) calculated for different non carcinogenic toxicological endpoints (adapted from Barocelli et al., 2011)

	3-MCPD mg/kg bw per day		3-MCPD dipalmitate mg/kg bw per day		BMD_{10} ratios mg/kg or moles/Kg
	$BMDL_{10}$	BMD_{10}	$BMDL_{10}$	BMD_{10}	
Kidney female			3.6	7.9	
Kidney male	2.5	5.6	17.4	41.1	7.3 or 1.4
Testis	6.0	8.4	44.3	64.4	7.7 or 1.4
Proteinuria male	2.7	6.4	18.7	47.2	7.4 or 1.4
RBC 5% loss male	3.5	7.2	24.8	53.5	7.4 or 1.4
RBC 5% loss female	2.6	4.5	90.4	187.0	41.6 or 7.8
Mortality female	2.3	7.4	< 157	< 157	< 21 or < 4.0
Mean ± SD Excluding kidney/female and mortality for MCPD palmitate)		6.58 ± 1.39 (5.19–7.97)		78.6 ± 61.2 (17.5–139.8)	

BMD: benchmark dose; BMDL: benchmark dose (lower confidence limit); bw: body weight; 3-MCPD: 3-monochloropropane-1, 2-diol; SD: standard deviation.

Appendix F – Derivation of T25 levels for glycidol

As reported in EFSA (2005), the T25 is a simplified method to estimate the carcinogenic potency of a given substance in the absence of dose–response data allowing for the application of the benchmark dose (BMD) approach.

T25 is defined as 'the chronic dose rate in mg/kg bw per day, which will give 25% of the animal tumours at a specific tissue site, after specific correction for the spontaneous incidence within the standard life time of that species'. The T25 approach was applied to the NTP studies on glycidol (NTP, 1990) in rats and mice.

The derivation of T25 levels was carried out using the methodology described by Dybing et al. (1997).

In particular, both for mice and rats, T25 levels were calculated for the tumour incidences reported at different tissues in separate sexes. T25 were calculated for all tested doses, although it is indicated by Dybing et al. (1997) that the lowest tumour incidence data showing a significant response are generally used.

Tumour incidences (reported in percentages) observed in the treated groups were corrected for the spontaneous incidences observed in controls, by applying the equation:

$$\mathrm{CI} = 100 \times (\mathrm{OI} - \mathrm{SI})/(100 - \mathrm{SI})$$

where: CI is the tumour incidence corrected for the spontaneous incidence; OI is the tumour incidence observed in the treatment group; andSI is the spontaneous incidence observed in the control group.The T25 was calculated by applying the following equation:

$$\mathrm{T25} = 25 \times \mathrm{D}/\mathrm{CI}$$

where CI is the corrected incidence (or the observed incidence in case there is no spontaneous tumour incidence observed) and D is the related dose at which the incidence is reported.

No additional corrections were included in the calculation (e.g. to correct for specific treatment regimes).

For each species and sex, the lowest T25 levels were considered.

Table F.1: Calculation of T25 for tumours reported in male rats

Dose (mg/kg bw per day)	Incidence (per number of animals)						Observed Incidence (%)			Corrected Incidence		T25 (mg/kg bw per day)	
	0		37.5		75		0	37.5	75	37.5	75	37.5	75
Peritoneal mesothelioma	3	(49)	34	(50)	39	(47)	6.12	68.00	82.98	65.91	81.87	14.22	22.90
Mammary gland fibroadenoma/ carcinoma	3	(45)	8	(39)	7	(17)	6.67	20.51	41.18	14.84	36.97	63.19	50.71
Brain glioma	0	(46)	5	(50)	6	(30)	0.00	10.00	20.00	10.00	20.00	93.75	93.75
Oral mucosa papilloma/ carcinoma	1	(46)	2	(50)	6	(32)	2.17	4.00	18.75	1.87	16.94	502.23	110.66
Intestine aden polyp or adenocarc	0	(47)	1	(50)	4	(37)	0.00	2.00	10.81	2.00	10.81	468.75	173.44
Skin sebaceous gland tumours	0	(45)	5	(41)	4	(18)	0.00	12.20	22.22	12.20	22.22	76.88	84.38
Zymbal gland carcinoma	1	(49)	3	(50)	6	(48)	2.04	6.00	12.50	4.04	10.68	231.96	175.61
Thyroid follicular cell adenoma/ carcinoma	1	(46)	4	(42)	6	(19)	2.17	9.52	31.58	7.51	30.06	124.78	62.38

Table F.2: Calculation of T25 for tumours reported in female rats

Dose (mg/kg bw per day)	Incidence (per number of animals)						Observed Incidence (%)			Corrected Incidence		T25 (mg/kg bw per day)	
	0		37.5		75		0	37.5	75	37.5	75	37.5	75
Mammary gland fibroadenoma/ carcinoma	14	(49)	32	(46)	29	(44)	28.57	69.57	65.91	57.39	52.27	16.34	35.87
Brain glioma	0	(49)	4	(46)	4	(46)	0.00	8.70	8.70	8.70	8.70	107.81	215.63
Oral mucosa papilloma/ carcinoma	0	(47)	4	(38)	11	(30)	0.00	10.53	36.67	10.53	36.67	89.06	51.14
Clitoral gland adenoma/ carcinoma	5	(49)	9	(47)	12	(45)	10.20	19.15	26.67	9.96	18.33	94.11	102.27
Thyroid follicular cell adenoma/ carcinoma	0	(49)	1	(38)	3	(35)	0.00	2.63	8.57	2.63	8.57	356.25	218.75
Hematopoietic system leukaemia	13	(49)	14	(44)	20	(41)	26.53	31.82	48.78	7.20	30.28	130.26	61.91

Table F.3: Calculation of T25 for tumours reported in male mice

Dose (mg/kg bw per day)	Incidence (per number of animals)						Observed Incidence (%)			Corrected Incidence		T25 (mg/kg bw per day)	
	0		25		50		0	25	50	25	50	25	50
Harderian gland adenoma	8	(46)	12	(41)	22	44	17.39	29.27	50.00	14.38	39.47	43.47	31.67
Forestomach squamous cell papilloma or carcinoma	1	(50)	2	(50)	10	50	2.00	4.00	20.00	2.04	18.37	306.25	68.06
Skin squamous cell papilloma or carcinoma	0	(50)	0	(50)	4	50	0.00	0.00	8.00	0.00	8.00	NA	156.25
Liver adenoma or carcinoma	24	(50)	31	(50)	35	50	48.00	62.00	70.00	26.92	42.31	23.21	29.55
Lung alveolar/ bronchiolar adenoma or carcinoma	13	(50)	11	(50)	21	50	26.00	22.00	42.00	−5.41	21.62	NA	57.81

Table F.4: Calculation of T25 for tumours reported in female mice

Dose (mg/kg bw per day)	Incidence (per number of animals)						Incidence (%)			Incidence over background		T25 (mg/kg bw Per day)	
	0		25		50		0	25	50	25	50	25	50
Harderian gland adenoma	4	46	11	43	17	43	8.70	25.58	39.53	18.49	33.78	33.79	37.01
Mammary gland adenoma, adenocarc	2	50	6	50	15	50	4.00	12.00	30.00	8.33	27.08	75.00	46.15
Uterus carcinoma or adenocarc	0	50	3	50	3	50	0.00	6.00	6.00	6.00	6.00	104.17	208.33
Subcutaneous tissue sarcoma or fibrosarcoma	0	50	3	50	9	50	0.00	6.00	18.00	6.00	18.00	104.17	69.44
Skin squamous cell papilloma or carcinoma	0	50	0	50	2	50	0.00	0.00	4.00	0.00	4.00	NA	312.50