Food and Agriculture Organization of the United Nations

25
FOOD SAFETY AND QUALITY SERIES

ISSN 2415-1173

FOOD SAFETY IN THE CONTEXT OF LIMITED FOOD AVAILABILITY

RISK ASSESSMENT OF 3-MCPD AND FATTY ACID ESTERS IN NUTRIENT SUPPLEMENTS AND THERAPEUTIC FOOD

FAO

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME, 2024

Required citation:
FAO. 2024. *Food safety in the context of limited food availability – Risk assessment of 3-MCPD and fatty acid esters in nutrient supplements and therapeutic food.* Food Safety and Quality Series, No. 25. Rome. https://doi.org/10.4060/cd0761en

ISSN 2415-1173 [Print]
ISSN 2664-5246 [Online]

ISBN 978-92-5-138777-1

Cover photos (from left to right): ©FAO/Ami Vitale, ©FAO/IFAD/WFP/Michael Tewelde.
Layout: Tomaso Lezzi

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ACKNOWLEDGEMENTS

This publication was prepared by Mark Feeley and Gordon Barrett of Health Canada under the technical leadership and guidance of Vittorio Fattori and Markus Lipp of the Agrifood Systems and Food Safety Division of the Food and Agriculture Organization of the United Nations (FAO).

The work was carried out in collaboration with the World Food Programme (WFP), the United Nations Children's Fund (UNICEF) and Médecins Sans Frontières (MSF). UNICEF would like to thank the United States Agency for International Development (USAID) for their generous support.

The authors are grateful to experts who provided their insightful comments during the peer review process, in particular Alan Boobis, Lutz Edler, Sue Barlow and Peter Cressey.

The authors acknowledge the kind support provided by Magdalena Niegowska Conforti during the publication process of the report.

The document was copy edited by Jeannie Marshall, and graphic design was provided by Tomaso Lezzi.

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ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ADAF	age dependent adjustment factor
AIF	apoptosis-inducing factor
ALARA	as low as reasonably achievable
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
BfR	German Federal Institute for Risk Assessment
BMD	benchmark dose
BMD_{10}	BMD corresponding to 10% extra risk relative to background
$BMDL_{10}$	95% lower confidence limit on the BMD_{10}
$BMDU_{10}$	95% upper confidence limit on the BMD_{10}
BMR	benchmark response
bw	body weight
CalEPA	California Environmental Protection Agency
CCCF	Codex Committee on Contaminants in Foods
CK	creatine kinase
C_{max}	maximal concentration
CONTAM	EFSA Panel on Contaminants in the Food Chain
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DAG	diacylglycerols
DHPMA	*S*-(2,3-Dihydroxypropyl)mercapturic acid
DHPV	*N*-(2,3-dihydroxypropyl)valine
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria
FA	fibroadenoma
FAO	Food and Agriculture Organization of the United Nations
FSCJ	Food Safety Commission of Japan

GEMS/Food	WHO's Global Environment Monitoring System/ Food Contamination Monitoring and Assessment Program
GEs	glycidyl fatty acid esters
GI	gastrointestinal
GSH	glutathione
GST	glutathione S-transferases
HBGV	health-based guidance value
HCT	hematocrit
Hgb	hemoglobin
Hmox1	heme oxygenase 1
HOTT	heterozygous transgenic heme oxygenase triple
HVP	hydrolyzed vegetable proteins
IARC	International Agency for Research on Cancer
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IL-18	interleukin-18
i.p.	intraperitoneal
i.v.	intravenous
ILCR	incremental lifetime cancer risk
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LADD	lifetime average daily dose
LB	lower bound
LNS	Lipid-based nutrient supplements
LNS-LQ	LNS - Large Quantity
LNS-MQ	LNS - Medium Quantity
LNS-PLW	LNS intended for lregnant and lactating women
LNS-SQ	LNS - Small Quantity
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantification
MAG	monoacylglycerols
3-MCPD	3-monochloropropane-1,2-diol
3-MCPD-d5	deuterated 3-MCPD
ML	maximum level

MOE	margin of exposure
ND	not detected
NOAEL	no observed adverse effect level
NSRL	no significant risk level
NTP	United States National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
P95	95th percentile
PMTDI	provisional maximum tolerable daily intake
PoD	point of departure
PTDI	provisional tolerable daily intake
RBC	red blood cell
RIVM	Netherlands National Institute of Public Health and the Environment
ROS	reactive oxygen species
RUSF	ready-to-use supplementary food
RUTF	ready-to-use therapeutic food
SAM	severe acute malnutrition
SCF	European Commission's Scientific Committee on Food
SD	Sprague-Dawley rats
SKLM	Senate Commission on Food Safety of the German Research Foundation
Sir3	Sirtuin 3
TAG	triacylglycerol
TDI	tolerable daily intake
TVM	mesotheliomas in the tunica vaginalis/peritoneum
WFP	United Nations World Food Programme
WHO	World Health Organization
UB	upper bound
UGT	UDP-glucuronosyltransferases
UNICEF	United Nations Children's Fund

EXECUTIVE SUMMARY

Lipid-based nutrient supplements (LNS) and ready-to-use therapeutic food (RUTF) are fortified foods intended to be eaten over a specified period to prevent and treat malnutrition in children 6 months and older. LNS/RUTF products are ready-to-eat, energy-dense pastes that may contain ≥ 36 percent fat by weight. They are often produced locally in regions experiencing food insecurity, and the source of dietary lipid may vary depending on availability. However, prior to consumption or use in formulating products such as LNS/RUTF, all edible oils obtained from oleaginous seeds or fruits must be refined to remove undesirable substances and create a palatable, shelf-stable product. This process typically involves the use of high temperature distillation to strip the oils of substances that may produce odours, off-flavour components, as well as other volatile contaminants (a step known as deodorization). In recent years, however, it has come to light that an undesired side-effect of the refining process of edible oils is the formation of heat-induced contaminants, including various chloropropanols and glycidyl fatty acid esters (GEs). Among the chloropropanols, 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters typically occur in the highest concentrations in edible oils. Therefore, 3-MCPD is the focus of the current assessment although it is acknowledged that additional related contaminants such as 2-chloro-1,2-propanediol (2-MCPD) can also be present but at lower levels compared to 3-MCPD. While the main form of 3-MCPD detected in edible fats and oils is as mono- or di- fatty acid esters, evidence to date supports the notion that these esters are effectively metabolized following ingestion resulting in systemic exposure primarily to the parent 3-MCPD compound. Similarly, GEs found in products are mainly in the form of monoesters that undergo de-esterification in the gastrointestinal (GI) tract prior to being systemically absorbed. The concentration of 3-MCPD, 3-MCPD fatty acid esters, and GEs in refined oils varies as a function of the refining process but also their fatty acid composition, with the highest levels typically observed in refined palm oil and palm olein. Due to its low cost, availability and physical properties (e.g. semi-solid at room temperature), palm oil is used extensively in the manufacture of LNS/RUTF products. As these substances or their metabolites (such as glycidol in the case of GEs) have been shown to possess toxic properties in experimental animals, including genotoxicity/carcinogenicity (glycidol), their presence in refined oils and fats as well as in foods containing these ingredients is of concern.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) have both recently delivered opinions on the risk to human health of 3-MCPD, 3-MCPD fatty acid esters and GEs in food. In 2018, the European Union introduced maximum limits for GEs expressed as glycidol in infant formula and foods for special medical purposes intended for infants and young children, as well as vegetable oils and fats intended for consumer use or as an ingredient in food.

In 2020, the European Union regulations were expanded to include free 3-MCPD and 3-MCPD fatty acid esters in these same products. Currently, the only Codex standard that has been developed for 3-MCPD is for liquid condiments containing acid hydrolyzed vegetable proteins, while no Codex standards are available for GEs.

Recently, the levels of 3-MCPD fatty acid esters and GEs were quantified in LNS products of various formats as well as RUTF, obtained from several suppliers. The results showed that exposure to these process-induced contaminants in finished LNS/RUTF products could lead to exceedances of the health-based guidance values (HBGVs) for 3-MCPD or the reference points for GEs established by JECFA and EFSA, indicating the potential for health concerns. While internationally, efforts are underway to reduce the concentrations of these process-induced contaminants in edible fats and oils, the use of high quantities of palm oil as the main fat source in food products as well as local production in regions that may lack the material resources and technological capabilities for remediation pose short-term challenges in reducing exposure. Therefore, an assessment was needed to characterize the risk of less-than-lifetime exposure to 3-MCPD (including 3-MCPD fatty acid esters) and GEs via LNS/RUTF in the context of limited food availability.

In the case of 3-MCPD and its esters (singly or in combination, expressed as 3-MCPD equivalents), JECFA (2016) has established a provisional maximum tolerable daily intake (PMTDI) of 4 µg/kg bw based on nephropathy and renal tubular hyperplasia in a chronic rat study (Cho *et al.*, 2008). Following a review of the literature, no new studies were identified that were considered more appropriate for the derivation of a reference point and thus the same critical effect as selected by JECFA was retained in the current assessment. However, guidance from both EFSA and the World Health Organization (WHO) for the use of benchmark dose (BMD) modelling in risk assessment has recently been updated and the use of Bayesian model averaging is now the preferred approach. The same rat data for nephrotoxicity selected by JECFA (2016) from the Cho *et al.* (2008) study were modelled, therefore, in accordance with the most recent guidance, which yielded a slightly more conservative one-sided 95 percent confidence bound ($BMDL_{10}$) on the BMD of 0.48 mg/kg bw/day in comparison with the value of 0.87 mg/kw bw/day derived by JECFA in 2016. Applying the same composite uncertainty factor of 200 as used by JECFA would result in a revised PMTDI of 2.4 µg/kg bw. Although consumption of LNS/RUTF is of limited duration and mainly restricted to infants and young children, 3-MCPD and its fatty acid esters are present in many other foodstuffs and most of the total lifetime exposure is attributed to foods other than LNS/RUTF. Therefore, it was considered appropriate to use a lifetime average daily dose (LADD) approach to characterize the potential risk of exposure to these substances from less-than-lifetime use of LNS/RUTF, under the assumption that short-term excursions above the PMTDI may be tolerable so long as the LADD was not exceeded. Although the typical intake periods for LNS and RUTF are two to three months and four to eight weeks, respectively, as a worst-case scenario for a LADD exposure, it was assumed that children are consuming these products as their sole source of nutrition for 0 to 1 years and for 1 to 5 years.

Using conservative estimates of LNS/RUTF consumption in young children and infants in combination with high (95th percentile) background consumer intakes for all other age categories, it was determined that the LADD would not exceed the updated PMTDI of 2.4 µg/kg bw if total 3-MCPD equivalent concentrations in LNS/RUTF did not exceed 382 µg/kg; decreasing either the duration of exposure or the amount consumed daily would increase the tolerance for 3-MCPD in these products.

When ingested, GEs are thought to undergo rapid and extensive presystemic hydrolysis to form glycidol. Therefore, for the purposes of human health risk assessment, it is assumed that exposure to GEs is equivalent to exposure to an equimolar quantity of glycidol. In contrast to 3-MCPD and its fatty acid esters, glycidol is an established genotoxic carcinogen and, therefore, previous assessments by EFSA and JECFA elected not to establish a HBGV to characterize risk but rather to use a margin of exposure (MOE) approach. As was the case for 3-MCPD and its esters, the reference point from JECFA (2016) of 2.4 mg/kg bw/day was recalculated using Bayesian model averaging to yield a $BMDL_{10}$ of 0.83 mg/kg bw/day. The MOE is the ratio of a critical effect level, usually obtained from an animal study, to the estimated level of exposure in humans. While the MOE is useful to characterize the magnitude of a risk, it cannot be used to directly quantify the increased probability of an adverse health effect. Therefore, an incremental lifetime cancer risk (ILCR) approach was used to estimate the increase in the lifetime cancer risk associated with GEs exposure from LNS/RUTF relative to background exposure from other dietary sources. Assuming the mean lower bound background exposure from JECFA (2016) and using a LADD approach, the estimated increase in ILCR attributable to GEs exposure from LNS/RUTF would not exceed 1 in 10^5 (1 in 100 000) provided the concentration of GEs in LNS/RUTF products does not exceed 164 µg/kg. This estimate is considered highly conservative as it is based on 12 months exposure to RUTF as the sole source of nutrition, which would be considered an extreme scenario.

Previous assessments by JECFA, EFSA and others have all characterized the risk of exposure to 3-MCPD and GEs based on chronic exposure. However, in contrast to other dietary sources containing these compounds, the use of LNS/RUTF is intended to be of finite duration and confined to a specific life stage. The thresholds identified herein for concentrations of 3-MCPD and glycidol equivalents in LNS/RUTF products are considered to represent a level of exposure that is of low concern for human health. Moreover, any theoretical risks attributable to process-induced contaminants in these products must be weighed against their benefits in the management of the significant morbidity and mortality associated with frank malnutrition in children and infants experiencing food insecurity.


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CHAPTER 1
BACKGROUND

The World Food Programme (WFP) is the largest humanitarian organization fighting hunger worldwide. WFP operates in more than 80 countries around the world, feeding people in areas affected by conflict and disaster, and laying the foundations for a better future. The United Nations Children's Fund (UNICEF) works in more than 190 countries and territories to save children's lives, to defend their rights and to help them fulfil their potential from early childhood through adolescence. Among other initiatives, the two agencies are dedicated to the treatment and prevention of child wasting.[1]

According to data from UNICEF, one in five deaths among children under age 5 is attributable to severe wasting, making it one of the top threats to child survival globally (UNICEF, n.d.). Lipid-based nutrient supplement (LNS) is a food supplement manufactured for WFP that is intended to be eaten over a specified period, as part of a nutritional programme, to treat moderate acute malnutrition for children aged 6 months and older. Ready-to-use therapeutic food (RUTF) is catalogued as a food for special medical purposes used by UNICEF as the sole source of nutrition to treat severely wasted children from 6 to 59 months of age with no other medical complications and is consumed over a period of 4 to 8 weeks. Horizon scanning and internal testing of RUTF and LNS by WFP and UNICEF have raised concerns over levels of the process-related contaminants 3-monochloropropane-1,2-diol (3-MCPD), 3-MCPD fatty acid esters (3-MCPD esters) and glycidyl fatty acid esters (GEs) in such products when exposure is compared against the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional maximum tolerable daily intake (PMTDI) of 4 µg/kg bw for 3-MCPD and 3-MCPD esters, singly or in combination, or when calculating a margin of exposure (MOE) for the suspected carcinogen glycidol, which is the hydrolysis product of GEs (JECFA, 2016). Current efforts with RUTF/LNS suppliers are ongoing to bring 3-MCPD, its fatty acid esters and GEs levels to meet limits indicated in Commission Regulation (EU) 2020/1322 Amending Regulation (EC) No 1881/2006 as regard maximum levels of 3-MCPD, 3-MCPD fatty acid esters, and GEs in certain foods. However, given the high fat content in RUTF

[1] Wasting, defined as low weight for height, is the most visible and lethal type of malnutrition. Severe wasting, also known as severe acute malnutrition, is its most deadly form. For additional details, see UNICEF. n.d. Child alert: Severe Wasting. In: *UNICEF*. New York. [Cited 14 March 2023] https://www.unicef.org/child-alert/severe-wasting

and LNS (≥ 26–36 g/100 g), this can be very challenging for a supplier, especially those using high quantities of palm oil as the main fat source. Given that the JECFA PMTDI relates to dietary exposure over a lifetime, whereas LNS/RUTF are intended to be provided for only a period of several weeks to a few months, an evaluation was undertaken to assess the risks associated with the target population in combination with the intended use.

During emergencies, LNS could be used interchangeably with another supplementary food known as super cereal plus (typically a corn/soy blend), although it must be noted that ready-to-use supplementary food such as LNS and super cereal plus have a very different macronutrient and slightly different micronutrient formulation. In such scenarios, it may happen that LNS is provided to young children for longer than three months. Thus, the assessment was expanded to include exposure scenarios in which children are provided LNS for up to 12 months to cover such situations and ensure continued provision of safe food. In addition, the daily consumption of a single 100 g sachet of LNS by infants/children 6 to 59 months of age was also considered in the risk characterization for 3-MCPD.

The objective of this evaluation was to perform a risk assessment to identify limits for 3-MCPD esters and GEs in RUTF/LNS, assuming oil to be the main contributor, for respective target populations when consumed as intended for a duration of three months, six months or one year for GEs and up to 5 years of age for 3-MCPD.

1.1 TERMS OF REFERENCE

1. Review current European Food Safety Authority (EFSA) and JECFA health-based guidance values (HBGVs) for 3-MCPD and GEs and identify appropriate points of departure (PoD) for risk characterization specific to this issue. For example, are there shorter-term toxicological reference values that could be developed for short-term intake scenarios? Is a cancer risk MOE appropriate for short-term exposure to GEs?
2. Develop 3-MCPD/GEs intake scenarios specifically for LNS/RUTF products as a sole source of nutrition. This would include consideration of 3-MCPD and its esters/GEs monitoring data provided by WFP and other agencies.
3. Perform risk assessment to identify limits for 3-MCPD esters and GEs in LNS/RUTF for respective target populations when consumed as intended for a duration of three months, six months or one year.

CHAPTER 2
INTRODUCTION

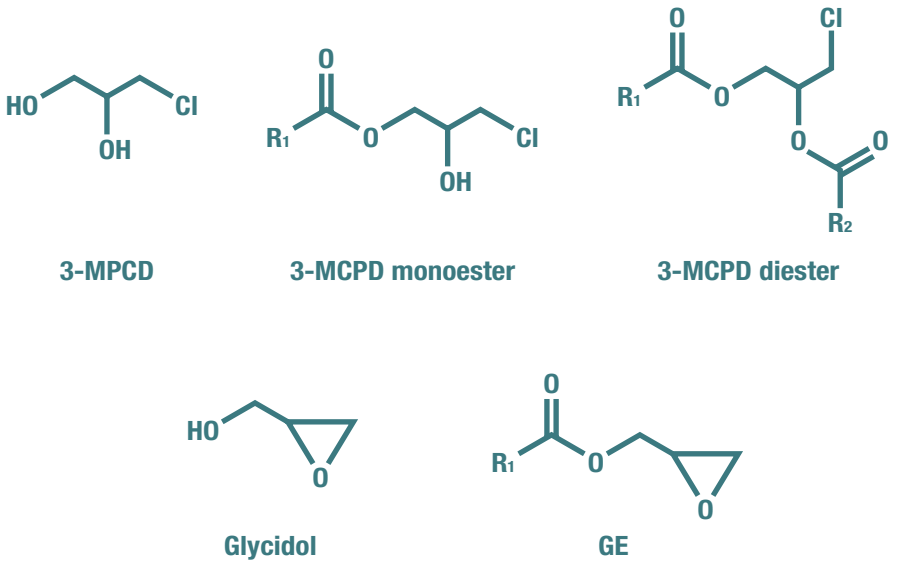
The use of heat during the various stages of food production and/or processing is an important step in improving its nutritional quality, stability and microbial safety. In the case of edible oils used as food ingredients, heat treatments are important steps in the extraction, refining and purification processes. However, over the past few decades, there has been increasing interest in understanding the formation, presence and potential risk to human health posed by the various compounds formed endogenously during the cooking and heat processing of different foods.

3-Monochloro-1,2 propanediol (also referred to as 3-chloro-1,2 propanediol, 3-chloropropane-1,2 diol, α-chlorohydrin or 3-MCPD) and GEs (Figure 1) are part of the large group of food process-induced contaminants that were initially detected in acid-hydrolyzed vegetable protein (acid-HVP) and soy sauces.[2] More recently, however, it has become apparent that these contaminants are also found in the majority of refined vegetable oils and fats, as well as in foods containing these products as ingredients, including infant formulas. Based on the analysis of foodstuffs in several countries, refined vegetable oils are considered to be a major contributor to the levels of 3-MCPD esters and GEs in food and there appears to be limited evidence that one or more of these is formed in food during cooking (EFSA, 2016). The majority of 3-MCPD and GEs detected in foods due to the use of refined edible oils as food ingredients is typically found as fatty acid esters, with 3-MCPD able to combine with mono- or diesters while glycidol occurs only as monoesters. Typical analysis involves the lipid extraction of a food and then treatment (de-esterification) to release the 3 MPCD and glycidol from the fatty acid components prior to quantification (indirect method). Alternatively, more specific analyses are now possible due to the availability of purified analytical standards for most commonly occurring mono- and diesters of both 3-MCPD and glycidol (direct method).

During refining, 3-MCPD and GE can form in edible oils when the oils are heated at very high temperatures to remove unwanted tastes, colours or odours and improve shelf-life stability and nutritional content (FDA, 2022). It is currently considered that the main formation of 3-MCPD and GE in edible fats and oils occurs during deodorization (Kuhlmann, 2011; Matthäus and Pudel, 2022), which is essentially a high-temperature steam distillation process under vacuum.

[2] While additional chloropropanols are also included in this group of contaminants, namely 2-chloro-1,2-propanediol (2-MCPD) and 1,3-dichloro-2 propanol (1,3-DCP), this assessment is specific to 3-MCPD.

FIGURE 1. **STRUCTURE OF THE PROCESS-INDUCED FOOD CONTAMINANTS 3-MCPD AND ITS ESTERS, AS WELL AS GLYCIDYL FATTY ACID ESTERS AND ITS HYDROLYSIS PRODUCT GLYCIDOL**



Note: R1 and R2 represent different ester side chains.

The highest concentrations of both contaminants typically occur in refined palm oil and palm olein (the liquid fraction obtained during fractionation of palm oil), with concentrations in other refined oils increasing incrementally as follows: rapeseed oil < soybean oil < sunflower oil < safflower oil < walnut oil < palm oil (Weisshaar, 2008). Likely due to the use of heat during processing steps, 3-MCPD and GEs have also been detected in a variety of marine oils from diverse origins (fish, algae, krill) (Beekmann *et al.*, 2022). In a recent survey of oils used in the manufacture of infant formulas, the highest concentrations of 3-MCPD were found in palm olein, followed by oils produced from safflower, fungal/algal oils, canola, soybean, sunflower and coconut (Beekman and MacMahon, 2020). Almoselhy *et al.* (2021) recently carried out an investigation of 3-MCPD in some edible oils purchased from local Egyptian markets using gas chromatography tandem triple quadrupole mass spectrometry (GC-MS/MS) with deuterated 3-MCPD (3-MCPD-d5) as an internal standard. Maximum values (range or averages not provided) detected were palm oil (5 634.1 µg/kg), palm olein (5 576.8 µg/kg), corn oil (2 447 µg/kg), sunflower oil (1 817.3 µg/kg), soybean oil (1 486.1 µg/kg), olive pomace oil (572.5 µg/kg), blend of 5 percent sunflower oil with extra virgin olive oil (210 µg/kg) and extra virgin olive oil (93.1 µg/kg).

MCPD and GEs have been detected in numerous food categories, including bread, coffee, coffee creamer, non-HVP seasonings, smoked, grilled and fried meat, fish, cheese, vegetable products, salami, infant formula, margarine, French fries (chips) and doughnuts. In a recent survey of various fish products, thermal treatment during processing or preparation was considered a prerequisite for the formation

of 3-MCPD and GEs in breaded and pre-fried frozen fish products and fried fish products (Ostermeyer *et al.*, 2021).

In daily duplicate diet samples collected from 40 healthy children aged between 26 and 36 months in China, 3-MCPD and glycidyl esters were detected in greater than 71% of the mixed diet and dairy products sampled (Jiang *et al.*, 2021).

As refined vegetable oils are typically the primary lipid source used in infant formula, special attention has been placed on gathering information on the concentrations of 3-MCPD and GEs present and in estimating the potential risk to formula-fed newborns/infants. One of the main edible oils used in the production of infant formulas, palm oil, is also the edible oil that tends to have the highest concentration of both contaminants. Palmitic acid, although not an essential fatty acid, is the main saturated fatty acid found in human milk (20–25 percent of all fatty acids) and it comprises up to 44 percent of total fatty acids in palm oil. In a review of the concentrations of both 3-MCPD and GEs found in various types of infant formula on the Canadian market in 2012–2013, concentrations of bound 3-MCPD ranged from <LOQ-89 µg/kg (average 39 µg/kg) while bound glycidol concentrations were <LOQ-70 µg/kg (average 13.9 µg/kg) (Becalski *et al.*, 2015). In infant formula samples collected from the United States of America market in 2015, levels of bound 3-MCPD ranged from 21–920 µg/kg (average 320 µg/kg) while bound glycidol concentrations ranged from 5–400 µg/kg, average 107 µg/kg) (MacMahon and Beekman, 2019). In an updated survey of 176 powdered infant formula samples collected across the United States in 2017–2019, the concentrations of 3-MCPD esters, measured by direct analysis of various mono- and di-esters, on average were 150 µg/kg formula, range of 13–950 µg/kg while glycidyl esters averaged 61 µg/kg, range <LOD-370 µg/kg (Grassi and MacMahon, 2020). If using a typical 7-fold dilution factor for powdered infant formula, as consumed 3-MCPD and GE concentrations would average approximately 21 µg/kg and 8.7 µg/kg, respectively. 3-MCPD esters were detected in approximately 64 percent of 852 infant formula samples collected from Chinese markets in 2015–2017. The mean concentration detected was 67 µg/kg (range <LOD-1469 µg/kg) (Cui *et al.*, 2021). The estimated mean daily dietary exposure for infants 0 to 6 months, 7 to 12 months and 13 to 36 months of age were 0.97, 0.54 and 0.39 µg/kg bw/day with 95th percentile (P95) estimates of dietary exposure of 2.52, 1.26 and 0.90 µg/kg bw/day, respectively. In liquid infant formula products collected in Denmark in 2020, mean concentrations of 3-MCPD esters and GEs were reported as 5.4 µg/kg (range 3.1–7.9 µg/kg) and 1.7 µg/kg (0.5–4.3 µg/kg), respectively (Nguyen and Fromberg, 2020), whereas powdered infant formulas had mean concentrations of 40.4 µg/kg (5.3–102.8 µg/kg) and 10.7 µg/kg (3.0–30.7 µg/kg), respectively. In a recent survey of commercially prepared foods collected from Japanese markets, 3-MCPD and GEs were detected in all foods by a direct method with GEs concentrations ranging from 1.6–418 µg/kg and 3-MCPD esters 0.08–59 µg/kg (Shimamura *et al.*, 2021). Oleate and palmitate tended to be the predominant fatty acid esters detected for both contaminants.

Declines in the concentrations of both groups of contaminants in infant formula products have been reported within the past 10 years. As an example, mean amounts of bound 3-MCPD and glycidol for infant formula products purchased in 2019 in Germany were approximately half the mean concentrations for infant formula products purchased in 2015 (54 vs 94 µg/kg for bound 3-MCPD and 6 vs 10 µg/kg for bound glycidol) (Beekman *et al.*, 2021). Similar declines have also been observed in infant formulas collected in Canada; in reconstituted powdered formula collected in 2015, the mean concentration of 3-MCPD was 48.6 µg/kg (3.7–91.9 µg/kg) compared to a mean of 28.6 µg/kg (3.9–74.8 µg/kg) for samples collected in 2019 (Schneider *et al.*, 2023).

While 3-MCPD esters and GEs are typically found together in food and food ingredients, their route(s) of formation during edible oil and fat processing can differ. 3-MCPD is formed during food processing at high temperatures in the presence of two reactants, a chlorine source and free glycerol, mono-, di- and triacylglycerols. *In vitro* experiments, under laboratory-controlled conditions, have demonstrated that triacylglycerols, not diacylglycerols, preferentially react with chlorine donors to form MCPD esters (Destaillats *et al.*, 2012a). Research has shown that 3-MCPD begins to form at temperatures as low as 180–200 °C in vegetable oils while most oil deodorization steps involve temperatures in excess of 240 °C. Depending on the fatty acid composition of the oil or fat, a variety of different 3-MCPD esters can be formed. For example, the majority of 3-MCPD in palm oil has been reported to be in the diester form compared to other vegetable oils (Weisshaar, 2011). Additional details regarding the formation of 3-MCPD, glycidol and their respective fatty acid esters can be found in the EFSA evaluation (EFSA, 2016).

GEs are also formed in edible oils during the refining process, in the deodorization step. In refined-bleached palm oil, the formation of GEs proceeds at an exponential rate above 220 °C (Craft *et al.*, 2012). GEs are formed primarily from diacylglycerols and monoacylglycerols (DAGs and MAGs), but not from triacylglycerols (TAGs) and, therefore, high levels of GE in an edible oil can be traced back to high levels of DAGs in certain oils such as crude palm oil (Destaillats *et al.*, 2012b). Commercially refined oils rich in DAG (87 percent of total acylglycerol content) can contain more than 10-fold greater GE levels relative to oils with lower DAG (3.9 percent to 6.8 percent) content but high in TAGs (Masukawa *et al.*, 2010). While oils such as rapeseed, sunflower, olive or soybean contain between 1 and 3 percent DAGs, in palm oil amounts between 6 and 10 percent can be found resulting from the activity of lipases after maturation and before inactivation. Crude coconut, palm and palm kernel oils are distinguished by high amounts of free fatty acids up to 7 percent, while the other oils contain between only 1 and 2 percent (Matthäus *et al.*, 2011). GE formation appears to increase exponentially when the DAG content of edible oils exceeds 3 to 4 percent of total lipids (Craft and Destaillats, 2022).

CHAPTER 3
PREVIOUS ASSESSMENTS – 3-MCPD AND ITS FATTY ACID ESTERS

3.1 JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

3-MCPD was initially evaluated by JECFA at its 41st meeting (JECFA, 1993), when, based on toxic effects (renal toxicity and inhibition of male fertility) observed in experimental animals, JECFA concluded that 3-MCPD should be considered an "undesirable" contaminant in food and that levels in hydrolyzed vegetable proteins should be reduced to the "lowest technologically achievable." Although certain tumours were observed in chronic rodent studies, JECFA concluded that there was insufficient information to support that the reported tumour response was related to organ toxicity and/or hormonal imbalance. A HBGV was not established at this time.

3-MCPD was re-evaluated by JECFA at its 57th meeting (JECFA, 2002). Short- and long-term studies in rodents showed that 3-MCPD is nephrotoxic in both sexes and also affects the male reproductive tract and male fertility. At that meeting, JECFA considered that the kidney was the main target organ and tubule hyperplasia in the kidney the most sensitive endpoint for establishing a HBGV. This effect was seen in a long-term study of toxicity and carcinogenicity in male and female Fischer 344 rats given drinking water containing 3-MCPD to provide intakes of 0, 1.1, 5.2 and 28 mg/kg bw/day for males and 0, 1.4, 7.0 and 35 mg/kg bw/day for females for 104 weeks (Sunahara, Perrin and Marchesini, 1993). JECFA concluded that 1.1 mg/kg bw/day, the lowest dose, was a lowest observed adverse effect level (LOAEL) and that this was close to a no observed adverse effect level (NOAEL). JECFA established a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw for 3-MCPD on the basis of this LOAEL, using a safety factor of 500. This factor was considered adequate to allow for the absence of a clear NOAEL and to account for the effects on male fertility and for inadequacies in the studies of reproductive toxicity. Data available to JECFA indicated that the estimated mean intake of 3-MCPD for consumers of soy sauce would be at or above this PMTDI.

3-MCPD was again evaluated by JECFA at its 67th meeting (JECFA, 2007). No new toxicology data were available and so JECFA retained the previous PMTDI of 2 µg/kg bw.

At its most recent evaluation at JECFA 83rd meeting (JECFA, 2016), JECFA considered the main form of 3-MCPD typically detected in foods, 3-MCPD fatty acid esters.[3] Substantial hydrolysis (de-esterification) of 3-MCPD esters (monoesters and diesters) to 3-MCPD in the gastrointestinal (GI) tract had been demonstrated, with complete hydrolysis of the 3-MCPD esters assumed. Following gavage dosing of male Wistar rats with equimolar doses of 3-MCPD and 3-MCPD dipalmitate, the relative amount of 3-MCPD bioavailable from the diester was 86 percent on average of the amount bioavailable following administration of 3-MCPD (Abraham *et al.*, 2013). One noticeable difference was that there was a five-fold lower maximal plasma concentration (C_{max}) observed for the diester (949 ng/mL) compared to 3-MCPD (4 850 ng/mL), which was interpreted as resulting from delayed hydrolysis during transit in the gastrointestinal tract. No intact parent 3-MCPD diester was detected in plasma or various organs. In a series of studies commissioned by EFSA to compare the toxicities of 3-MCPD and the palmitic acid esters, it was reported that, based on measurement of 3-MCPD metabolites in urine in rats, the bioavailability of 3-MCPD originating from the diester of palmitic acid was 70 percent (Barocelli *et al.*, 2011). JECFA also concluded that the capacity of the neonate to hydrolyze fatty acids in the gut is efficient, and therefore the same assumption of substantial hydrolysis could be extended to this age group.

In a more recent two-year oral carcinogenicity study in Sprague Dawley (SD) rats in which 3-MCPD was administered in the drinking water (Cho *et al.*, 2008), the incidences of nephropathy and renal tubular hyperplasia were significantly greater than in controls at all doses tested (1.97, 8.27 and 29.5 mg/kg bw/day) in males, with the hyperplasia more frequently observed in males.

The main target organs for 3-MCPD and its esters in rats and mice are the kidneys and the male reproductive organs. 3-MCPD was carcinogenic in two rat strains, but not in mice. 3-MCPD has shown no genotoxic potential *in vivo*. Two long-term carcinogenicity studies with 3-MCPD in rats were identified as pivotal studies, and renal tubular hyperplasia was identified as the most sensitive endpoint. The lowest $BMDL_{10}$ for renal tubular hyperplasia was calculated to be 0.87 mg/kg bw/day for male rats from the Cho *et al.* (2008) study. After application of a 200-fold uncertainty factor, JECFA established a revised group PMTDI of 4 µg/kg bw for 3-MCPD and 3-MCPD esters, singly or in combination, expressed as 3-MCPD equivalents. The overall uncertainty factor of 200 incorporates an additional factor of 2 to take into account inadequacies in the studies of reproductive toxicity.

[3] Note that the 83rd JECFA meeting occurred in 2016 and the summary and conclusions were published at that time, although the full monograph was not published until 2018.

3.2 EUROPEAN FOOD SAFETY AUTHORITY

In 2001, the European Commission's Scientific Committee on Food (SCF) evaluated 3-MCPD and concluded that it is a non-genotoxic carcinogen on the basis of the long-term study in rats by Sunahara, Perrin and Marchesini (1993) (SCF, 2001). Nephropathy and renal tubular hyperplasia were considered critical effects and while there were increased incidences of various tumours observed (renal, Leydig cell, mammary), the carcinogenic effects were considered secondary to chronic progressive nephropathy and hormonal imbalance. The LOAEL of 1.1 mg/kg bw/day for renal hyperplasia was selected for establishing a tolerable daily intake (TDI) of 2 µg/kg bw.

The initial evaluation conducted by EFSA (2016) was specific to the question of the presence of 2/3-MCPD and their fatty acid esters, and GEs in food. The EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that fatty acid esters of 3-MCPD undergo rapid presystemic de-esterification in the GI tract and the toxic effects observed with 3-MCPD were considered equivalent to those with the 3-MCPD esters. In experimental animal studies, kidney and testes were regarded as the main target organs with equimolar multiple doses of 3-MCPD and 3-MCPD dipalmitate producing similar (pattern and magnitude) biochemical changes associated with renal toxicity. Similar to the earlier JECFA evaluation, while some tumours in experimental animals developed following chronic exposure to 3-MCPD in drinking water, there was no evidence to suggest 3-MCPD was genotoxic *in vivo*. Considering a range of both renal and testes toxicity, EFSA selected the lowest $BMDL_{10}$ value of 0.077 mg/kg bw/day for increased renal tubular hyperplasia in male rats from the chronic drinking water study of Cho *et al.* (2008) as the basis for establishing their HBGV (TDI) of 0.8 µg/kg bw/day, which is applicable to 3-MCPD and its fatty acid esters.

Following their 2016 review, EFSA provided an updated assessment for 3-MCPD and its fatty acid esters with specific emphasis on reproductive and developmental toxicity that incorporated new guidance on dose-response modelling adopted by the Scientific Committee of EFSA in 2017 (EFSA, 2018). Renal and testicular toxicity were again noted as the main effects associated with exposure of experimental animals to 3-MCPD and its fatty acid esters. Tubular hyperplasia was reconfirmed as the key effect in kidneys of rats and using the updated guidance for dose-response modelling (model averaging), a $BMDL_{10}$–$BMDU_{10}$ interval for 3-MCPD of 0.20–1.95 mg/kg bw/day was obtained from the same critical study used in the 2016 assessment. Various male fertility parameters were also assessed in detail with a dose-related decrease in the percentage of motile sperm observed, with concurrent decreases in a series of sperm motility parameters following short-term (nine days) exposure to 3-MCPD doses up to 10 mg/kg bw/day. BMD modelling of sperm velocity resulted in a $BMDL_{05}$–$BMDU_{05}$ confidence interval of 0.44–3.88 mg/kg bw/day, while modelling the decrease in sperm counts seen after 3-MCPD exposure at doses up to 16 mg/kg bw/day for 90 days resulted in a $BMDL_{23}$ (benchmark response [BMR] set to one SD of control group) of 1.23 mg/kg bw/day.

It was also noted that 3-MCPD exposures of up to 30 mg/kg bw/day for less than two weeks seemed to have minimal effect on sperm counts while significant decreasing trends in epididymal sperm count could be seen in rats after 13 weeks of exposure to 3-MCPD at doses >4 mg/kg bw/day.

As with the previous assessment, renal tubular hyperplasia in male rats following chronic exposure to 3-MCPD was selected as the critical effect for establishing the HBGV. The lowest $BMDL_{10}$ obtained from using a model averaging approach, 0.20 mg/kg bw/day, was selected along with a composite uncertainty factor of 100 to establish a TDI of 2.0 µg/kg bw.

CHAPTER 4
PREVIOUS ASSESSMENTS – GLYCIDOL AND ITS FATTY ACID ESTERS

4.1 GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

In 2009, following the detection of GEs in refined vegetable oils, the German Federal Institute for Risk Assessment (BfR) conducted an evaluation to determine whether ester-bound glycidol could pose a risk to human health (BfR, 2009). At the time, the levels of GEs in oils could not be precisely quantified, therefore the assessment was based on the assumption that one kilogram of edible fat contains one milligram of glycidol. The two-year (chronic) bioassay in rats and mice conducted by the United States National Toxicology Program (NTP, 1990) was selected as the critical study, and the formation of mesotheliomas in the tunica vaginalis/peritoneum (TVM) in male rats was identified as the most sensitive endpoint. Based on this endpoint, BfR derived a T_{25} value[4] of 10.2 mg/kg bw/day and concluded that formula-fed infants may be exposed to harmful levels of glycidol. More recently, BfR issued an updated opinion on the possible health risks due to high concentrations of GEs in certain foods, including infant formula (BfR, 2020). The BfR did not conduct a *de novo* assessment of the hazard potential of GEs but rather elected to use the reference point previously derived. A potential health risk due to chronic glycidol exposure was identified for certain subpopulations, including formula-fed infants, as well as under various consumption scenarios (such as frequent consumers of frying fat).

[4] The T_{25} is the chronic daily dose which results in 25 percent of the animals developing tumours at a specific tissue site, after correction for spontaneous incidence, within the standard life span of that species (Dybing *et al.*, 1997). MOEs greater than 25 000 from a T_{25} value are generally considered to be of low priority for risk management.

4.2 CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

The human cancer potency of glycidol was estimated and used to calculate a "no significant risk level" (NSRL) by the California Environmental Protection Agency (CalEPA, 2010). CalEPA also selected the NTP (1990) bioassay as the critical study, but, as glycidol is a multi-site carcinogen, rather than selecting the most sensitive tumour type, Monte Carlo modelling was used to probabilistically sum the potencies from the different tissue types. Allometric scaling was then applied to derive a human-equivalent multisite cancer potency estimate of 1.3 $(mg/kg\ bw/day)^{-1}$. Based on this potency estimate, the NRSL (defined as the intake associated with a lifetime cancer risk of 10^{-5}) was determined to be 0.54 µg/day. However, the potency estimate assumes that tumours arising at different sites/tissues are independent. Given that many, if not most, tumours likely depend on the same mechanism of action (i.e. DNA adduct formation and incomplete repair or misrepair) as well as the fact that they occur in the same animals, in the opinion of the current authors the assumption of statistical independence across tumour sites may overstate the true cancer risk.

4.3 FOOD SAFETY COMMISSION OF JAPAN

The Food Safety Commission of Japan (FSCJ) also conducted a safety assessment of glycidol and its fatty acid esters in food (FSCJ, 2015). A $BMDL_{10}$ of 1.6 mg/kg bw/day was derived from the two-year NTP (1990) bioassay using TVM in male rats as the critical effect and uncertainty factors totaling 1 000 were applied to the $BMDL_{10}$ to obtain a TDI of 1.6 µg/kg bw/day. Daily intakes of GEs were estimated for the general population as well as for males aged 15–19, who were determined to be the greatest consumers of edible oils. FSCJ concluded that while MOEs in these populations were acceptable, due to the genotoxic potential of glycidol an "as low as reasonably achievable" (ALARA)[5] approach was recommended. Formula-fed infants were estimated to have the highest dietary exposure to glycidol on a per kilogram basis, although given that intake of infant formula is limited to the period of infancy it was concluded that it would be inappropriate to use these dietary exposure estimates for risk assessment of chronic exposure.

4.4 JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

JECFA evaluated GEs in response to a request from the Codex Committee on Contaminants in Foods (CCCF) at their 83rd meeting (JECFA, 2016; 2018). As GEs are substantially hydrolyzed to glycidol in the GI tract and elicit toxicity as glycidol, JECFA based its evaluation on the conservative assumption of complete hydrolysis of GEs to glycidol. JECFA noted that glycidol was clearly genotoxic *in vitro* in many bacterial and mammalian cell assays on mutagenicity with and without an

[5] ALARA refers to the goal of maintaining contaminants in food, particularly those that are genotoxic, to the lowest level practical when current agricultural or manufacturing practices cannot completely eliminate their presence.

exogenous metabolic system. Glycidol also tested mostly positive for genotoxicity *in vivo*, inducing DNA strand breaks in rat liver and urinary bladder, as well as induction of micronuclei, chromosomal aberrations and sister chromatid exchanges in mouse bone marrow. Therefore, JECFA concluded that glycidol is a genotoxic compound and considered its carcinogenicity as the most sensitive endpoint on which to base a point of departure. The lowest $BMDL_{10}$ was 2.4 mg/kg bw/day for TVM in male rats observed in the NTP (1990) carcinogenicity study. JECFA used an MOE approach and concluded that margins were low for a compound that is genotoxic and carcinogenic and may indicate a human health concern.

4.5 EUROPEAN FOOD SAFETY AUTHORITY

The EFSA CONTAM Panel was also asked to deliver an opinion on GEs in food (EFSA, 2016). However, CONTAM considered the dose–response data from the NTP (1990) bioassay to be inadequate for BMD modelling on account of having only two dose levels. As such, CONTAM elected to use the same approach as BfR (2009), using the T_{25} of 10.2 mg/kg bw/day for TVM in male rats as the reference point. CONTAM concluded that although there is a high uncertainty in the reference point used as a basis for the calculation of the MOEs, in view of the genotoxic and carcinogenic potential of glycidol a health concern was indicated, particularly in the case of formula-fed infants.

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CHAPTER 5
CURRENT FOOD GUIDELINES AND REGULATIONS

Following the 2006 JECFA evaluation of 3-MCPD in which a focus was placed on possible exposures from foods containing acid-HVP and soy sauces (JECFA, 2007), it was stated that high consumers may exceed the HBGV established in 2001, which was retained at the subsequent meeting (2 µg/kg bw). In response, the CCCF developed a *Code of Practice for the Reduction of 3-Monochloropropane-1,2 diol (3-MCPD) during the production of Acid-HVPs and Products that Contain Acid-HVPs* (CXC 64-2008) (CCCF, 2008) and set a maximum level for 3-MCPD at 0.4 mg/kg in liquid condiments containing acid hydrolyzed vegetable proteins.

After the 2016 JECFA evaluation of 3-MCPD and its esters (JECFA, 2018), it was concluded that, for the general population, even consumers in the high dietary exposure category (typically 90–95th percentile) for total diet would be unlikely to exceed the revised HBGV of 4 µg/kg bw. However, it was estimated that formula-fed infants from certain countries might exceed the HBGV by up to 2.5-fold (dietary exposures up to 10 µg/kg bw/day) based on estimated median infant formula consumption. As such, JECFA recommended that appropriate efforts to reduce concentrations of 3-MCPD and its fatty acid esters in infant formula should continue to be implemented. In response to the JECFA recommendations, the CCCF initiated the development of a *Code of Practice for the Reduction of 3-Monochloropropane-1,2 Diol Esters (3-MCPDEs) and Glycidyl Esters (GEs) in Refined Oils* and Food Products Made with Refined Oils (CXC 79-2019) (CCCF, 2019). To date, no Codex maximum levels (MLs) have been established for 3-MCPD in any food category other than those related to the use of acid-HVP.

A similar conclusion was reached by EFSA in that their 2016 evaluation identified the age groups infants, toddlers and other children with dietary exposures likely to exceed the existing HBGV for 3-MCPD and its esters (0.8 µg/kg bw/day) (EFSA, 2016). The maximum of the range of dietary exposures for the latter age groups were 1.5 µg/kg bw/day and 2.6 µg/kg bw/day for mean and high (P95)

estimates, respectively. If considering infants consuming only infant formula products (170 g/kg bw/day), the mean and P95 dietary exposure estimates (based on mean and P95 3-MCPD concentrations in infant formula) were 2.4 µg/kg bw/day and 3.2 µg/kg bw/day, respectively. After establishing a new HBGV of 2.0 µg/kg bw/day in 2018, the conclusions were that adults in the mean and high dietary exposure groups were unlikely to be exceeding the HBGV, while estimated dietary exposure for younger age groups, including formula-fed infants, would slightly exceed the HBGV. Previously, in 2006, the European Commission had set a maximum level of 20 µg/kg for 3-MCPD in HVP and soy sauces, which was expanded in 2020 to include additional MLs for vegetable oils and fats, fish oils and oils from other marine organisms destined for the production of baby food and processed cereal-based food for infants and young children (750 µg/kg), infant formula, follow-on formula and foods for special medical purposes intended for infants and young children, and young-child formula, powder (125 µg/kg) and liquid (15 µg/kg) (Table 1).

As described above, in 2016 both JECFA and CONTAM also evaluated GEs in food. As GEs are substantially hydrolyzed in the GI tract and elicit toxicity as glycidol, both committees based their evaluation on the conservative assumption of complete hydrolysis of GEs to glycidol. A two-year bioassay of glycidol exposure in rats and mice (NTP, 1990) was selected as the critical study, and TVM in male F344 rats was identified as the most sensitive endpoint. Although the selection of a reference point differed slightly between the two assessments, both used an MOE approach and concluded that, in view of the genotoxic and carcinogenic potential of glycidol, margins were low and may indicate a human health concern, particularly in the case of formula-fed infants. In response to the updated guidance from EFSA, the European Commission published Commission Regulation (EU) 2023/915, which amended Regulation (EC) No 1881/2006 regarding MLs of glycidyl fatty acid esters in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. With the aim of excluding any possible health concerns related to dietary exposure to GEs and taking into account dietary exposure of infants solely fed on infant formula, MLs for 3-MCPD and GEs, expressed as 3-MCPD and glycidol equivalents, were established for these product categories as set out in Table 1.

TABLE 1 **EUROPEAN UNION MAXIMUM LEVELS FOR 3-MCPD, 3-MCPD FATTY ACID ESTERS AND GLYCIDYL FATTY ACID ESTERS IN SPECIFIC FOOD PRODUCTS**

	FOODSTUFFS	MAXIMUM LEVEL (µg/kg)
4.1	**3-Monochloropropane-1,2-diol (3-MCPD)**	
4.1.1	Hydrolyzed vegetable protein	20
4.1.2	Soy sauce	20
4.2	**Glycidyl fatty acid esters expressed as glycidol**	
4.2.1	Vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food with the exception of the foods referred to in 4.2.2	1000
4.2.2	Vegetable oils and fats destined for the production of baby food and processed cereal-based food for infants and young children	500
4.2.3	Infant formula, follow-on formula and foods for special medical purposes intended for infants and young children (powder)	50
4.2.4	Infant formula, follow-on formula and foods for special medical purposes intended for infants and young children (liquid)	6
4.3	**Sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD**	
4.3.1	Vegetable oils and fats, fish oils and oils from other marine organisms placed on the market for the final consumer or for use as an ingredient in food falling within the following categories, with the exception of the foods referred to in 4.3.2 and of virgin olive oils: — oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils (composed of refined olive oil and virgin olive oil) and mixtures of oils and fats with oils and fats only from this category, — other vegetable oils (including pomace olive oils), fish oils and oils from other marine organisms and mixtures of oils and fats with oils and fats only from this category, — mixtures of oils and fats from the two above mentioned categories.	 1 250 2 500 Applies to the mixture
4.3.2	Vegetable oils and fats, fish oils and oils from other marine organisms destined for the production of baby food and processed cereal-based food for infants and young children	750
4.3.3	Infant formula, follow-on formula and foods for special medical purposes intended for infants and young children and young-child formula (powder)	125
4.3.4	Infant formula, follow-on formula and foods for special medical purposes intended for infants and young children and young-child formula (liquid)	15

Source: European Commission published Commission Regulation (EU) 2023/915.[6]

[6] Available at https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32023R0915

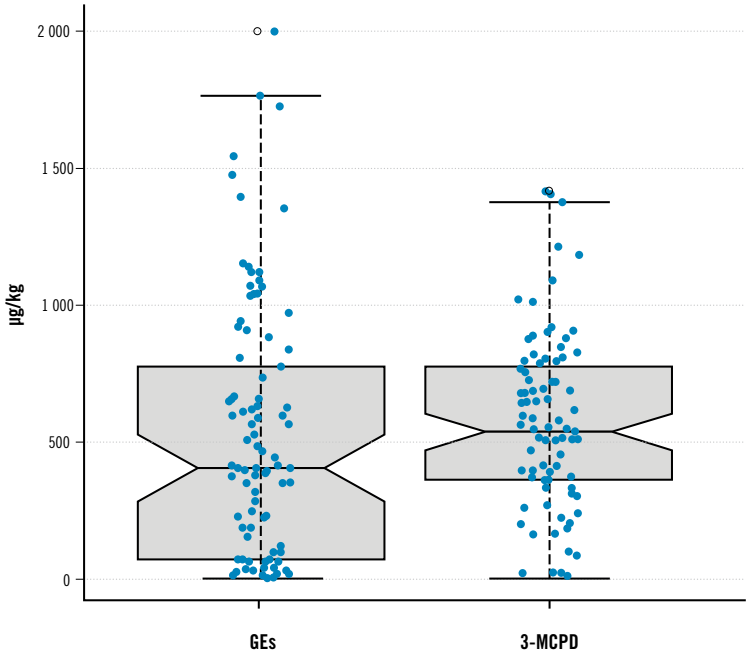

FAO/Giulio Vitale

CHAPTER 6

LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD DATASETS

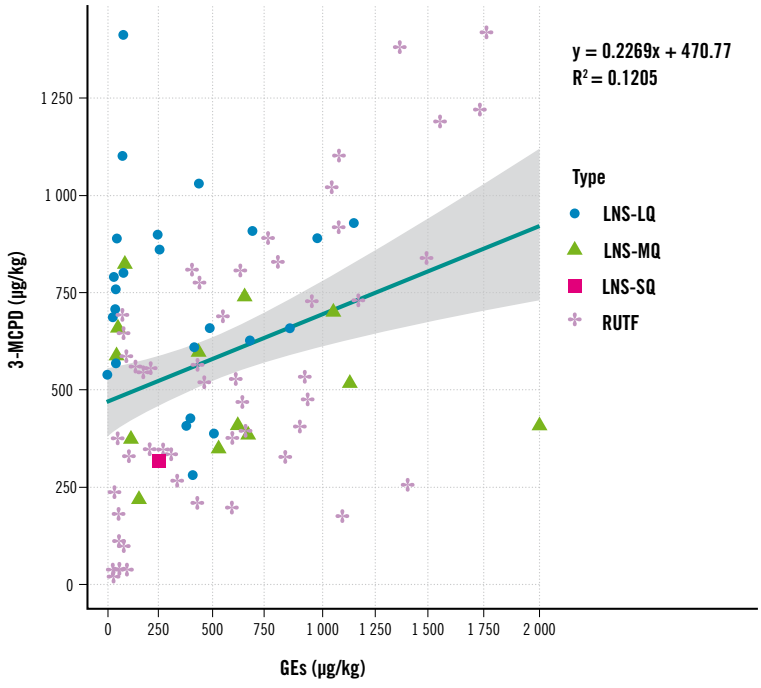
Data concerning concentrations of 3-MCPD and its esters and GEs in LNS/RUTF products ($n = 97$) were received from the two United Nations organizations (WFP and UNICEF) that use these products in the field to treat and prevent malnutrition in populations experiencing food insecurity and acute malnutrition. The products tested were from various manufacturers and consisted of LNS – Large Quantity (LNS-LQ; $n = 26$), LNS – Medium Quantity (LNS-MQ; $n = 18$), LNS – Small Quantity (LNS-SQ; $n = 1$) and RUTF ($n = 52$). 3-MCPD and its fatty acid esters and GE concentrations in these products were measured by third-party laboratories. Neither GEs nor 3-MCPD ester concentrations were significantly different between RUTF and LNS and only a weak correlation was observed between levels of 3-MCPD esters and GEs in the same products (Figure 2 and 3). Summary statistics for the concentrations of 3-MCPD esters (as 3-MCPD equivalents) and GEs in the LNS products sampled may be found in Table 2.

FIGURE 2. **BOXPLOT OF GLYCIDYL FATTY ACID ESTERS (GEs) AND 3-MCPD ESTERS (AS 3-MCPD EQUIVALENTS) CONCENTRATIONS IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD PRODUCTS (N = 97)**



Note: The shaded notched box represents the IQR (inter-quartile range) of the data, therefore the bottom of the box is Q1 (25th percentile), the top is Q3 (75th percentile) and the line in the middle is the median (50th percentile). The horizontal lines on the vertical "whiskers" represent the max and min values, excluding potential outliers. The closed circles represent the GE or 3-MCPD content in the 97 individual LNS/RUTF products tested, while the open circles indicate suspected outliers (i.e. data points that are more than 1.5 IQR above Q3).

FIGURE 3. **ASSOCIATION BETWEEN 3-MCPD LEVELS AND GES IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD PRODUCTS**



Note: Shaded area represents the 95 percent confidence region of the linear regression line.

TABLE 2 SUMMARY STATISTICS FOR THE CONCENTRATION OF 3-MCPDS AND GLYCIDYL FATTY ACID ESTERS IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD PRODUCTS (N = 97)

SUMMARY STATISTIC	3-MCPD (µg/kg)	GEs (µg/kg glycidol equivalent)
Minimum	30	8.3
Maximum	1 420	2 010
Mean	588	517
50th percentile (median)	550	420
95th percentile	1 118	1 416

LNS/RUTF products come in various formats for various indications, as shown in Table 3. The analytical data received were based on LNS and RUTF, which have the highest recommended daily doses and therefore the greatest potential for exposure to process-induced contaminants.

TABLE 3 INTENDED USES AND DAILY CONSUMPTION LEVELS OF LIPID-BASED NUTRIENT SUPPLEMENTS, READY-TO-USE SUPPLEMENTARY FOOD AND READY-TO-USE THERAPEUTIC FOOD

PRODUCT	INTENDED USE	TYPICAL DOSAGE/DAY	PACK SIZE
RUTF	During the period of SAM treatment, RUTF is the sole source of food, except for breast milk in the case of breast-fed infants.	184 g	92 g
LNS-LQ	Part of a nutritional programme to treat moderate acute malnutrition for children 6 months and older.	100 g	100 g
LNS-MQ	Prevent malnutrition for children 6 months and older.	50 g	50 g
LNS-SQ	Complement the diet of children aged 6 months and older with essential nutrients.	20 g	20 g
LNS-PLW	To supplement the diet of pregnant and lactating women as part of a nutritional programme.	75 g	75 g

Note: Dosage may vary depending on the weight of the child and the severity of wasting; SAM refers to severe acute malnutrition.


IS PREVENTABLE
If I had taken Oncho Tablet I would not be blind
Always cover food to prevent diarrhoea
Kaba yu it oltem fo mek yu no get Ronbele
IMMUNIZATION ROOM
FOR A POLIO FREE SIERRA LEONE
If I had taken Oncho tablet, I would not be BLIND
Oncho tablet is FREE
Go for HIV Test during pregnancy
BURY ALL EMPTY TINS TO PREVENT MALARIA
Berr N Emti tin fo mek yu no get MALERIA
MCH/ANC/FP
Protector
Sight is Life
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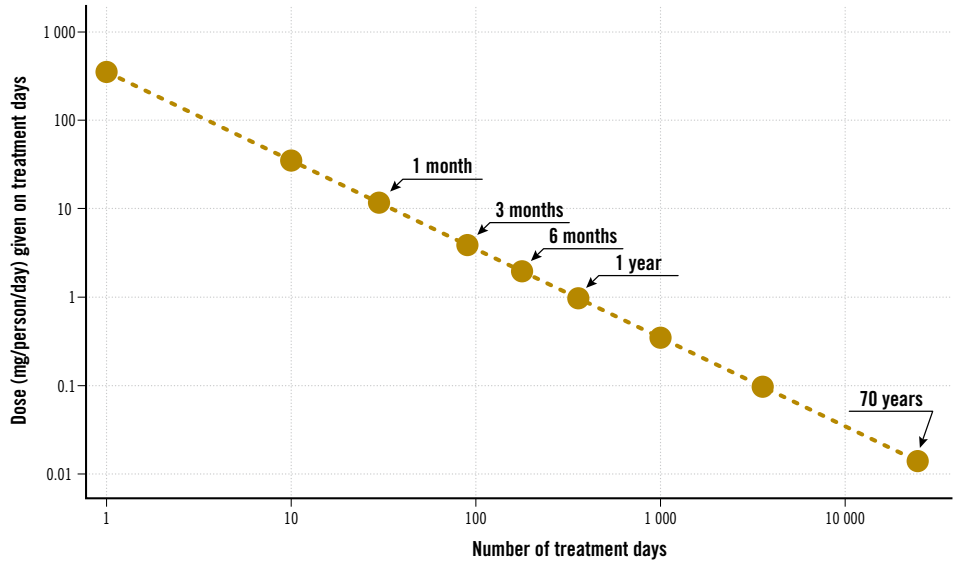
CHAPTER 7
LESS-THAN-LIFETIME EXPOSURE

It should be noted that the term "less-than-lifetime" exposure could describe many distinct scenarios, but in the current context refers to daily exposure over a period of weeks to months to a maximum duration of one year. All previous human health risk assessments of GEs and 3-MCPD by international expert committees selected a reference point based on chronic dietary exposure, as these substances are common constituents of a range of foods to which humans are exposed throughout life. Despite the fact that dietary exposure to GEs and 3-MCPD and its esters is chronic, some potentially important sources of exposure to these substances such as infant formula (and LNS/RUTF) are only consumed for a limited period and at a particular life stage. In the case of GEs, the toxicity of which is mediated by the genotoxic carcinogen glycidol, previous assessments (e.g. JECFA, 2016) have compared a reference point derived from a chronic animal study to less-than-lifetime exposure, such as dietary exposure during infancy, and concluded that MOEs indicate a potential cause for concern. This approach is highly conservative, as it is generally assumed that carcinogenic effects are proportionate to both the dose and the duration of exposure. Therefore, cancer risk estimates for genotoxic carcinogens are typically based on the LADD, which is derived from the total cumulative exposure (Gaylor, 2000), and high doses received over short periods of time are amortized in accordance with Haber's rule.

According to this principle, the product of dose and time is equal to a constant ($d * t = k$) and thus the cancer risk of a continuous low dose over a lifetime would be equivalent to an identical cumulative exposure averaged over a shorter duration (ICH, 2018; Figure 4). The applicability of Haber's law to genotoxic carcinogens is premised on the notion that carcinogenesis is a stochastic process, and therefore the probability of a tumour occurring is proportional to the number of molecules of the genotoxic substance at the target site, i.e. the total cumulative dose (Felter *et al.*, 2011). Application of Haber's rule might also be considered appropriate for non-genotoxic carcinogens, particularly when exposure has to be prolonged for carcinogenic effects to become manifest (CoC, 2019). Although Haber's rule is a theoretical consideration, the principle of averaging the cumulative dose received over a lifetime to derive a LADD as an appropriate measure of exposure does

have some empirical support and may be considered a health-protective option (EPA, 2005; Felter *et al.*, 2011). It is also important to note that risk-benefit considerations are not generally accounted for when assessing genotoxic carcinogens in food, as in the previous assessments of 3-MCPD and its esters and GEs by JECFA (2016) and EFSA (2016; 2018). However, in the case of therapeutic foods such as LNS/RUTF, which are consumed for a prescribed period of time to treat and prevent childhood malnutrition, the significant health benefits in terms of reduced morbidity and child mortality must be weighed against any potential adverse effects associated with process-induced contaminants.

FIGURE 4. **DOSE CORRESPONDING TO A 1:100 000 CANCER RISK (MG/PERSON/DAY)**



Note: Haber's rule states that the incidence or severity of a toxic effect depends on the cumulative dose (i.e. the total combined exposure). The dashed line represents the relationship between the daily intake of glycidol corresponding to a 1:100 000 cancer risk and the number of treatment days, with the linearization being a function of the double-logarithmic scale for time and exposure.

CHAPTER 8
HAZARD CHARACTERIZATION UPDATE ON 3-MCPD

8.1 ACUTE TOXICITY

Male and female Swiss mice (4 weeks old; *n* = 5 per sex) were administered single doses of 1 000, 1 428, 2 040, 2 914 or 4 162 mg/kg bw of 1-stearic, 1-oleic, 1-linoleic, 1-linoleic-2-palmitic and 1-palmitic-2-linoleic acid esters of 3-MCPD orally by gavage and observed for 14 days. LD_{50} values for 3-MCPD esters of steric, oleic and linoleic acids ranged from 2 016–2 973 mg/kg bw (597–871 mg/kg bw 3-MCPD equivalent dose) while those of the diesters were >5 000 mg/kg bw (Liu *et al.*, 2017). In comparison, the LD_{50} for 3-MCPD in ICR mice has been reported to be 190.7 mg/kg bw (Qian *et al.*, 2007).

8.2 SHORT-TERM STUDIES

Heterozygous transgenic heme oxygenase triple (HOTT) reporter male mice of a C57BL/6NTac background (*n* = 20), approximately 25 g bw, were randomly allocated to four dose groups (*n* = 5 per dose group) and given 3-MCPD orally by gavage at doses of 0, 1, 10 or 100 mg 3-MCPD/kg bw/day for 28 days. These transgenic mice have a bacterial *lacZ* gene encoding β-galactosidase that is under the control of the heme oxygenase 1 (*Hmox1*) promoter. The *Hmox1* promoter is activated by pro-oxidant stimuli, predominantly via the Nrf2 transcription factor. At study termination, the mice were terminated, and various organs collected for assessment of toxicity and indications of oxidative stress (Schultrich *et al.*, 2020). Mice in the highest dose group were euthanized at day seven due to severe weight loss (>20 percent) while all other dose groups showed growth comparable to the controls. Relative organ weights for kidneys, testes, spleen and liver were not affected by 3-MCPD treatment in the low- and mid-dose groups, with the mid-dose animals showing a minimal, though statistically significant, increase in relative heart weight.

No increase in oxidative stress was observed in liver, heart, or spleen, while animals in the mid-dose group showed an increase in β-galactosidase in kidney. Also, primarily in the kidney, increased expression of various genes associated with detoxification of reactive oxygen species was observed in both low- and mid-dose animals, but this showed no dependency with dose in the low- and mid-dose groups.

Male C57 mice (25–30 g bw; $n = 6$ per group) were exposed to 3-MCPD via drinking water to provide doses of 0, 2, 4, 8 or 32 mg/kg bw/day, five days per week for eight weeks. At the end of the dosing period, the mice were terminated, and kidneys collected for subsequent gene expression analysis (Khosrokhavar *et al.*, 2021). No significant change in bodyweight gain was observed and histopathological analysis of kidney tissue was not undertaken. Expression of the proinflammatory cytokine interleukin-18 (IL-18) mRNA was upregulated in all dose groups but with no significant difference among the three highest dose groups. Similar results were seen with expression of Nrf2 mRNA in the kidney; all dose groups showed up regulation compared to controls but with no significant difference among the three highest dose groups. *Nrf2* is activated in response to cellular stress and regulates the transcription of components of the glutathione and thioredoxin antioxidant systems. Supporting this observation were results showing that Sirtuin 3 (*Sir3*) gene expression was also upregulated in all dose groups compared to controls. The *Sir3* gene is located in the mitochondrial matrix and functions to control homeostasis during periods of stress that would disrupt mitochondrial function.

Male Wistar rats ($n = 5$) were exposed to 10 mg/kg bw/day 3-MCPD by gavage for 28 days. Kidney, testes and liver were collected at termination and subjected to comparative RNA transcriptomic analysis. No histopathological alterations were detected in liver and kidney, while two out of six rats showed multifocal slight or moderate tubular degeneration in the testes. The number of up- or down-regulated transcripts was highest in kidneys, followed by the liver and then testes. The highest degree of down regulation (76-fold) was observed for the mRNA encoding CYP24A1 whereas the strongest up regulation (40-fold) was observed for *Ugt2b4*, a gene coding for an enzyme from the UDP-glucuronosyltransferase family (Buhrke *et al.*, 2017).

In a similar study, male SD rats ($n = 6$ per dose group) were dosed by gavage with 0, 15, 30 or 40 mg/kg bw/day of 3-MCPD for 28 days. At termination, blood samples and kidneys were collected for histopathological and proteomic analysis. Relative kidney weights were increased in a dose-related manner compared to controls (approximately 28 percent in the low-dose animals, increasing to 91 percent in the high-dose group) with significant increases in blood urea nitrogen and creatinine also seen in the high-dose animal groups. Dysregulated proteins detected in the kidney tissue of animals from the high-dose group included several enzymes related to the metabolism of amino acids, lipids and carbohydrates, as well as proteins involved in pathways including oxidative stress, oxidative phosphorylation, apoptosis and autophagy (Jin *et al.*, 2021). Loss of mitochondrial homeostasis and function and cell death pathways were described as being involved in the development of renal damage induced by 3-MCPD.

Male Wistar Albino rats, 8 weeks old (n = 10 per dose group), were exposed daily by gavage to 0, 0.87 or 10.0 mg/kg bw/day 3-MCPD for 28 days. At termination, blood and brain tissue were collected from all animals for histochemical evaluation and gene expression analysis (Sevim *et al.*, 2021). No significant changes in blood aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) levels were noted. Relative gene expression levels of miR-21 in the brain were significantly reduced in both dose groups to the same extent, while immunoreactivity of caspase-3 and apoptosis-inducing factor (AIF) in cerebellum was increased only in the high-dose animals. miR-21 is an oncogenic miRNA that plays a key role in tumour progression and apoptosis balance and which has an up regulation that has been reported to lead to tumour suppressor gene down regulation and subsequent cell proliferation. Caspase-3 and AIF are both involved with cellular and nuclear apoptosis.

Male Wistar rats (45 days old; prepubertal; n = 10 per dose group) received 3-MCPD by gavage at doses of 0, 2.5, 5.0 or 10 mg/kg bw/day for 30 days. At termination, various organs were collected for a range of analyses, including testicular and epididymal histology and sperm parameters (Vieira and Favareto, 2017). No significant effects were noted for body weight gain or relative organ weights compared to the controls. Sperm number per gram of testis and daily sperm production per gram of testis were significantly reduced ($p < 0.05$) in mid- and high-dose groups when compared to the control group (maximum decrease approximately 18 percent), however sperm number in the caput/corpus epididymis, epididymal transit time and sperm morphology were similar among all experimental groups. The number of sperm with progressive movement was significantly decreased ($p < 0.05$) resulting in the percentage of immotile sperm being increased ($p < 0.05$) in the mid- and high-dose groups. Spermatid changes were not associated with any observable pathological changes in the reproductive organs. While the stages of spermatogenesis were not affected, the number of Sertoli cells and germ cells was slightly lower in the mid- and high-dose groups when compared to controls. No fertility assessment was conducted.

Male SD rats, 6 weeks old, 20 per dose group, were exposed to 3-MCPD by gavage at doses of 0, 36 or 72 mg/kg bw/day for four weeks. At the end of the exposure period, 12 animals per dose group were terminated and various organs collected for histopathological analysis and sperm assessed for numbers and motility. The additional animals in each dose group were held on control diets for an additional seven weeks before being terminated and assessed in a similar manner to the rats terminated at four weeks (Xing *et al.*, 2019). Body weight gain during the exposure period was significantly reduced in the high-dose animals compared to controls, however all animals showed a similar weight gain during the seven-week recovery period. Various hematological parameters (red blood cell [RBC] count, hemoglobin [Hgb] and hematocrit [HCT]) showed decreases in both 3-MCPD dose groups during the exposure period but were similar to controls by the end of the recovery period. Relative liver and kidney weights were also increased in both dose groups but again were similar to control values at the end of the recovery period.

3-MCPD induced a significant decrease in the weight of the testes and epididymis, and in the total sperm concentration in the high-dose group along with significant decreases in the percentage of motile sperm and progressive sperm, and a significant increase in the percentage of sperm with abnormal morphology in rats exposed to both doses of 3-MCPD for four weeks, which were still different from control values at the end of the recovery period.

Male SD rats, 6 weeks old (n = 12 per dose group), were maintained on diets designed to deliver doses of 0, 10 or 100 mg/kg bw/day 3-MCPD monooleate, or 15 or 150 mg/kg bw/day 3-MCPD monostearate, for 90 days. Equivalent 3-MCPD doses would be 2.8 and 28.0 mg/kg bw/day and 4.2 and 42.0 mg/kg bw/day, respectively. At the end of the treatment period, the rats were terminated, and various tissues were collected for histological and molecular analysis (Yang *et al.*, 2020). No difference in body weight gain was observed for any dose group and only absolute kidney weights were changed (increased) at all doses, with the exception of the low-dose 3-MCPD monooleate group (no effect). While the study authors reported no change in body weights, relative organ weights were not provided. Similar degrees of histopathological changes in kidney tissues were observed with both esters, with the majority of animals in both high-dose groups showing effects. A greater number of proteins were differentially expressed in the high-dose monostearate kidney samples (151) compared to the high-dose monooleate kidney samples (83), with the major pathways involving ion transport, apoptosis and xenobiotic metabolism.

Male F344 rats (n = 10 per dose group) were exposed to 3-MCPD via drinking water for 13 weeks at concentrations providing doses of 0, 0.7, 2.2, 6.7, 21.3 or 54.0 mg/kg bw/day (Toyoda *et al.*, 2017). Final body weight was decreased in only the high-dose animals, while various mild haematological and serum biochemical effects were seen in animals mainly from the two highest dose groups, with the exception of serum creatinine, which was significantly decreased in the three highest dose groups. It should be noted that only the high-dose animals had serum creatinine outside of the reference range reported by Loeb and Quimby (1999) for F344 rats. Relative liver and kidney weights increased in a dose-dependent fashion in animals from the three highest dose groups while histopathological changes were limited to kidneys (high-dose group only) and epididymis (two highest dose groups). The NOAEL was determined to be 2.2 mg/kg bw/day.

Previous work reviewed by the 83rd JECFA meeting (Sawada *et al.*, 2015; 2016) attempted to identify cellular targets responsible for the kidney and testicular toxicity observed as sensitive endpoints in experimental animals for 3-MCPD. The oxidative metabolites of 3-MCPD, β-chlorolactaldehyde and β-chlorolactic acid, have been shown to inhibit glycolytic enzymes and cause oxidative stress, which in turn contributes to the toxic effects in both the kidney and male reproductive system. Proteomic analysis of liver, kidney or testes had revealed that the DJ-1 protein was one of the most affected proteins in rats treated by gavage, either with 10 mg/kg bw/day 3-MCPD or with a molar equivalent dose of 3-MCPD dipalmitate for 28 days.

The DJ-1 protein is a small (20 kDa) protein that is ubiquitously expressed in more than 20 human tissues, including kidney and testes, and, among other diverse functions, acts as a reactive oxygen species (ROS) scavenger mainly due to the presence of three redox-sensitive cysteine residues. The reduced form of DJ-1 functions to eliminate excessive ROS and is over-expressed in the presence of various oxidative agents (Zhang *et al.*, 2020). In the studies of Sawada *et al.* (2015; 2016), while the total amount of DJ-1 protein in the animals was not affected by 3-MCPD treatment, approximately 90 percent of DJ-1 was present in kidney and liver in its inactive or oxidized form, compared with only around 10 percent in controls (Buhrke *et al.*, 2018).

8.3 CHRONIC TOXICITY AND CARCINOGENICITY

Two principal studies were previously identified by both JECFA and EFSA as critical in their evaluation of the carcinogenic potential of 3-MCPD. Cho *et al.* (2008) and Sunahara, Perrin and Marchesini (1993) both exposed different rat strains (SD and F344, respectively) to drinking water containing 3-MCPD over 104 weeks. While various tumour types, including kidney, testis and mammary, were reported to be significantly increased compared to the control animals, these increases were considered to be secondary to organ damage and/or hormonal imbalance. Combined with the absence of *in vivo* genotoxicity potential for 3-MCPD, both JECFA and EFSA concluded that the tumourigenic response was due to a non-genotoxic mode of action.

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

8.4 GENOTOXICITY

While various *in vitro* tests of mutagenicity have yielded positive results with 3-MCPD and 3-MCPD fatty acid esters (bacterial reverse mutations, SCE V79 cells, gene mutations in mouse lymphoma cells), *in vivo* test results for chromosomal aberrations in the micronucleus assay with bone marrow, Pig-a gene mutation assay with red blood cells and the *gpt* assay for mutant frequencies of *gpt* and *red/gam* (Spi–) genes in kidneys and testes were negative.

Female BalbC mice (n = 4 to 5 per dose group) were administered 3-MCPD dissolved in 0.9 percent saline at dose levels of 0, 50, 75, 100 or 125 mg/kg bw via intraperitoneal (i.p.) injection (15 uL/g bw). The positive control mice were administered acrylamide at 60 mg/kg bw, dissolved in 0.9 percent saline. Forty-five hours after dosing, the mice were terminated and the peripheral blood erythrocytes were collected for micronuclei detection. Only acrylamide showed a clear genotoxic effect (Aasa, Törnqvist and Abramsson-Zetterberg, 2017).

8.5 REPRODUCTIVE/DEVELOPMENTAL TOXICITY

While there is currently a lack of single or multi-generation reproductive toxicity studies conducted with either 3-MCPD or related fatty acid esters, there are numerous investigations into the effects on sperm parameters and/or declines in fertility after treatment of male rats for varying periods of time. As described by the 57th JECFA meeting report, short-term exposure (up to two weeks) of male rats to 3-MCPD at doses typically exceeding 3–5 mg/kg bw/day results in impaired sperm mobility in the epididymis, increases in testicular and epididymal lesions and decreased sperm counts. However, in a developmental toxicity study in which pregnant SD rats, n = 5 to 6 per dose group, were treated from gestational day 11.5 through day 18.5 by gavage with daily doses of 0 (vehicle control), 5, 10 or 25 mg/kg bw/day of 3-MCPD, testicular morphology of either fetuses or 3–5-day old neonates was described by the authors as being comparable to the controls. Only maternal, not fetal, weight gain was reduced in the mid- and high-dose group (El Ramy *et al.*, 2006). In addition to the lack of histopathological effects, intratesticular testosterone levels and testicular secretion in response to human chorionic gonadotropin stimulation were unchanged in the high-dose group compared to controls when measured in the 19.5-day old fetuses. Gene expression in fetal testes for markers of Leydig and Sertoli cell function, Ah receptor, peritubular and germ cells, as measured by semi-quantitative RT-PCR, were also unaffected by 3-MCPD treatment. This would imply that the developmental and perinatal periods are not overly sensitive compared to post-puberty for 3-MCPD-induced testicular effects. As nephrogenesis is essentially complete in humans by term (Frazier, 2017), it could also be theorized that neonates and infants would not be more susceptible to 3-MCPD-induced renal effects compared to post-pubertal individuals.

In a study previously reviewed by EFSA (2018), male SD rats (n = 15 per dose group) were exposed to 3-MCPD at doses of 0, 1, 3 or 10 mg/kg bw/day by gavage for nine consecutive days. On the last dosing day, the animals were placed with untreated females for approximately 12 hours for fertility assessment. At sacrifice, male sexual organs and sperm were collected for analysis (Ban *et al.*, 1999). A slight increase in relative epididymis weights in the high-dose animals was the only reproductive organ weight affected by treatment while there were no treatment-related histopathological findings in either testes or epididymis. The fecundity index was significantly decreased in the mid- and high-dose groups only, with no females in the 10 mg/kg bw/day dose group becoming pregnant. While no significant change in sperm numbers were observed, sperm motility (velocity and direction) was decreased in the mid- and high-dose groups. Sperm numbers reaching the oviducts of females was on average decreased by almost 99 percent in the high-dose animals. The dose of 1 mg/kg bw/day was considered by EFSA to be the NOAEL (no change in sperm numbers, sperm motility or fertility index) while a $BMDL_{10}$ of 1.4 mg/kg bw/day for a 10 percent decline in curvilinear sperm velocity was calculated as part of this review (Annex 1).

In a similar study, male SD rats (n = 6 per dose group) were exposed to 3-MCPD at doses of 0, 3, 10 or 30 mg/kg bw/day by oral gavage for seven days and then assessed for spermatotoxicity at termination. The only significant change in reproductive organ weights was a slight increase in epididymis weights in the high-dose group while there was a dose-dependent increase in the number of rats with evidence of abnormal histopathological findings in the epididymis (vacuolization of epididymal epithelium in 100 percent of rats in the high-dose group). Sperm motility was significantly reduced only in the mid- (25 percent) and high-dose animals (31 percent) (Kim *et al.*, 2012).

In the study by Hoyt *et al.* (1994), increases in testes weights were observed in male CD rats (n = 10 per dose group) exposed by oral gavage to 25 mg/kg bw/day of 3-MCPD for 14 days. However, the testicular weights were not different from control values after 14 days of control diet. The same dose produced decreases in sperm numbers and motility (path, amplitude, velocity), which were still different from control values after two weeks on a 3-MCPD-free diet. Similar effects on sperm motility were observed in rats from the same study dosed with 5 mg/kg bw/day, with both the 5 and 25 mg/kg bw/day dose groups showing significant declines in fertility. While the sperm motility was almost the same as in the controls in the 5 mg/kg bw/day dose group after two weeks on control diet, fertility was still partially reduced compared to controls. The low dose of 1 mg/kg bw/day produced no effect on sperm number, sperm motility or fertility.

In the study by Xing *et al.* (2019), 3-MCPD induced a significant decrease in the weight of the testes and epididymis, and in the total sperm concentration in the high-dose group along with significant decreases in the percentage of motile sperm and progressive sperm, and a significant increase in the percentage of sperm with abnormal morphology in rats exposed to 3-MCPD at 36 or 72 mg/kg bw/day for four weeks, which were still different from control values at the end of a seven-week recovery period. 3-MCPD also caused significant decreases in serum testosterone and significant increases in serum progesterone levels in both dose groups, but values were similar to control levels after the recovery period. The intratesticular testosterone level was not affected by either 3-MCPD dose while testosterone production was increased in Leydig cells. Both dose groups of 3-MCPD showed significantly down regulated gene expression of Rec8, which is a meiosis-specific component of the cohesion complex that binds sister chromatids in preparation for the two divisions of meiosis (Xing *et al.*, 2022). The authors concluded that 3-MCPD caused spermatogenesis failure by down regulating the expression of meiosis regulators (NRG1, NRG3 and RA) and interfering with androgen-receptor signalling in Sertoli cells.

Testicular samples were also collected from the animals in the study by Yang *et al.* (2020) and assessed for hormonal, proteomic and histopathological changes (Yang *et al.*, 2021). Decreases in serum testosterone levels were observed in all dose groups compared to the controls with no significant differences between the two monoesters. Similar results were seen for increases in serum interferon γ (IFN γ) levels; both 3-MCPD esters tested produced increases compared to the controls with

no significant difference in response between esters. Relative testes weights were slightly, but statistically significantly reduced in both high-dose groups and in the low-dose monooleate group, compared to the controls while both compounds caused similar histopathological damage, resulting in a decrease of spermatids in a single seminiferous tubule in the low-dose monostearate group, and atrophic and irregular seminiferous tubules, with severe aspermatogenesis in both high-dose groups. As with the results from the kidney samples, a greater number of proteins were differentially expressed in the testes samples in the high-dose monostearate animals (305) compared to the high-dose monooleate samples (84) with the main pathways involving inflammatory necrosis.

In male Han Wistar rats (n = 10 per dose group) administered 0, 5, 10 or 20 mg/kg bw/day 3-MCPD by gavage for five days, no significant change in testes or epididymis weights were observed, however, decreases in sperm motility, as scored by visual observation, was dose-dependent and statistically significant in all dose groups compared to controls (Woods and Garside, 1996). No females mated with treated males (any dose) became pregnant, while histopathological changes (including tubular degeneration and/or atrophy, and interstitial hyperplasia in the testes or epididymis) were seen only in the high-dose animals.

In male SD rats (n = 7 to 14 per dose group) treated by gavage with 0, 2 or 8 mg/kg bw/day 3-MCPD for 14 days, there were no effects observed on sperm number, viability, maturation, or number of abnormal sperm. No abnormal histopathological findings were observed in either the testes or epididymis, however, none of the females bred with males from the 8 mg/kg bw/day dose group became pregnant, while the low dose had no effect on fertility (Yamada *et al.*, 1995). While the percent motile sperm was not affected by either dose, sperm activity immediately and two hours after collection was significantly reduced in the high-dose group while sperm activity was reduced in the low-dose group only two hours after collection. After two weeks on a 3-MCPD-free diet, all sperm parameters, including fertility, were similar to control values in the high-dose group (only dose tested). In a range finding study in which the same animals were treated with 10 or 20 mg/kg bw/day 3-MCPD for 14 days, no effects were seen on sperm numbers but sperm activity was decreased to a similar extent in both dose groups and no females became pregnant following mating.

Male rats (strain not provided; n = 15 rats per dose) were given doses of 0, 3.0, 7.5 or 15.0 mg/kg bw/day 3-MCPD via gavage five days per week for 30 or 90 days. At the end of the treatment periods, the rats were terminated, with blood, semen and tissue samples collected for analysis (Moustafa *et al.*, 2022). Following both 30 or 90 days of exposure, there was a dose-dependent reduction in glutathione (GSH) in testicular tissue with a corresponding increase in malondialdehyde concentration, suggesting increased oxidative stress and reduced antioxidant capacity. Sperm motility, as scored by visual estimation, was low to very low in the mid- and high-dose animals following 30 days of exposure and in all dose groups in the 90-day exposure groups. Sperm counts were significantly reduced in all dose groups, with the exception of the low-dose, 30-day exposure animals.

Various degrees of testicular degeneration were described as being more frequently detected and more severe in the 90-day exposure groups. Relative testicular weight, described as testicular somatic index, was not significantly changed in either duration exposure group.

Male SD rats (15 rats per dose) were dosed by gavage with 0, 0.01, 0.05, 0.25, 1 or 5 mg/kg bw/day 3-MCPD for 28 days (Kwack *et al.*, 2004). At the end of the administration period, the animals were mated with untreated females and then, once pregnancy in females had been ascertained, the respective males were terminated for assessment of sperm parameters, spermatogenesis and reproductive organ histopathology. Pregnant females were terminated on gestation day 20. No significant effect on body weight gain was observed in treated males, although there was a minor decrease in relative testicular weight in rats from the 0.05 and 1.0 mg/kg bw/day dose groups which was not associated with any pathologic changes in either the testes or epididymides. Sperm counts and total sperm motility were reduced in the three highest dose groups to a similar extent, however, only rats from the highest dose group showed decreased fertility. No specific effects were noted for histopathological changes in the testes or epididymis. It was proposed by the authors that the spermatotoxic effect of 3-MCPD is mediated via reduced H^+-ATPase expression in the cauda epididymis, which results in altered pH levels and perturbation of sperm maturation and motility. The NOAEL was considered by the authors to be 0.05 mg/kg bw/day based on reduced sperm counts and total sperm motility reported for the three highest dose groups. However, the only significant effects noted in the 1 mg/kg bw/day dose group was reduced epididymal sperm number and motility, which had no effect on fertility.

In the study by Onami *et al.* (2014), male and female F344 rats (n = 10 per dose group) were treated with 3-MCPD palmitate diester, 3-MCPD palmitate monoester or 3-MCPD oleate diester by oral gavage for 13 weeks, five days per week, with dosing designed to be equimolar to 0, 2.5, 10.0 or 40.0 mg/kg bw/day free 3-MCPD. The lowest dose tested for all of the esters, 1.8 mg/kg bw/day 3-MCPD equivalents (averaged over a seven-day week), had no effect on body weight gain, relative kidney or liver weights or increased histopathological lesions of testes, epididymis and kidney, which were affected at both the mid and high doses for all three esters. This study is considered to be relevant for exposure from formula and other related products as the primary form of 3-MCPD would be as a mono- or diester.

8.6 OBSERVATIONS IN HUMANS

Twelve breastmilk samples collected from Czech mothers contained a mean concentration of 3-MCPD esters of 35.5 µg/kg milk (<11–76 µg/kg milk) (Zelinková *et al.*, 2008). No free 3-MCPD was detected above the limit of detection (LOD, 3 µg/kg milk). The main 3-MCPD fatty acid esters detected were symmetric diesters with lauric, palmitic, and oleic acids, and asymmetric diesters with palmitic acid/oleic acid. It was suggested by the authors that the likely source of 3-MCPD in human milk was from the maternal diet. This study was included in the latest JECFA assessment (JECFA, 2016), where

JECFA noted that in animal experiments, 3-MCPD esters undergo metabolism in the GI tract and are not absorbed intact. In addition, there was no evidence to show free 3-MCPD undergoes re-esterification *in situ*. Subsequent to this Czech study, it has been reported that 3-MCPD, measured using an indirect method, was not detected above the limit of detection (2 µg/kg) in 193 human breastmilk samples collected across Canada from 2008–2011 (Becalski *et al.*, 2018). In a randomly selected subset of these samples (n = 11), free 3-MCPD was also not detected. In the most recent analysis, 30 human breastmilk samples, collected from Chinese volunteers, were analyzed for both 3-MCPD and GEs (Li *et al.*, 2022). 3-MCPD esters were detected above the limit of quantification (LOQ) in 100 percent of the samples while GEs were detected in 60 percent of samples. The concentrations were in the range 1.2–71.3 µg/L (mean 29.5 µg/L) for 3-MCPD and not detected (ND) to 21.0 µg/L (mean 8.3 µg/L) for GEs. For 23 samples of infant formula, 100 percent were positive for 3-MCPD esters (1.7 to 362.9 µg/kg; mean 53.3 µg/kg) while 65 percent of the samples were positive for GEs (ND to 30.9 µg/kg; mean 12.9 µg/kg). Estimated dietary exposures for breast-fed infants were reported by the authors to be comparable to those of formula-fed infants up to 6 months of age for 3-MCPD and GEs.

8.7 SUMMARY

Previous 3-MCPD fatty acid ester evaluations have identified both kidney and testes as critical target organs. From two chronic (104 weeks) bioassays where 3-MCPD was delivered to rats via drinking water, the lowest dose ($BMDL_{10}$) associated with a significant increase in renal tubular cell hyperplasia was 0.87 mg/kg bw/day (JECFA, 2016). Use of this dose in establishing a HGBV for 3-MCPD presumes 100 percent of 3-MCPD esters in food are metabolized in the GI tract and release free 3-MCPD. The majority of experimental studies to date support the notion that the toxicological effects induced by 3-MCPD fatty acid esters are similar to those seen with free 3-MCPD. It should, however, be noted that in most foods, the majority of the 3-MCPD present is in the form of fatty acid esters and only a small percentage is present as free (or unesterified) 3-MCPD (Svejkovska *et al.*, 2004).

In comparison to longer duration exposure periods (chronic studies), in the study by Toyoda *et al.* (2017), in which F344 rats were exposed to 3-MCPD by drinking water for 13 weeks, the lowest dose producing no histopathological effects in the kidneys was 21.3 mg/kg bw/day. The overall NOAEL was 2.2 mg/kg bw/day, based on increased relative liver and kidney weights. While not directly comparable to the chronic studies due to the different rat strains used, this study supports the notion that higher exposures would be required for shorter term exposure periods to produce similar effects to those seen with chronic dosing, in which the LOAELs for kidney tubular hyperplasia were in the range of 1–2 mg/kg bw/day (Sunahara, Perrin and Marchesini, 1993; Cho *et al.*, 2008). Similar to previous evaluation conclusions, daily doses of 3-MCPD greater than approximately 1–3 mg/kg bw/day for up to 30 days are required in order to cause decreased fertility. This effect, depending on the exposure duration, can be transient and is typically not associated with any histopathological changes in male reproductive organs.

CHAPTER 9
RISK CHARACTERIZATION OPTIONS FOR 3-MCPD

The following default intakes (EFSA, 2016), unless indicated otherwise, were applied to the various options for risk characterization:

1. up to 1 month of age, average body weight = 4.2 kg, average daily formula intake is 170 mL/kg bw (total 714 mL).
2. 2 to 5 months of age, average bw = 7.3 kg, average daily formula intake is 110 mL/kg bw (total 803 mL).

9.1 OPTION 1 - STATUS QUO

Retain the reference point and HBGV as established at the most recent JECFA meeting.

Applying the current European Union ML value for 3-MCPD in liquid formula (15 µg/kg) to the infant formula consumption scenarios outlined above would result in dietary exposure to 3-MCPD of 1.65 (2 to 5-month infants) and 2.55 (0 to 1-month infants) µg/kg bw/day (corresponding to 41–63 percent of the JECFA HBGV of 4 µg/kg bw/day). Alternatively, if infant formula or similar products were regarded as the sole source of dietary exposure to 3-MCPD, at the scenario consumption levels, any ML value less than approximately 30 µg/kg (23.5 or 36.3 µg/kg for the two scenarios considered) should result in exposures that do not exceed the current JECFA HBGV. In comparison, in 97 samples of LNS/RUTF products analyzed by UNICEF and WFP from 2020 to 2022, the mean concentration of 3-MCPD was 588 µg/kg, with a 95th percentile of 1 118 µg/kg. Using the average daily formula intakes as described would result in mean exposures of 99 µg/kg bw/day and 65 µg/kg bw/day, respectively.

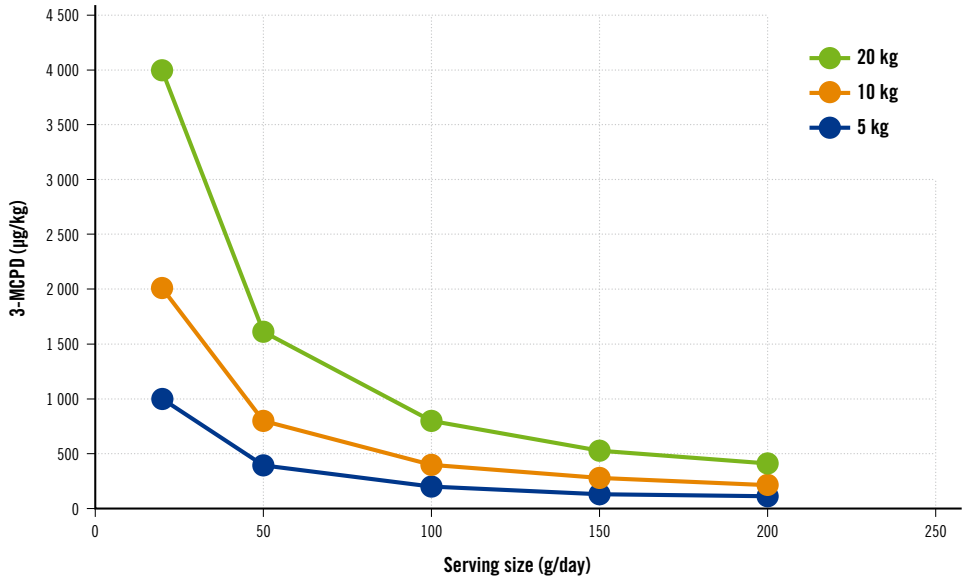
LNS-Large Quantity (LNS-LQ) is designed for children 6–59 months of age and can be provided as a daily ration of approximately 100 g for the prevention of malnutrition. For a 5 kg child, the tolerated daily 3-MCPD dietary exposure based on the current JECFA HBGV would be 20 µg. Assuming daily consumption of a single 100 g sachet (20 g/kg bw), in order not to exceed the current JECFA HBGV,

the maximum 3-MCPD concentration in the product as consumed would be 200 µg/kg, or approximately 645 µg/kg in the oil ingredient (assuming 31 percent lipid w/w). In comparison, the most stringent European Union 3-MCPD maximum level (ML) for oil when used as an ingredient destined for the production of baby food and processed cereal-based food for infants and young children is 750 µg/kg. Approximately 90 percent of the LNS/RUTF samples analyzed by WFP and UNICEF exceeded 200 µg/kg 3-MCPD.

At an average body weight of up to 14 kg for the age range of 6–59 months, a child consuming a single 100 g sachet of LNS-LQ/day would not exceed the current JECFA HBGV if the total 3-MCPD concentration in the product as consumed was less than 560 µg/kg. If the total amount of 3-MCPD in LNS-LQ originates from the fat component (assuming a fat content of 31 percent or 31 g per 100 g), the vegetable/marine oil ingredient could contain a maximum of approximately 1 800 µg/kg of 3-MCPD in order for the finished product not to exceed a 3-MCPD concentration of 560 µg/kg. In comparison, LNS-Small Quantity (LNS-SQ) is designed for the same age categories but is provided in 20 g sachets with instructions to consume one sachet/day. As such, daily consumption of five-fold less of product would permit 3-MCPD concentrations in the finished product to be five-fold greater than LNS-LQ, if LNS-SQ represented the only dietary exposure source for 3-MCPD.

An example of the influence daily serving size and body weight has on maximum tolerated 3-MCPD concentration is illustrated in Figure 5.

FIGURE 5. EFFECT OF SERVING SIZE AND BODY WEIGHT ON MAXIMUM 3-MCPD CONCENTRATION IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD (µG/KG) RELATIVE TO THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES HEALTH-BASED GUIDANCE VALUES OF 4 µG/KG BW/DAY



Variable body weights of 5, 10 or 20 kg and daily serving sizes of 20–200 g are used to derive maximum 3-MCPD concentrations in order for dietary exposures not to exceed the current JECFA HBGV. A consistent upper range intake of 42 g/kg bw[7] across all age categories would result in the most conservative scenario of a 3-MCPD concentration no more than 102 µg/kg in order not to exceed the JECFA HBGV of 4 µg/kg bw/day.

9.2 OPTION 2 – UPDATE BENCHMARK DOSE MODELLING

Retain the same critical study used by JECFA but adjust the reference point using the most recent dose-response modelling guidelines as per Environmental Health Criteria (EHC) 240 (WHO, 2020).

Although the JECFA (2016) assessment is relatively recent, current guidance for BMD modelling no longer recommends the selection of individual models (WHO, 2020). The previous approach was to fit a number of models to the data, and then select one based on various criteria (such as most conservative, goodness of fit, correspondence to a biological mechanism, etc.) to estimate the reference point. However, it is often the case that multiple models provide a reasonable fit to the observed data yet produce different BMDL estimates. As all models are estimates, and none is assumed to be the "true" model, this variance among estimates is referred to as model uncertainty (WHO, 2020). The use of model averaging to account for the fit of all models through a weighted average is now the preferred approach to address model uncertainty. Bayesian methods in particular are generally preferred, as Bayesian model averaging includes informative prior information for the parameters of the constituent models, resulting in more accurate and reproducible estimates (Wheeler *et al.*, 2020).

At the 83rd JECFA (2016), the chronic drinking water study of Cho *et al.* (2008) conducted in SD rats provided daily 3-MCPD exposures of 0, 1.97, 8.27 and 29.50 mg/kg bw/day for males and 0, 2.68, 10.34 and 37.03 mg/kg bw/day for females. Chronic progressive nephropathy and testicular atrophy were significantly increased in all dose groups for male rats. Renal tubular hyperplasia was selected as the most sensitive endpoint with $BMDL_{10}$s in the range 0.87–4.60 mg/kg bw/day based on the restricted models. The lowest BMDL of 0.87 mg/kg bw/day provided by the log-logistic model (restricted) was selected and used to establish a HBGV of 4 µg/kg bw/day. In the previous JECFA opinion, although model averaging was done and the result was considered similar to the lowest $BMDL_{10}$ obtained from the log-logistic model (restricted), the latter was selected as the PoD or reference point for use in establishing the HBGV.

Based on the most recently recommended dose-response model averaging approach where the models are unconstrained, BMDs for renal hyperplasia in male rats from the Cho *et al.* 2008 study ranged from 0.93–5.68 (average 2.01 mg/kg bw/day)

[7] Per guidance from UNICEF, the RUTF label includes a dose recommendation of 135 to 220 kcal/kg bw/day for the treatment of acute malnutrition and RUTF provides 520 to 550 kcal per 100 g. Therefore, assuming a dose of 220 kcal/kg bw/day and an energy content of 520 kcal/100 g, the indicated dose of RUTF is equivalent to 42 g/kg bw/day.

while the BMDL–BMDU range was 0.29–6.99 mg/kg bw/day. The model average for the 90th percentile confidence limit for the latter is 0.48–3.04 mg/kg bw/day) (Annex 1). Using the same 200-fold uncertainty factor as applied by JECFA and the model average $BMDL_{10}$ of 0.48 mg/kg bw/day, the HBGV would be 2.4 µg/kg bw/day, approximately two-fold lower than the current value. Based on the infant formula consumption scenarios for 0–1 and 2–5 month infants outlined above, a maximum 3-MCPD limit of approximately 15–20 µg/kg would be required in order not to exceed the HBGV of 2.4 µg/kg bw/day.

Based on LNS-LQ consumption as described for Option 1 (100 g/day), for infants (with an average body weight of 5 kg), if the product contained less than 120 µg/kg 3-MCPD, dietary exposure would not exceed the revised HBGV and would not be considered to represent a significant health risk. Based on an average fat content of 31 percent in ready-to-use supplementary food (RUSF), in order not to exceed the revised HBGV, this ingredient would need to contain less than approximately 387 µg/kg 3-MCPD. For an average body weight of 14 kg applied to the 6–59 months old age category, but retaining the same 100 g/day of product consumption, in order not to exceed the revised JECFA HBGV of 2.4 µg/kg bw/day, the product would need to contain maximum 336 µg/kg 3-MCPD or 1 100 µg/kg if based on the lipid ingredient.

Similar to Option 1, if the product in question was LNS-SQ, the maximum concentration of 3-MCPD in the product as consumed (20 g/day) could be five-fold higher.

9.3 OPTION 3 - USE OF A LIFETIME AVERAGE DAILY DOSE APPROACH

In 2021, the United Kingdom of Great Britain and Northern Ireland's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) recommended principles on assessing risk from less-than-lifetime exposure to non-genotoxic chemicals. COT recognized that a chronic HBGV, such as a TDI, might result in an overly conservative risk assessment when considering a less-than-lifetime exposure scenario. One of the steps recommended in considering risk from less-than-lifetime exposure was to better define the exposure period in question as it relates to a chronic HBGV.

Haber's rule states that the incidence and/or severity of a chemical's toxic effect depends on the total exposure, i.e. the product of exposure concentration and the duration of exposure (c x t) (Gaylor, 2000). According to Haber's rule, if the exposure period is reduced, for example, by eight-fold (experimental subchronic vs chronic study duration), the dose to achieve a similar effect seen in a chronic study could be increased by eight-fold. For chemicals producing toxic effects not directly related to achieving a maximum blood or tissue concentration (C_{max}), the total dose and not the concentration is assumed to be the determining factor.

Based on Haber's rule, total lifetime exposure (70 years) if less than 6.3 g (JECFA HBGV of 4 µg/kg bw/day x 70 years x 365 days/year x lifetime average bw of 61.3 kg) would not be considered to represent an appreciable health risk.

In terms of possible increased sensitivity or susceptibility of younger age groups, two of the major phase II enzyme systems involved with detoxication of various xenobiotics, including 3-MCPD and glycidol, are the UDP-glucuronosyltransferases (UGT) and glutathione S-transferases (GST). Both glucuronidated and glutathione conjugates of 3-MCPD fatty acid esters have been identified as metabolites in rats following dosing with 3-MCPD dipalmitate (Huang *et al.*, 2018). Glucuronidated 3-MCPD has previously been identified as one of the common metabolites of 3-MCPD mono- and dipalmitate (Gao *et al.*, 2017).

UGT are membrane-bound enzymes involved with important aspects of xenobiotic metabolism. UGT show a relatively wide substrate specificity with high activity particularly in the GI tract. Conjugation of the glucuronic acid moiety to a hydroxyl, carboxyl, amino, or sulfhydryl group of a target compound is mainly considered to be a detoxification mechanism with the resulting compound made more polar (water soluble) and excreted via the kidneys (Meech *et al.*, 2018). One of the major human forms of UGT, UGT1A1, is active at birth with levels increasing to those in adults after 3.8 months. In contrast, UGT1A6 has comparatively higher activity at birth compared to UGT1A1 and reaches adult levels by 14 months (Miyagi and Collier, 2011). Other UGT isozymes have been reported to be present at birth and to increase to near adult levels within a month after birth (UGT2B4, UGT2B7, UGT2B10 and UGT2B15) or during infancy (UGT1A3, UGT1A4 and UGT1A9) (Badée *et al.*, 2019).

GST catalyze reduced glutathione (GSH) conjugation to a wide variety of electrophilic compounds and reactive oxygen species, as the first step in a detoxification process. As with UGT, diverse functions of GST are due to multiple forms of the enzyme being present, mainly located in the cytosol of kidney, liver, adrenal glands and blood, with broad and/or overlapping substrate specificities (Tsuchida, 1997). GST also function to maintain the internal GSH pool that provides protection of cell structures against oxidative stress (Hayes, Flanagan and Jowsey, 2005). GST activity in fetal tissues is able to catalyze the conjugation of various substrates at rates approaching or exceeding the adult values (Pacifici *et al.*, 1988). Based on an extensive literature review performed on *in vivo* GST activity in healthy humans, Buratti *et al.* (2021) concluded that while GST activity in humans demonstrates wide inter-individual variability, existing data suggest a similar variability, which overlaps with adult activities and exists during fetal development.

Based on the development of both important phase II metabolism enzyme systems, it can be presumed that phase II metabolism of 3-MCPD esters in human newborns/young infants would not be significantly impaired as compared to the post-pubertal period.

Using default body weights and P95 intakes from the 2016 EFSA assessment, total lifetime intake of 3-MCPD would be approximately 2.5 g or a LADD of 1.72 µg/kg bw/day for a mean body weight of 61.3 kg over a 70-year life span (43 percent of the JECFA HBGV). As such, higher exposures relative to a shorter period may be tolerated, depending on comparison to short-term toxicological reference points.

For example, increasing the exposure to 25 µg/kg bw/day[8] for the age categories 0–1 year and 1–5 years (mean 3-MCPD 588 µg/kg) would increase the LADD to 3.6 µg/kg bw/day or approximately 89 percent of the JECFA HBGV. At the 95th percentile concentration of 3-MCPD (1 118 µg/kg) based on current survey results, the LADD would be 5.44 µg/kg bw/day or 136 percent of the JECFA HBGV. Any 3-MCPD concentration greater than approximately 720 µg/kg in foods consumed by infants/children up to the age of 5 years would result in a LADD exceeding 4 µg/kg bw/day (additional details concerning the LADD calculation may be found in Annex 3 and the impact of alternate exposure scenarios is explored in Annex 4).

Support for using a cumulative dose approach was seen in earlier studies on the effects of 3-MCPD on male fertility, in which it was observed that the number of days of treatment required to achieve an effect suggested that the cumulative level of the compound has to exceed a certain threshold (Vickery, Erickson and Bennett, 1974). In this study, decreased fertility was observed in rats one day after treatment of males with 25 mg/kg bw/day 3-MCPD, tw days after 10 mg/kg bw/day and five days after 2.5 mg/kg bw/day. As previously reported in the evaluation of chloropropanols conducted by JECFA (1993), the lowest doses shown to cause infertility in male rats following daily orally treatment with 3-MCPD were 6.5 mg/kg bw for ten days, 5 mg/kg bw for 14 days and 2.5 mg/kg bw upon continuous treatment.

In comparison to an exposure of 25 µg/kg bw/day estimated from infants/young children consuming RUTF/LNS at 42 g/kg bw/day with a concentration of 3-MCPD of 588 µg/kg, exposures of up to 1 mg/kg bw/day 3-MCPD for 30 days have not been associated with spermatid effects (numbers, motility, etc.), histopathological changes in male reproductive organs or decreased fertility in experimental animals (EFSA, 2018). Longer exposure to higher equimolar doses of 3-MCPD esters has also been shown not to produce effects on male reproductive organ weights or increase apoptosis in epididymal epithelium. In the study by Onami *et al.* (2014), the lowest dose tested for all esters, 1.8 mg/kg bw/day 3-MCPD equivalents (averaged over a seven-day week) had no effect on body weight gain, relative kidney or liver weights or incidence of histopathological lesions of testes, epididymis and kidney, which were affected at both the mid and high doses for all three esters. This study is considered to be relevant for exposure from formula and other related products as the primary form of 3-MCPD would be as a mono- or diester. In the study by Toyoda *et al.* (2017) in which male rats were exposed to 3-MCPD doses up to 54 mg/kg bw/day for 13 weeks, a strong linear relationship ($R^2 = 0.9974$) was observed between increasing dose and increases in relative kidney weights. The average $BMDL_{10}$ for increase in relative kidney weights was calculated as 2.13 mg/kg bw/day; over 13 weeks this dose would result in an approximate cumulative exposure to 57.5 mg 3-MCPD. While not directly comparable (different rat strain and gavage dosing), Vieira and Favareto (2017) reported no significant change in kidney weights with a cumulative 3-MCPD dose of approximately 90 mg/rat.

[8] Presumes RUTF/LNS intake at 42 g/kg bw for 0–1 (10 kg) and 1–5-year (18.5 kg) categories.

Similar observations have been seen with subchronic dosing of 3-MCPD monoesters; in the study by Yang *et al.* (2020), cumulative dosing over 90 days to 90–110 mg 3-MCPD equivalents produced no significant change in relative kidney weights. Relative kidney weights in the study of Jin *et al.* (2021) were significantly increased at a cumulative 3-MCPD dose of 126 mg delivered over 28 days.

9.4 OPTION 4 – LIFETIME AVERAGE DAILY DOSE USING UPDATED HEALTH-BASED GUIDANCE VALUES BASED ON REVISED DOSE-RESPONSE MODELLING

As described in Option 2, updating the dose-response modelling according to EHC 240 guidance (WHO, 2020) results in a more conservative PoD (0.48 mg/kg bw/day) for the same toxicological endpoint and therefore a lower HBGV (2.4 µg/kg bw/day) if applying the same composite uncertainty factor. Similar to Option 3, total lifetime exposure, if at the revised HBGV, would result in exposure to approximately 3.8 g of 3-MCPD over 70 years. The same conservative background EFSA dietary exposures would represent 72 percent of the revised HBGV. Increasing exposures for the 0 to 1 and 1 to 5-year-old age groups using the mean 3-MCPD concentration from the WFP/UNICEF data of 588 µg/kg and a daily LNS/RUTF intake of 36 g/kg bw would result in an average lifetime exposure of 3.57 µg/kg bw/day or approximately 150 percent of the revised HBGV. A maximum concentration of 3-MCPD in RUTF/LNS products would be approximately 382 µg/kg in order for the LADD not to exceed the revised HBGV (Table 4).

TABLE 4 SUMMARY OF THE MAXIMUM ACCEPTABLE CONCENTRATION OF 3-MCPD EQUIVALENTS IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD PRODUCTS BASED ON THE FOUR PROPOSED OPTIONS FOR RISK CHARACTERIZATION

OPTION 1*	OPTION 2**	OPTION 3***	OPTION 4****
560 µg/kg (ppb)	336 µg/kg	720 µg/kg	382 µg/kg

Notes: *Current JECFA PTDI and average body weight of 14 kg for 6–59 months; compliance = 51 percent of current products tested ($n = 97$): **Revised JECFA provisional tolerable daily intake (PTDI) based on updated DR modelling and average body weight of 14 kg for 6–59 months; compliance = 17 percent of current products tested ($n = 97$); ***LADD approach for cumulative exposure over 70 years based on current JECFA HBGV (4 µg/kg bw/day); compliance = 70 percent of current products tested ($n = 97$); ****LADD approach for cumulative exposure over 70 years based on revised JECFA HBGV (2.4 µg/kg bw/day); compliance = 17 percent of current products tested ($n = 97$).

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CHAPTER 10
HAZARD CHARACTERIZATION UPDATE FOR GLYCIDYL FATTY ACID ESTERS

Glycidol was most recently evaluated by EFSA (2016) and JECFA (2016) and these assessments were reviewed and served as a starting point for the current evaluation. Both previous assessments selected the NTP (1990) two-year bioassays in rats and mice as the critical studies from which to derive a reference point. A search for additional relevant toxicological and toxicokinetic studies in animals or humans was undertaken by research librarians of the Government of Canada's Health Library in order to identify any critical new data for the assessment of human health risks subsequent to the EFSA and JECFA assessments (2016 to present). Following the removal of duplicates, 202 unique references were identified and subjected to screening, of which 20 were considered relevant to the present assessment. Upon review of these studies, however, no new data were identified that were deemed to be more suitable for the derivation of a reference point and thus the NTP (1990) bioassays were retained as the critical studies. The following is not intended to be a comprehensive overview of glycidol or GEs toxicity, but rather to highlight some of the key studies that inform the hazard characterization of these substances.

10.1 TOXICOKINETICS

The comparative disposition of glycidol in rats was investigated by Nomeir *et al.* (1995) following oral and intravenous (i.v.) administration. Male Fischer 344 rats were administered [^{14}C]glycidol by gavage at doses of 37.5 mg/kg bw (n = 8) or 75 mg/kg bw (n = 11), corresponding to those doses used in the chronic cancer bioassay by the National Toxicology Program (NTP, 1990). For the i.v. study, the same doses were administered via a caudal vein (n = 8 per group). Approximately 87 to 92 percent of the orally administered dose was systemically absorbed from the

gastrointestinal tract. After 72 hours, [^{14}C]glycidol equivalents were excreted mainly via the urine (40–48 percent of the dose) or feces (5–12 percent) with the remaining exhaled as CO_2 (26–32 percent). The highest concentrations of radioactivity were observed in blood cells, thyroid, liver, kidney and spleen, whereas the lowest levels occurred in adipose tissue, skeletal muscle and plasma, with the pattern of distribution being similar with the two routes of administration.

Similar results in terms of excretion were observed in a study in which male Wistar rats were administered either a corn oil vehicle control (n = 2), 50 mg/kg bw glycidol (n = 16) or an equimolar amount of glycidyl palmitate (209.4 mg/kg bw, n = 16) by gavage (Appel *et al.*, 2013). In this study, however, high concentrations of the retained radiolabel were observed in skeletal muscle and bone in addition to liver and erythrocytes. Both glycidol and glycidyl palmitate administration led to the same steady-state level of the glycidol-derived hemoglobin adduct *N*-(1,2 dihydroxypropyl)valine in blood, although in the case of the ester the level was reached after a delay of approximately four to eight hours, which is likely attributable to the kinetics of the presystemic enzyme-mediated hydrolysis.

The toxicokinetics of glycidol and glycidyl linoleate were also compared in three male Sprague Dawley rats and three male cynomolgus macaques (Wakabayashi *et al.*, 2012). Following oral administration of 75 mg/kg bw glycidol (equivalent to approximately 1 mmol/kg bw), the bioavailability was 68.8 percent in rats and 34.3 percent in monkeys, with the maximum blood concentration (C_{max}) and area under the curve (AUC, a measure of overall exposure) 3.9- and 2.0-fold greater in rats than monkeys, respectively. Both species were also administered an oral dose of 341 mg/kg glycidyl linoleate (equivalent to approximately 1 mmol/kg body weight). Glycidol measurements in plasma following exposure to the GE indicated similar kinetics as observed following glycidol exposure. The C_{max} and AUC in blood corresponded to 77 percent and 128 percent, respectively, relative to those observed following the equimolar exposure to glycidol in rats. In the primates, however, the C_{max} and AUC in blood after glycidyl linoleate were just 17 percent and 56 percent, respectively, of those achieved after glycidol administration.

In rats, GEs undergo rapid and efficient presystemic hydrolysis to form glycidol following oral dosing and it is generally assumed that for the purposes of risk assessment human exposure to GEs should be regarded as an exposure to the same molar quantity of glycidol (Appel *et al.*, 2013). However, based on the data of Wakabayashi *et al.* (2012) described above (albeit in a limited number of animals), de-esterification appears to be more extensive in rats than in monkeys, suggesting potential species differences in the bioavailability of GEs assessed as glycidol equivalents (EFSA, 2016; FSCJ, 2015). The glycidol moiety is rapidly metabolized by several enzymatic pathways, including glutathione conjugation, upon which it is excreted predominantly in urine. It may also be hydrolyzed to glycerol by epoxide hydrolases (JECFA, 2016) and this step is required for elimination by exhalation in the form of CO_2. The metabolism of glycidol in rats has been described in greater detail by Scholz and Schilter (2022).

10.2 MODE OF ACTION

The toxic effects of GEs are attributable to their rapid presystemic hydrolysis to glycidol. Glycidol is a highly reactive molecule due to its epoxide moiety and is a direct-acting alkylating agent that may bind covalently to DNA and produce mutagenicity.

10.3 ACUTE TOXICITY

JECFA (2016) reported the oral LD_{50} of glycidol was 450 mg/kg bw in mice and 420–850 mg/kg bw in rats. Following oral administration (gavage) in acute toxicity studies, glycidol produced central nervous system depression in rats and mice as evidenced by incoordination, ataxia, depressed motor activity and loss of consciousness (Hine *et al.*, 1956).

10.4 SHORT-TERM STUDIES

Prior to the two-year bioassay, NTP (1990) conducted 16-day and 13-week (~90-day) oral toxicity studies in male and female F344 rats and $B6C3F_1$ mice. In the 16-day study, groups of five mice of each sex were administered glycidol by oral gavage at doses of 0, 37.5, 75, 150, 300 or 600 mg/kg bw/day on 14 of the 16 days. All mice that received the 600 mg/kg bw/day dose of glycidol died within four days, and 3/5 males and 2/5 females in the 300 mg/kg bw/day group also died before the end of the study. Diarrhea was observed in male and females that received glycidol at the dose of 150 mg/kg bw/day and at the 300 mg/kg bw/day dose focal demyelination in the medulla and thalamus of the brain was observed in all female mice.

Groups of five rats of each sex were also administered glycidol by oral gavage at doses of 0, 37.5, 75, 150, 300 or 600 mg/kg bw/day on 14 of the 16 days. All rats in the 600 mg/kg bw/day group died before the end of the study. Body weights of male rats in the 150 and 300 mg/kg bw/day groups were 10 percent and 21 percent lower than controls, respectively, although body weights of treated females were similar to controls. Evidence of reproductive toxicity was observed in male rats in the 300 mg/kg bw/day group, including edema and degeneration of the epididymal stroma, testicular atrophy and granulomatous infiltration of the epididymis.

Thirteen-week studies in rats and mice were conducted to evaluate the toxic effects of repeated glycidol exposure and determine appropriate doses for the two-year study. Groups of ten mice of each sex received 0, 19, 38, 75, 150 or 300 mg/kg bw/day glycidol five days per week for 13 weeks. All mice in the highest dose group (300 mg/kg bw/day) died by the second week, and 4/10 males and 3/10 females that received 150 mg/kg bw/day glycidol died by the end of the study. Reductions in final body weight (6–10 percent) were observed in all treated mice with the exception of males in the 38 mg/kg bw/day group. In male mice, sperm count was reduced in a dose-dependent fashion although the magnitude of the changes was not as great as seen in rats.

Groups of ten rats of each sex were administered glycidol at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw/day by gavage, five days/week for 13 weeks. As observed in mice, all rats in the 400 mg/kg bw/day group died by week two, and 3/10 males and 1/10 females in the 200 mg/kg bw/day group died before the end of the study. Body weights of male and female rats were lower than that of controls at doses of 50 mg/kg and above. In male rats, a dose-dependent reduction in sperm counts from the cauda epididymis relative to controls was observed from the lowest dose, reaching 4 percent of counts in vehicle control males at the 200 mg/kg bw/day dose. In both rats and mice, multiple treatment-induced histopathological lesions in brain, kidney, thymus and testes were present at the highest dose levels.

10.5 CHRONIC TOXICITY AND CARCINOGENICITY

No chronic studies of the oral toxicity and carcinogenicity of GEs were identified. However, the United States NTP conducted a chronic oral bioassay of glycidol in rats and mice (NTP, 1990) that is applicable to the risk assessment of GEs. It should be noted that although the basal diets (NIH-07 Rat and Mouse Ration[9]) used in these studies were formulated with potential sources of GEs such as fish meal and soy oil, the presence of GEs in the diet was not accounted for as dietary GEs exposure was largely unrecognized at the time of the study.

Male and female B6C3F mice (50 per sex) were administered glycidol in distilled water at doses of 0, 25 or 50 mg/kg bw by oral gavage five days per week for 104 weeks (equivalent to 0, 17.9 or 35.7 mg/kg bw/day, adjusted for non-continuous dosing). Survival of male mice and low-dose female mice was comparable to vehicle controls although survival of high-dose females was significantly lower (final survival in males: vehicle control, 33/50; low dose, 25/50; high dose, 27/50; females: 29/50; 27/50; 17/50). Non-neoplastic, treatment-related lesions included hyperkeratosis and epithelial dysplasia of the forestomach in both sexes, while male mice exhibited cysts in the preputial gland and kidney. Glycidol exposure was observed to induce tumours in multiple tissue sites of both sexes. The NTP concluded that under the conditions of the study there was clear evidence of carcinogenic activity of glycidol for both male and female mice, based on increased incidences of neoplasms in multiple tissues.

Male and female F344 rats (50 per sex per species) were administered glycidol in distilled water by oral gavage at doses of 0, 37.5 or 75 mg/kg glycidol five days per week for 104 weeks (equivalent to 0, 26.8 or 53.6 mg/kg bw/day, adjusted for non-continuous dosing). Almost all rats that received glycidol died or were euthanized in moribund condition as a result of neoplastic disease prior to the end of the study (final survival in males: vehicle control, 16/50; low dose, 0/50; high dose, 0/50; females: 28/50; 4/50; 0/50). As in mice, non-neoplastic,

[9] The ingredients of NIH-07 rat and mouse cation may be found at National Library of Medicine. n.d. NTP Developmental and Reproductive Toxicity Technical Report on the Prenatal Development Studies of Dimethylaminoethanol Bitartrate (CASRN 5988-51-2) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies): DART Report 04 [Internet]. In: *National Center for Biotechnology Information*. [Cited 27 September 2022] https://www.ncbi.nlm.nih.gov/books/NBK562916/table/t-2-B.1

treatment-related lesions included hyperkeratosis and epithelial dysplasia of the forestomach. Fibrosis of the spleen was observed in rats of both sexes, as were tumours in multiple tissue sites. In male rats, TVM were the predominant cause of early mortality, whereas in females, mammary neoplasms were most frequently fatal. The NTP concluded that under the conditions of the study, there was clear evidence of carcinogenic activity of glycidol for both male and female rats, based on increased incidences of neoplasms in multiple tissues.

The carcinogenicity of glycidol was also investigated in a study in Syrian golden hamsters (Lijinsky and Kovatch, 1992). In this study, ten-week old male and female hamsters (n = 20 per sex) were administered glycidol by oral gavage at a dose of 12 mg per animal twice weekly for 60 weeks, for a total dose per animal of 1.45 g (equivalent to approximately 100 mg glycidol/kg bw per gavage treatment or 29 mg/kg bw/day, doses adjusted for non-continuous dosing) and then observed for the remainder of their lifespan. Control groups (12 of each sex) where given an equivalent volume of the corn oil/ethyl acetate vehicle for 90 weeks. The median week of death was 92 weeks in males and 84 weeks in females, which was not significantly different than the vehicle controls (97 weeks for males and 84 weeks for females). Most of the non-neoplastic lesions observed, such as amyloidosis, nephrosis, thrombosis of the heart (especially in females) and bile duct hyperplasia were common to both treated and untreated animals. There were very few tumours in the control animals with the exception of the adrenal cortex (7 of 12 animals in both the male and female groups), which occur spontaneously in this strain. In the glycidol-treated groups, no statistically significant increase in the incidence of tumours was observed, although a wider variety of tumour types was present, particularly in females. The authors stated that there appeared to be no indication of a predilection for any tissue site or cell type other than potentially the spleen, where hemangiomas or hemangiosarcomas were observed in two treated males and four females but not in control animals. While the difference was not statistically significant, these cancers are very rare in control animals but commonly seen in hamsters treated with alkylnitrosoureas, which are another class of direct-acting alkylating agents. The authors concluded that glycidol does not have a potent carcinogenic effect in Syrian hamsters.

While the two-year rodent bioassay is often considered the gold standard for assessing chemical carcinogenicity, it requires considerable resources and efforts have been undertaken to develop alternative assays that are faster, less costly and more efficient for identifying human carcinogens. Transgenic rodent models in which oncogenes are constitutively or conditionally expressed or null mutations of tumour suppressor genes are introduced have emerged as important tools to investigate the mutagenicity and carcinogenic potential of chemicals. Glycidol was tested in two different transgenic mouse models that were haploinsufficient for either the tumour suppressor gene *p53* or two other tumour suppressor genes ($p16^{Ink4a}$/$p19^{Arf}$). In the first study, no tumours were reported after six months of oral administration of glycidol at 25–50 mg/kg bw/day in $p53^{+/-}$ mice (Tennant *et al.*, 1999 as cited in NTP, 2007). In the second study, male and female

haploinsufficient *p16*^{Ink4a}/*p19*^{Arf} mice (15 males and 15 females per group) were administered glycidol by oral gavage at doses of 0, 25, 50, 100 or 200 mg/kg bw/day, five days per week for 40 weeks (NTP, 2007). The NTP concluded that there was clear evidence of carcinogenic activity of glycidol in male haploinsufficient *p16*^{Ink4a}/*p19*^{Arf} mice based on the occurrence of histiocytic sarcomas, and that incidences of alveolar/bronchiolar adenomas in male mice were also treatment related. In haploinsufficient *p16*^{Ink4a}/*p19*^{Arf} female mice, there was some evidence of carcinogenic activity based on the occurrence of alveolar/bronchiolar adenoma. While these studies are useful for hazard identification, they are less useful for hazard characterization due to the limited understanding of how dose-responses in transgenic models correspond to those in wild type animals or humans. Therefore, it was considered that the results of the two-year chronic bioassay (NTP, 1990) remain the most appropriate carcinogenicity data for human health risk assessment, in agreement with recent evaluations by EFSA (2016) and JECFA (2016).

10.6 GENOTOXICITY

No studies were identified that evaluated the potential for GEs to spontaneously form adducts with DNA. However, the genotoxic potential of glycidol and glycidyl linoleate, a GE commonly detected in edible oils, was investigated by Ikeda *et al.* (2012) in a bacterial reverse mutation test, an *in vitro* chromosomal aberration test, and an *in vivo* bone marrow micronucleus test. All tests were conducted under GLP conditions and in accordance with Organisation for Economic Co-operation and Development (OECD) test guidelines. In contrast with glycidol, only weak responses were detected with glycidyl linoleate in the bacterial reverse mutation test in specific strains of bacteria that detect point mutations, which the authors attributed to its bioconversion to glycidol. The GE did not induce chromosomal aberrations in cytogenetic tests in CHO cells in the presence or absence of metabolic activation, whereas glycidol did so under both conditions. Neither glycidol nor glycidyl linoleate induced significant increases in micronucleated polychromatic erythrocytes in bone marrow of male ICR mice. The authors concluded that glycidyl linoleate and GEs generally are unlikely to pose appreciable genotoxic concerns in the absence of de-esterification. In contrast to GEs, however, it is well established that glycidol can directly alkylate DNA in the absence of exogenous metabolic activation. Glycidol possesses a highly reactive epoxide moiety that likely accounts for its mutagenicity and genotoxicity, as observed in a wide variety of *in vitro* and *in vivo* test systems (for review see EFSA, 2016; JECFA, 2018).

10.7 REPRODUCTIVE/DEVELOPMENTAL TOXICITY

As described in the section Short-term studies, there was clear evidence of glycidol-induced testicular toxicity in both rats and mice in the NTP (1990) 13-week study. In male rats, sperm counts and sperm motility were reduced at the lowest dose of 25 mg/kg bw/day and at all higher doses. Similar effects were observed in male mice administered glycidol at doses of 75 and 150 mg/kg bw/day.

There were no histopathological lesions in female reproductive tissues. In mice, 4 of 10 males and 3 of 10 females that received 150 mg glycidol/kg bw/day died before the end of the study.

The potential for developmental toxicity of glycidol was investigated in CD-1 mice by Marks, Gerling and Staples (1982). Groups of pregnant female mice received either vehicle control (n = 32) or glycidol at doses of 100, 150 or 200 mg/kg bw/day (n = 37, 31 and 30, respectively) by oral gavage on gestation days 6–15. On gestational day 18, mice were terminated and examined for evidence of embryotoxicity and teratogenicity. No reduction in the total number of implantations and no increase in the numbers of resorptions or fetal deaths per pregnancy were observed and there was no indication of morphological variations that could be directly attributed to the treatment. At the highest dose tested of 200 mg/kg bw/day, glycidol was lethal in 5 of 30 dams. No evidence of teratogenicity was observed, although stunted fetuses (defined as live fetuses weighing less than two-thirds the mean of their larger littermates) were observed in one dam receiving glycidol at the highest dose. The authors concluded that glycidol was not teratogenic or otherwise toxic to the mouse embryo and fetus at doses that were not lethal to dams.

No other studies on the reproductive or developmental toxicity of glycidol administered via the oral route were identified.

10.8 OBSERVATIONS IN HUMANS

Several studies have evaluated the formation of glycidol-hemoglobin adducts in blood or conjugation products in urine as potential biomarkers of glycidol exposure (Honda *et al.*, 2012; Abraham *et al.*, 2019; Aasa, Granath and Törnqvist, 2019; Göen *et al.*, 2021). Following consumption of GEs in rats and humans, the formation of adducts at the N-terminal valine of the hemoglobin protein complex (*N*-(2,3-dihydroxypropyl)valine) has been observed. Although it has been suggested that DHPV adducts are not formed uniquely in response to glycidol exposure and that several other substances such as MCPDs, epichlorohydrin and cigarette smoke may also be precursors (Hindso Landin *et al.*, 2000), experimental data suggest that the adduct level/dose ratio is far lower than that observed with glycidol (Abraham *et al.* 2019). Honda *et al.* (2012) studied the formation of DHPV adducts in consumers of oils rich in diacylglycerols (DAG, a precursor of GEs) versus non-consumers in a matched case-control study. Glycidol-DHPV adducts were quantifiable in all samples, but no significant difference was observed between DAG oil-exposed (n = 15) and non-exposed subjects (n = 42). The authors speculated that GE exposure via DAG oil intake was relatively low and insufficient to significantly increase adduct concentrations above background levels.

More recently, Abraham *et al.* (2019) conducted a study in which 11 adult volunteers (six males and five non-pregnant females) consumed a weekly portion of 250 g palm fat (35.7 g fat daily on average) over four weeks. The palm oil product (labelled as 100 percent palm fat) was identified in a survey of GEs and MCPDs in food items from the German market as having the highest level of GEs, equivalent to

8 700 µg glycidol/kg of fat. Consumption of 250 g of the product per week corresponded to a mean additional dietary exposure of 4.2 µg/kg bw/day (median 4.3, range 2.7–5.2 µg/kg bw/day), which is comparable to the upper bound of the high percentile exposure estimate for infants of 4.9 µg/kg bw/day determined by JECFA (2016). Two blood samples were drawn prior to consumption of the palm fat, followed by draws at the end of each week of exposure and every 3 weeks thereafter for an additional 15 weeks (total = 11 blood samples per participant). Prior to the intervention, there was relatively little inter-individual variation in DHPV concentrations. DHPV adduct levels continuously increased during the period when palm fat was consumed and were approximately three-fold higher than baseline at the end of the four-week exposure period. Adduct levels declined thereafter, commensurate with the lifespan of erythrocytes, and were not significantly different from baseline at the end of the 15-week washout period. It was determined that the DHPV concentration decreased following zero-order elimination kinetics with a half-life of 104 days.

S-(2,3-Dihydroxypropyl)mercapturic acid (DHPMA), a secondary product of glutathione conjugation with glycidol, is excreted in urine following glycidol exposure in rats and has also been investigated as a potential biomarker of exposure. Spot urine samples were collected from 108 employees of a university in Germany that did not have occupational exposure to alkylating substances (Eckert *et al.*, 2011). Background DHPMA excretion was detected in all urine samples. Only a small range of variation was observed, and DHPMA levels were closely associated with urinary creatinine excretion. To date, no study has been performed that demonstrates that urinary DHPMA levels increase in response to glycidol exposure and DHPMA has not been unequivocally identified as a unique or specific human glycidol metabolite (Göen *et al.*, 2021). Indeed, Monien and Abraham (2022) recently reported that urinary DHPMA is only a minor metabolite of 3-MCPD and glycidol, and daily DHPMA excretion greatly exceeds human exposure to these substances. Therefore, the source(s) of most urinary DHPMA is unknown, and it cannot be considered a useful human biomarker for exposure to 3-MCPD and/or GEs.

The presence of GEs in human milk has been reported in one study (Li *et al.*, 2022). Human breastmilk samples were collected from lactating Chinese volunteers with infants of various ages: birth to <1 month (n = 4), 1 to <3 months (n = 7), 3 to <6 months (n = 7) and 6 to <12 months (n = 12). GEs were detected in 60 percent of samples with a mean concentration of 8.3 µg/L (range ND to 21.0 µg/L). Presumably, the presence of GEs in human milk is a result of dietary exposure as there is no evidence to suggest that GEs are synthesized endogenously (Rietjens *et al.*, 2022). However, GEs are generally assumed to be hydrolyzed to glycidol prior to being systemically absorbed and *de novo* synthesis by re-esterification has not been observed *in vivo*. Thus, the finding of GEs in human milk is somewhat enigmatic, although the possibility exists that the assumption of complete presystemic hydrolysis in humans is not valid and at least some esters have the potential to be absorbed intact.

No epidemiological studies were identified that investigated the relationship between human dietary exposure to glycidol and carcinogenicity. However, one study investigated the association between urinary DHPMA excretion and metabolic syndrome in 2 290 Chinese volunteers aged 45 to 75 years (Wan *et al.*, 2022). The authors considered that "urinary DHPMA could effectively evaluate the internal exposure" to both 3-MCPD and GEs from vegetable oils. Urinary DHPMA concentrations were positively associated with metabolic syndrome after adjustment for a number of known confounders. In a secondary analysis, urinary DHPMA was positively associated with hypertriglyceridemia, which is a lipid abnormality commonly associated with metabolic syndrome. Due to the cross-sectional design, however, a causal association between urinary DHPMA and metabolic syndrome cannot be established on the basis of these findings. Moreover, as described above, urinary DHPMA does not appear to be a suitable human biomarker for dietary exposure to 3 MPCD esters, GEs or their sum.

10.9 SUMMARY

The available evidence indicates that the toxicological effects of GEs are mediated by free glycidol following presystemic hydrolysis. GEs appear to be rapidly and efficiently hydrolyzed in the gastrointestinal tract to liberate glycidol, although some differences in the extent of hydrolysis have been observed between rats and nonhuman primates, suggesting the possibility of species-specific differences in oral bioavailability. Glycidol is a direct-acting alkylating agent that can form adducts with DNA in the absence of metabolic activation. It has been shown to be mutagenic in a wide variety of *in vitro* and *in vivo* assays. In a two-year bioassay, glycidol was a multisite carcinogen in both male and female rats and mice. Based on the carcinogenic and genotoxic properties of glycidol, it has been classified by various competent authorities or expert panels as "probably carcinogenic to humans" (class 2A) (IARC, 2000), a "confirmed animal carcinogen with unknown relevance to humans" (A3) (ACGIH, 2010) and "reasonably anticipated to be a human carcinogen" (NTP, 1990). In short-term studies, reproductive toxicity in male rats appears to be the most sensitive endpoint, with higher doses producing multiple treatment-induced histopathological lesions in brain, kidney, thymus and testes.


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CHAPTER 11
RISK CHARACTERIZATION OF GLYCIDYL FATTY ACID ESTERS

There are several important considerations when characterizing the risk of GEs exposure in LNS/RUTF products. As shown in Table 3 (refer to LNS/RUTF datasets above), a number of therapeutic and supplemental food products exist that are intended for various indications, populations and durations of use. Among these, RUTF represents the greatest potential for exposure to process-induced contaminants such as GEs, as it is indicated as a sole source nutrition for children aged 6 to 59 months and has the largest recommended daily intake. Per guidance from the WHO, infants and children 6 to 59 months of age with severe wasting and/or nutritional oedema who are enrolled in outpatient care should be given RUTF in a quantity that will provide 150–185 kcal/kg/d and RUTF provides 520 to 550 kcal per 100 g. Therefore, assuming an energy intake of 185 kcal/kg bw/day and an energy content of RUTF of 520 kcal/100 g, the indicated consumption of RUTF is equivalent to 36 g/kg bw/day. As described above, RUTF represents a "reasonable worst case" and GEs levels that are determined to be of low concern for RUTF would also be considered of low concern for all LNS products, including LNS-PLW (intended for pregnant and lactating women).

11.1 BACKGROUND EXPOSURE CONSIDERATIONS

Another consideration in understanding the potential significance of GEs exposure via LNS/RUTF is how dietary exposure from these products compares to dietary exposure to GEs from all other sources. Although dietary exposure to GEs from consumption of LNS/RUTF is of limited duration and restricted to infants and young children (with the exception of LNS-PLW), GEs are present in many other foodstuffs and dietary exposure is chronic in nature. It was therefore considered appropriate to use a lifetime average daily dose (LADD) approach to characterize the potential added risk of GEs exposure

from less-than-lifetime use of LNS/RUTF. In its 2016 assessment, JECFA calculated dietary exposure estimates for infants, children and adults (Table 5). Due to the presence of left-censored (ND) values in the concentration data set used, estimates were in the form of ranges, with upper (UB) and lower bounds (LB) for a mean exposure scenario as well as a high percentile exposure scenario (90th to 95th percentile). Occurrence data from Japan and the United States of America were used to estimate national dietary exposures, as consumption data were also available for these countries (JECFA, 2016). The WHO's Global Environment Monitoring System/Food Contamination Monitoring and Assessment Program (GEMS/Food) has developed the GEMS/Food Consumption Cluster Diets for 17 groups of countries (Sy *et al.*, 2013). However, for international estimates of dietary exposure to glycidol, occurrence data were available for only one of the 17 clusters (cluster G10) and these data were used for all cluster estimates by JECFA (2016). Data on GEs in infant formula were combined with infant formula feeding rates to estimate infant dietary exposure for fully formula-fed infants.

TABLE 5 **DIETARY EXPOSURE VALUES FOR GLYCIDOL ESTIMATED BY THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES**

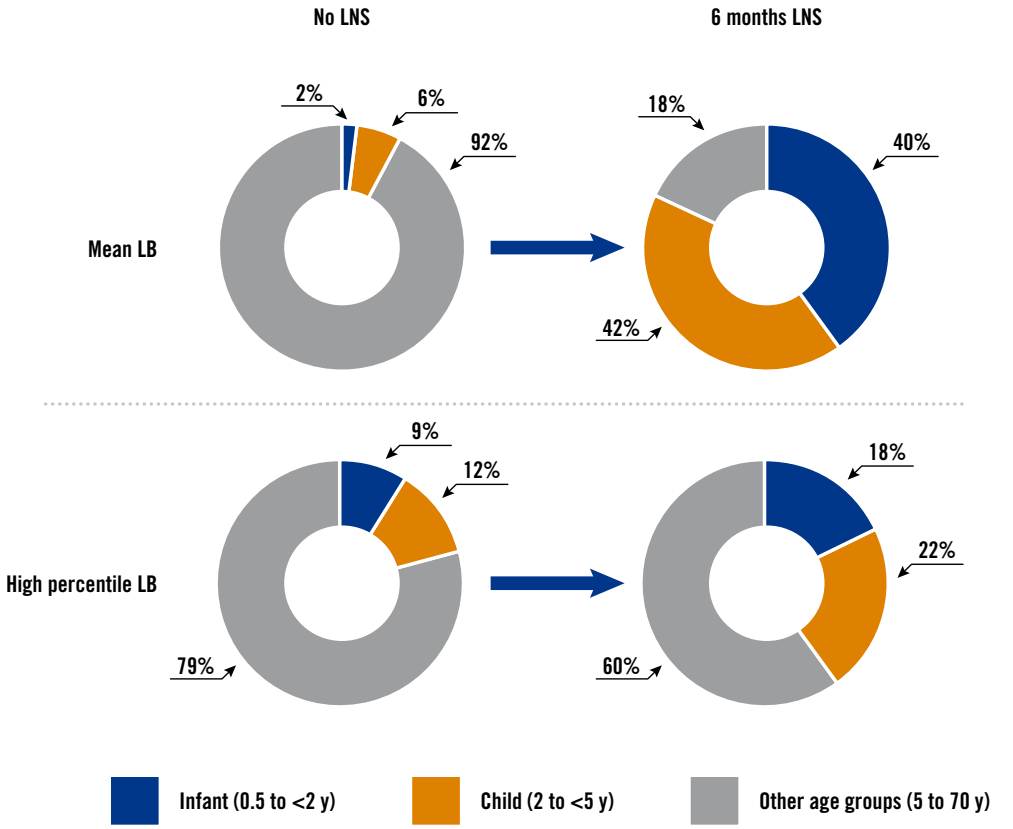
POPULATION GROUP	RANGE OF ESTIMATED DIETARY EXPOSURE TO GLYCIDOL (µg/kg bw/day)[a]	
	MEAN	HIGH PERCENTILE
Adults	0.1–0.3	0.2–0.8
Children	0.2–1.0	0.4–2.1
Infants	0.1–3.6	0.3–4.9

Source: JECFA. 2016. Joint FAO/WHO Expert Committee on Food Additives. Summary and Conclusions. Eighty-third meeting of JECFA, Rome, 08–17 November 2016. https://cdn.who.int/media/docs/default-source/food-safety/jecfa/summary-and-conclusions/jecfa83_8-17-november-2016_summary-and-conclusion.pdf?sfvrsn=ca027114_5

Note: [a] Includes LB and UB estimates from a range of national estimates of dietary exposure.

The relative contribution of LNS/RUTF to the total lifetime exposure to GEs will vary as a function of the background exposure estimate used. This is illustrated in Figure 6, where in the absence of LNS/RUTF, GEs exposure in children 6 to 59 months accounts for approximately 8 percent (mean LB scenario) to 21 percent (high percentile UB scenario) of the total lifetime exposure (infant + child exposure combined). However, after six months of LNS/RUTF exposure, assuming the median GEs concentration of 420 µg/kg, exposure during infancy and childhood increases by an order of magnitude for the lower bound exposure scenario but only roughly doubles in the upper bound scenario. Therefore, the mean lower bound background exposure estimate is a more sensitive scenario under which to assess any additional risk due to GE exposure from consumption of LNS/RUTF.

FIGURE 6. EFFECT OF BACKGROUND EXPOSURE SCENARIO ON LIFE STAGE EXPOSURE

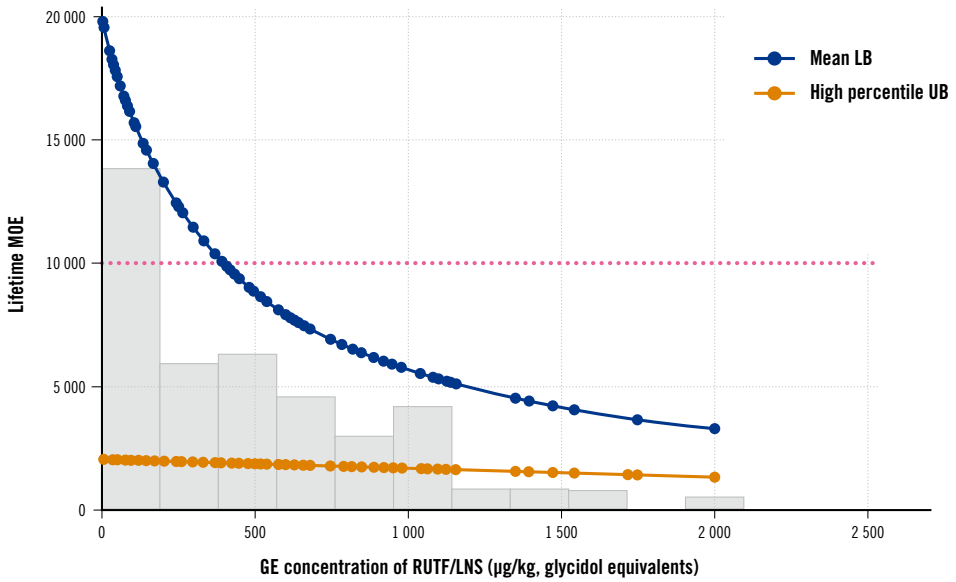


Note: Assuming LNS/RUTF contains GE at the median level of 420 µg/kg. The mean lower bound (Mean LB) and high percentile upper bound (High percentile UB) scenarios are contrasted in the case of no LNS/RUTF exposure versus exposure for six months.

11.2 SELECTION OF A REFERENCE POINT

JECFA evaluated GEs in 2016 and concluded that glycidol is a genotoxic compound. JECFA considered its carcinogenicity as the most sensitive endpoint on which to base a PoD for chronic exposure. The lowest $BMDL_{10}$ was 2.4 mg/kg bw/day for TVM in male F344 rats observed in the NTP (1990) carcinogenicity study. It is generally accepted that an MOE of 10 000 or greater, based on a $BMDL_{10}$ from an appropriate animal study and estimated lifetime exposure, is of low concern for human health. Figure 7 depicts the MOEs for glycidol exposure via GEs in LNS/RUTF at a consumption level of 36 g/kg bw/day for six months, based on the LADD. MOEs are calculated for the range of GEs concentrations reported in products under the two different background exposure scenarios from JECFA (2016) described above (mean LB and high percentile UB).

FIGURE 7. **EFFECT OF BACKGROUND EXPOSURE SCENARIO ON MARGINS OF EXPOSURE**



Note: MOEs for glycidol exposure following exposure to LNS/RUTF for six months, assuming background exposure corresponds to the mean LB estimate of JECFA (blue) or the high percentile UB scenario (orange). The dashed line represents an MOE of 10 000 from the JECFA (2016) $BMDL_{10}$ of 2.4 mg/kg bw/day and the histogram in grey represents the GE content of products tested ($n = 97$).

Notably, under the high percentile UB scenario, MOEs for glycidol dietary exposure in the absence of LNS/RUTF are roughly an order of magnitude lower than in the mean LB scenario and already indicate a potential concern, as concluded by JECFA. However, in the case of low GEs concentration in LNS/RUTF products (~100 µg/kg or less), consumption of these products as a sole source of nutrition improves the MOE in this scenario as it displaces intakes that contribute to the high background exposure estimates. The slope of the corresponding curves also differs significantly depending on the background scenario, being almost linear under the upper bound scenario but conforming to a power function in the case of the lower bound exposure, which has implications when extrapolating these results to an acceptable level in products. If the JECFA mean lower bound scenario is used to estimate background exposure, it is possible to estimate the GE concentration in LNS/RUTF that corresponds to an MOE of 10 000 for any given exposure duration. However, in the high percentile upper bound scenario, MOEs are less than 10 000 based on background exposure alone, and therefore it is not possible to extrapolate to a GE level that would be considered of low concern.

Although the JECFA (2016; 2018) evaluation is relatively recent, as previously discussed current guidance for BMD modelling no longer recommends the selection of individual models and Bayesian BMD modeling is preferred (WHO, 2020). The same male rat data for TVM selected by JECFA (2016) from the NTP (1990) bioassay were therefore modeled using Bayesian model averaging

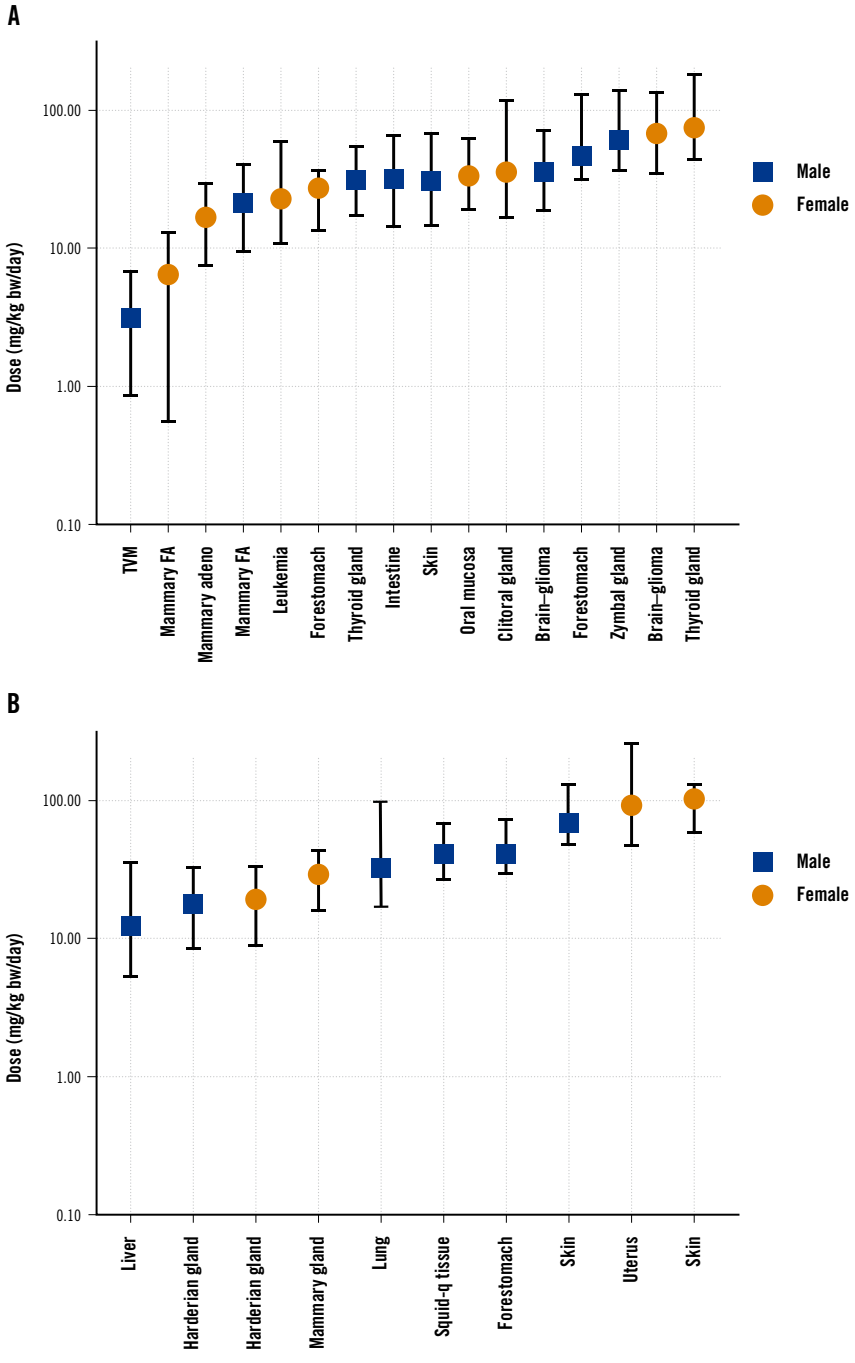
in the R package "ToxicR", which is based on WHO guidelines for model averaging (Wheeler and Lim, 2022). The resulting model average $BMDL_{10}$ was 0.83 mg/kg bw/day, or roughly three-fold lower than the reference point of 2.4 mg/kg bw/day selected by JECFA (see Annex 2 for additional details). Using the more conservative 0.83 mg/kg bw/days as the reference point, the MOE for glycidol exposure would be approximately 6 900 in the absence of LNS/RUTF consumption in the mean LB background exposure scenario; therefore, it is not possible to estimate a GE concentration in LNS/RUTF that would correspond to an MOE of 10 000 with this reference point.

11.3 OTHER OPTIONS CONSIDERED

In their respective assessments, both JECFA (2016) and EFSA (2016; 2018) selected TVM in male rats, which was the most sensitive tumour type observed in the chronic rodent bioassay (NTP, 1990), as the critical effect for hazard characterization. This is consistent with standard risk assessment practices for dietary exposure to genotoxic carcinogens, where the most conservative tumour site is generally used to derive a reference point. However, EFSA (2015) states that TVM are "almost unknown in humans" and are thought to be etiologically linked to asbestos exposure, at least in many cases (Vimercati *et al.*, 2019; Butnor *et al.*, 2019; Marinaccio *et al.*, 2020; but see Anderson *et al.*, 2022). There is also evidence to suggest that xenobiotic-induced TVM is a male F344 rat-specific event that is not relevant to humans (Maronpot *et al.*, 2009; 2016; Edler *et al.*, 2014). TVM is a spontaneous, age-associated tumour in male F344 rats and substance-induced increases have been observed in F344 rats but not in any other rat strain or in mice (Laube *et al.*, 2019). Given their high spontaneous background incidence and species-/strain-specific biology, Maronpot *et al.* (2016) concluded that TVM responses in F344 rat carcinogenicity studies "are inappropriate tumor types for human health risk assessment and lack relevance in predicting human carcinogenicity."

In the chronic rodent bioassay in male and female rats and mice (NTP, 1990), glycidol was observed to be a multisite carcinogen in both sexes of both species. The dose-response data from each tumour site was modelled using Bayesian model averaging in the R package "ToxicR" to generate BMDs and their corresponding confidence intervals for each tissue (Figure 8). After TVM in male rats, mammary fibroadenoma (FA) in female F344 rats was observed to be the next most sensitive tumour type. However, the low $BMDL_{10}$ for mammary FAs in female rats (0.55 mg/kg bw/day) is largely attributable to poor model fit due to a high incidence at baseline and lack of dose-response (greater incidence at the low dose than the high dose), resulting in a large confidence interval around the BMD. Mammary FA are also benign tumours that are not considered precursors of malignancy in either humans or rats (Cohen *et al.*, 2010), and rat FAs are regarded as species- and strain-specific responses with specific modes of action that are not relevant to women and therefore not likely predictive of human cancer risk (Eisenbrand, 2020).

FIGURE 8. **GLYCIDOL IS A MULTISITE CARCINOGEN IN BOTH RATS AND MICE. BAYESIAN MODEL AVERAGE BENCHMARK DOSES (TOXICR) FOR TUMOURS OBSERVED IN THE TWO-YEAR CHRONIC BIOASSAY IN (A) F344 RATS AND (B) B6C3F1 MICE**



Note: Symbols represent the BMD and the lower and upper error bars the $BMDL_{10}$ and $BMDU_{10}$, respectively. Blue squares represent values in males and orange circles represent those in females. Note that the doses are plotted on a log scale, and this should be kept in mind when considering the width of the confidence intervals. For example, in Figure 8A, the width of the CI for thyroid gland tumours in female rats appears smaller than that for mammary FAs but is actually more than 10 times larger (mammary FA BMDL to BMDU interval = 0.55 to 12.88 mg/kg bw/day; thyroid gland = 43.67 to 179.92 mg/kg bw/day).

Source: based on data from **NTP (National Toxicology Program)**. 1990. *National Toxicology Program, Toxicology and carcinogenesis studies of glycidol (CAS No. 556-52-5) in F344/N rats and B6C3F1 mice (gavage studies).* Technical Report Series No. 374. National Institutes of Health Publication No. 90-2829. Research Triangle Park, NC.

Based on the NTP (1990) study, F344 rats appear to be more sensitive to glycidol exposure than B6C3F1 mice in both the chronic bioassay and the subchronic (90-d) range-finding study that preceded it. Increased incidence of mammary adenocarcinoma in female F344 rats is the third most sensitive endpoint identified in the two-year cancer bioassay and may represent a more human-relevant tumour from which to derive a reference point. These tumours have a low background incidence and displayed clear evidence of treatment-related effects in both female rats and mice. Rats were generally the more sensitive species, and the Bayesian model average $BMDL_{10}$ in female F344 rats was 7.3 mg/kg bw/day, which is approximately three-fold higher than the $BMDL_{10}$ of 2.4 mg/kg bw/day selected by JECFA and nine-fold higher than the $BMDL_{10}$ for TVM in male F344 rats based on Bayesian model averaging. Alternatively, an increase in combined incidence of liver adenoma and carcinoma in male mice could be considered, with a $BMDL_{10}$ based on Bayesian model averaging of 5.42 mg/kg bw/day. However, liver tumours were observed in almost half (24 of 50) of male mice in the vehicle control group, increasing to 31/50 and 35/50 at the low and high dose, respectively. In contrast, the incidence of mammary adenocarcinoma in female rats was 1/50 in controls and 11/48 and 16/48 at the low and high dose, respectively, indicating a greater absolute increase as well as a larger reduction in the proportion of tumour-free animals relative to liver tumours in male mice. Caution should also be taken in interpreting one particular tumour site as being more sensitive than another, as the data shown in Figure 8 are a snapshot from a single experiment and their reproducibility is unknown. There is also considerable overlap in the confidence intervals for many tumour types and, therefore, the order shown above may be the result of chance. Lastly, the incidence of a tumour at one site may mask the potential observation of tumours at other sites due to mortality as a result of the primary tumour. Nevertheless, the GEs concentration in LNS/RUTF that corresponds to a lifetime MOE of 10 000 based on the various potential reference points described above and over different durations of exposure are shown in Table 6.

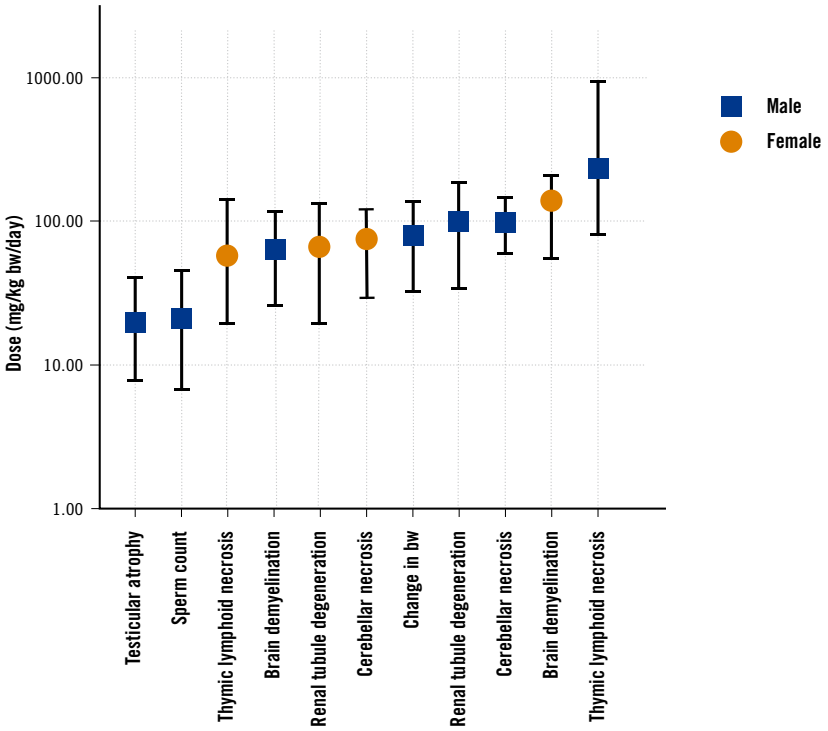
TABLE 6 **GE CONCENTRATION IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOODS (µG/KG) CORRESPONDING TO A LIFETIME MOE OF 10 000 (ASSUMING JECFA MEAN LB BACKGROUND EXPOSURE SCENARIO AND LNS/RUTF CONSUMPTION OF 42 G/KG BW/DAY)**

REFERENCE POINT	3 MONTHS	6 MONTHS	12 MONTHS
JECFA (2016) $BMDL_{10}$ (2.4 mg/kg bw/day)	797	400	201
Revised JECFA BMDL using Bayesian model averaging (0.83 mg/kg bw/day)	N/A	N/A	N/A
Use of a tumour type that may be of greater human relevance (7.3 mg/kg bw/day)	4 064	2 033	1 017

11.4 SHORT-TERM (NON-CANCER) EFFECTS

All previous assessments of GEs used reference points based on cancer and chronic exposure. However, given that exposure to GEs from consumption of LNS/RUTF is of limited duration, consideration was given to characterizing the risk specific to these products by using a reference point derived from a short-term study. Prior to initiating the chronic bioassay, the NTP (1990) conducted a 13-week range-finding study. F344 rats (ten per sex per group) were administered glycidol by gavage at doses ranging from 25 to 400 mg/kg bw/day, whereas B6C3F$_1$ mice (ten per sex per group) received doses ranging from 19 to 300 mg/kg bw/day (vehicle controls received distilled water). In both rats and mice, the highest dose led to 100 percent mortality. A number of non-neoplastic, treatment-related effects were observed, with rats being more sensitive than mice (data not shown). These non-cancer effects were modelled to derive BMDs and their corresponding confidence intervals for dichotomous or continuous effects using Bayesian model averaging in the R package "ToxicR" (Figure 9).

FIGURE 9. BAYESIAN MODEL AVERAGE BENCHMARK DOSES (TOXICR) FOR NON-CANCER EFFECTS IN F344 RATS OBSERVED IN A 90-DAY RANGE-FINDING STUDY



Note: Bayesian model average BMDs (ToxicR) for non-cancer effects in F344 rats observed in a 90-d range-finding study (NTP, 1990). Markers represent the BMD and the lower and upper error bars the BMDL$_{10}$ and BMDU$_{10}$, respectively. Blue squares represent values in males and orange circles represent those in females. As in Figure 8, doses are plotted on a log scale and this should be kept in mind when considering the width of the confidence intervals.

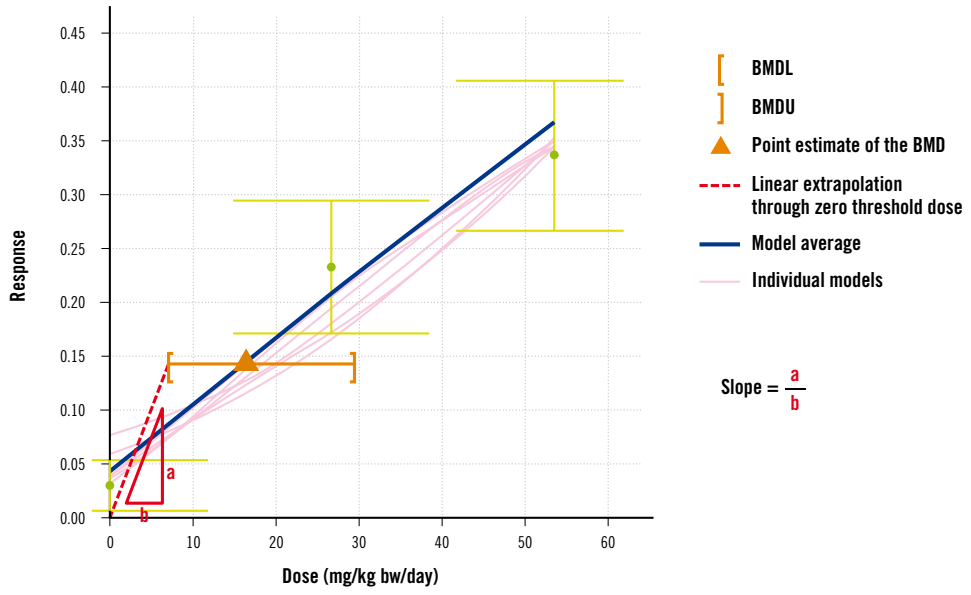
Source: based on data from **NTP (National Toxicology Program)**. 1990. *National Toxicology Program, Toxicology and carcinogenesis studies of glycidol (CAS No. 556-52-5) in F344/N rats and B6C3F1 mice (gavage studies).* Technical Report Series No. 374. National Institutes of Health Publication No. 90-2829. Research Triangle Park, NC.

Testicular toxicity in male rats appeared to be the most sensitive endpoint, with BMDLs for reduced sperm count of 6.69 mg/kg bw/day and testicular atrophy of 7.64 mg/kg bw/day. Although based on the application of standard uncertainty factors (e.g. for interspecies extrapolation and inter-individual variation) the MOEs that would be considered acceptable for a short-term, non-cancer endpoint would be lower than those for carcinogenicity, it is important to consider that while exposure to LNS/RUTF is limited to early life, exposure to GEs is chronic in nature. Therefore, reliance on a non-cancer endpoint to characterize the risk of short-term exposure in isolation may not be adequately protective of carcinogenicity as it does not account for the contribution via LNS/RUTF to the total combined exposure. However, it is important to note that using carcinogenicity as a reference point to characterize risk would also be protective of short-term, noncancer effects in infants and children.

11.5 INCREMENTAL LIFETIME CANCER RISK

While the MOE approach is commonly used in the risk assessment of carcinogens and is useful to characterize the magnitude of a risk, it cannot be used to quantify the increased probability of an adverse health effect. An alternative approach is to calculate the ILCR, which refers to the estimated increase in lifetime cancer risk above the risk associated with background exposures. The cancer potency of a given substance is proportional to the slope of the dose-response curve at low doses, referred to as the cancer slope factor (Figure 10).

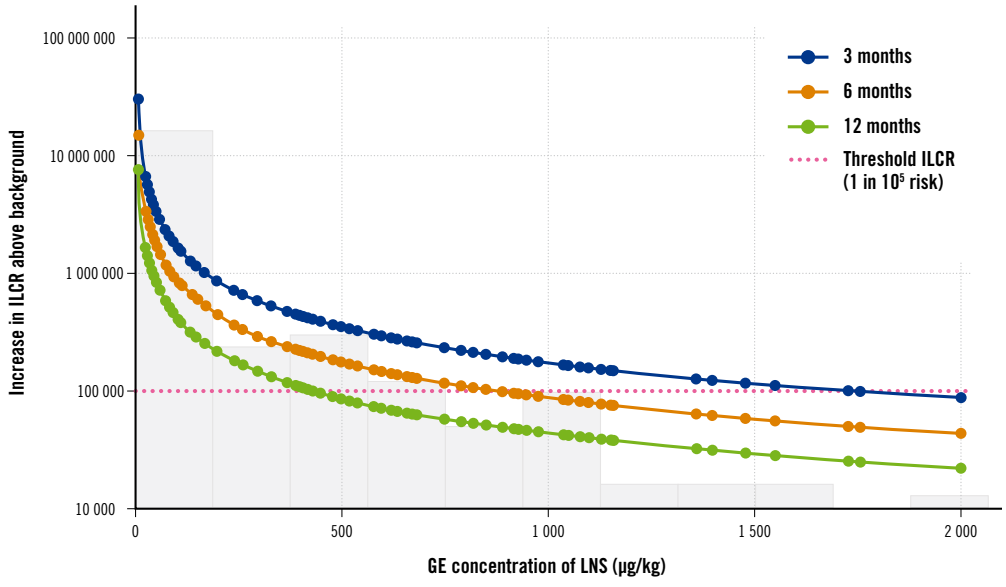
FIGURE 10. **DERIVATION OF A CANCER SLOPE FACTOR**



The cancer slope factor corresponds to "an upper bound, approximating a 95 percent confidence limit, on the increased cancer risk from a lifetime exposure to an agent by ingestion" (EPA, n.d.). There is an inherent assumption in this approach that observations in animals treated at relatively high doses are relevant to humans at lower doses, and that potency may be extrapolated linearly outside the range of experimental observations. ILCR may then be calculated as the product of the LADD and the cancer slope factor, and an ILCR of 1 in 100 000 (1 in 10^5) is generally considered negligible. This method has the advantage of being able to estimate the increase in cancer risk due to exposure to GEs from LNS/RUTF consumption specifically, relative to the risk associated with background exposure.

In the absence of LNS/RUTF consumption, the background lifetime cancer risk due to glycidol exposure via the diet is estimated to be approximately 1 in 200 000 in the JECFA (2016) mean lower bound exposure scenario. The contribution of any additional exposure to GEs from LNS/RUTF specifically can then be estimated (Figure 11) and in turn extrapolated to a GE concentration in products that would not be expected to exceed a 1 in 10^5 increase in ILCR (Table 7). An example of how the change in ILCR attributable to LNS/RUTF exposure is calculated may be found in Annex 5.

FIGURE 11. **EFFECT OF GLYCIDYL FATTY ACID ESTERS (GE) CONCENTRATION IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD ON INCREMENTAL LIFETIME CANCER RISK BASED ON THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES $BMDL_{10}$ OF 2.4 MG/KG BW/DAY AND ASSUMING MEAN LOWER BOUND BACKGROUND EXPOSURE SCENARIO**



Note: The histogram in grey represents the GE content of products (n = 97) and the dashed horizontal line indicates a 1 in 10^5 increase in ILCR. Note that the parallelism in the curves for the different exposure durations is a reflection of Haber's rule.

TABLE 7 GLYCIDYL FATTY ACID ESTERS CONCENTRATION IN LIPID-BASED NUTRIENT SUPPLEMENTS/ READY-TO-USE THERAPEUTIC FOOD (µG/KG) CORRESPONDING TO A 1 IN 10^5 INCREASE IN INCREMENTAL LIFETIME CANCER RISK ABOVE BASELINE IN THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (2016) MEAN LOWER BOUND BACKGROUND EXPOSURE SCENARIO, ASSUMING A DAILY INTAKE OF 36 G/KG BW/DAY

REFERENCE POINT	3 MONTHS	6 MONTHS	12 MONTHS
JECFA (2016) $BMDL_{10}$ (2.4 mg/kg bw/day)	1 890 (99%)	946 (80%)	474 (57%)
Revised JECFA BMDL using Bayesian model averaging (0.83 mg/kg bw/day)	648 (70%)	325 (40%)	164 (32%)
Use of a tumour type that may be of greater human relevance (7.3 mg/kg bw/day)	5 767 (100%)	2 884 (100%)	1 443 (95%)

Note: The numbers in parentheses indicate the percentage of current products for which data are available ($n = 97$) that contain GEs in glycidol equivalents at or below the proposed thresholds.

It should be emphasized that the "acceptable" levels of GEs in LNS/RUTF products noted above are specific to the assumptions under which they are derived (i.e. daily dose, duration of exposure, background exposure scenario and point of departure). If chemical-specific differences between adults and juveniles are known to exist that indicate early life susceptibility, then age dependent adjustment factors (ADAFs) may also be applied to the cancer slope factor that are specific to a particular age group. Given that LNS/RUTF exposure occurs almost exclusively during infancy and childhood, the application of ADAFs would result in more conservative thresholds than those identified above. However, if toxicokinetic and toxicodynamic evidence does not suggest that children are inherently more susceptible to glycidol-induced genotoxicity than adults, the application of ADAFs may not be justified. Recently, the Netherlands National Institute of Public Health and the Environment (RIVM) conducted a series of studies to determine whether exposure to genotoxic substances induced DNA mutations or chromosomal damage at higher rates in animals exposed at a young age compared to animals exposed as adults (RIVM, 2014). The experimental agents used included acrylamide, which is an alkylating agent that shares a similar mechanism of action with glycidol, and no evidence of increased susceptibility to mutagenic effects was observed when exposure occurred early in life. The authors concluded that "young animals do not appear to be more susceptible than adult animals to mutagenic effects of environmental chemicals". The Senate Commission on Food Safety (SKLM) of the German Research Foundation also critically reviewed the toxicity of acrylamide with a focus on its toxicological characteristics at low versus high doses (Guth *et al.*, 2023). Like glycidol, acrylamide is tumourigenic in animal studies and was concluded to be a genotoxic carcinogen by EFSA (2015; 2022b) and thus the MOE approach was considered appropriate for safety assessment. However, the SKLM argued that the evidence from low dose studies, which more closely reflect dietary exposure, suggest the existence of a sublinear, threshold dose-response and therefore genotoxic effects resulting in carcinogenicity are not anticipated at dietary levels of exposure. Although similar data are not available for glycidol, the use of linear, low dose (no threshold) extrapolation is inherently conservative.

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CHAPTER 12
DISCUSSION

While food is a basic necessity for life, foods can also be associated with adverse health effects, including those resulting from dietary exposure to chemical substances, whether naturally occurring, process-induced, or in the form of additives or contaminants. However, the health benefits of adequate food and nutrition, particularly in key windows of growth and development, are such that some degree of risk may be considered acceptable provided it is clearly outweighed by the benefits. Supplemental and therapeutic foods such as LNS and RUTF in particular are at the interface between food and medicines, as they are intended to treat and prevent severe acute malnutrition, a serious and debilitating condition that contributes to an estimated one to two million child deaths every year (Kassaw *et al.*, 2021). Therefore, although exposure to potentially harmful substances from consumption of these products should be mitigated to the extent feasible, their obvious benefits must be considered when determining the level of concern associated with process-induced contaminants.

12.1 3-MCPD

Although the majority of studies have been conducted with 3-MCPD, the predominant forms of 3-MCPD found in foodstuffs containing edible fats and oils are 3-MCPD mono- and di- fatty acid esters. However, evidence to date indicates substantial hydrolysis of the fatty acid ester is expected to occur in the GI tract leading to release and absorption of the free 3-MCPD compound. While close to 100 percent hydrolysis has been shown to occur with simple monoesters, 3-MCPD release from diesters is slightly lower. Current evaluations have concluded that the toxic effects noted for both 3-MCPD and equimolar doses of the corresponding fatty acid esters are similar.

To date, the critical studies used in 3-MCPD risk assessment have been chronic in duration where animals were exposed for their lifetime to 3-MCPD in drinking water. The most sensitive effect noted was a dose-dependent increase in renal tubular hyperplasia primarily in male rats. The lowest $BMDL_{10}$ reported by JECFA was 0.87 mg/kg bw/day, using the restricted log-logistic model, while EFSA selected a $BMDL_{10}$ of 0.2 mg/kg bw/day, using the same study and effect but based on use of an updated model averaging approach.

Updated toxicological data support the conclusions from the previous evaluations, which identified adverse effects in kidney and testes following chronic exposure to 3-MCPD as sensitive endpoints in experimental animal models. While the critical study and effect are still relevant, updated dose-response modelling guidance according to EHC 240 recommends using a model averaging approach, compared to the arbitrary selection of the lowest BMDL, with all adequate models fit through a weighted average. Applying this approach to the previous JECFA 3-MCPD evaluation would result in lowering the $BMDL_{10}$ for renal tubular hyperplasia in male rats from 0.87 mg/kg bw/day to 0.48 mg/kg bw/day. Retaining the same 200-fold composite uncertainty factor would result in an updated HBGV of 2.4 µg/kg bw/day. An additional recommendation would be for JECFA to reconsider if an additional factor of two is still required due to the described inadequacies in reproductive toxicity studies. As the updated BMD dose-response modelling guidance according to EHC 240, including the use of model averaging, is now recommended for generating BMD estimates, the dose-response analysis as described in Option 2 and 4 would be preferred.

RUTF and LNS are specially prepared food products which are generally provided to children aged 6 to 59 months in situations where under-nutrition or acute malnutrition is a concern. Due to the limited exposure period associated with the use of these products, comparison of lifestage-specific exposure with HBGVs developed based on chronic or lifetime exposure, as described in Option 2, may be overly conservative. However, due to the widespread occurrence of similar processing-induced contaminants in numerous food categories containing edible fats and oils, development of a short-term HBGV was not considered appropriate. While typically used in the assessment for genotoxic carcinogens, the LADD metric, or the cumulative dose received over a lifetime, might also be an appropriate measure of dose or exposure for some threshold toxicants.

Although consumption of LNS/RUTF is of limited duration and mainly restricted to infants and young children, most of the total lifetime exposure to 3-MCPD and its fatty acid esters is attributed to foods other than LNS/RUTF. It was therefore considered appropriate to use a LADD approach to characterize the potential risk of exposure to these substances from less-than-lifetime use of LNS/RUTF, under the assumption that short-term excursions above the PMTDI may be tolerable so long as the LADD was not exceeded. Although the typical intake periods for LNS/RUTF are recommended for durations of up to three months, depending on the malnutrition status, as a worst-case scenario for a LADD estimation, it was assumed that children are exposed to these products as sole source nutrition for 0–1 years or for 1–5 years. Using conservative estimates of LNS/RUTF consumption in young children and infants in combination with high (95th percentile) background consumer intakes for all other age categories, it was determined that the LADD would not exceed the updated PMTDI of 2.4 µg/kg bw if total 3-MCPD equivalent concentrations in LNS/RUTF did not exceed 335 µg/kg.

Most of the lifetime exposure to 3-MCPD (esters) occurs during age periods where LNS/RUTF are not used (ages 6–70 years), which would imply that exposures

greater than a chronic HBGV such as a TDI may be tolerated over shorter periods of time as long as the average total lifetime exposure did not exceed the TDI. As presented in Option 4, using LNS consumption of 36 g/kg bw/day for the age categories of 0–1 and 1–5 years plus background high consumer (95th percentile) intakes for the 6–10, 11–19 and 20–70 years age categories would not exceed the updated JECFA TDI if total 3-MCPD concentrations in LNS/RUTF did not exceed 335 µg/kg (ppb). While this would result in shorter-term exposure to 3-MCPD, which exceeds the chronic HBGV by approximately 10-fold, this exposure duration is less than 10 percent of a 70-year lifespan. Based on these factors, Option 4 is recommended for use in risk characterization of 3-MCPD in LNS products.

12.2 GLYCIDYL ESTERS

Several recent assessments of dietary GE exposure have been conducted by expert committees, notably JECFA (2016; 2018) and EFSA (2016). As the toxicologically relevant metabolite of these substances is a genotoxic carcinogen, both expert groups elected to use an MOE approach rather than establishing an HBGV. Both EFSA and JECFA selected the formation of mesotheliomas in the tunica vaginalis/peritoneum (TVM) in male F344 rats in the NTP (1990) chronic bioassay as the critical effect, with JECFA determining a $BMDL_{10}$ of 2.4 mg/kg bw/day and EFSA selecting the T_{25} of 10.2 mg/kg bw/day as reference points for risk characterization, respectively, at that time. Generally, an MOE of ≥10 000 based on a $BMDL_{10}$ from an appropriate animal study, when compared to an estimate of chronic human exposure, is considered to be of low concern for human health, whereas an MOE of 25 000 is typically applied to a T_{25}, and thus the two approaches lead to similar acceptable intakes.

The BMD approach is subject to less bias and less variation in estimating the potency of carcinogens compared to the T_{25} (Van Landingham *et al.*, 2001) and is the approach now preferred by JECFA and EFSA for dose-response modelling. Updated guidance on the use of the BMD approach in risk assessment has recently been published by both the WHO (2020) and EFSA (2022a) and Bayesian model averaging is recommended for estimating the BMD and calculating its credible interval. Therefore, in the current assessment, data from the same endpoint selected by EFSA and JECFA were subjected to dose-response modelling in accordance with the updated guidance. The Bayesian model average $BMDL_{10}$ for TVM in male F344 rats in the NTP (1990) chronic bioassay was 0.83 mg/kg bw/day, or roughly 3-fold lower than the reference point derived by JECFA. Although this is a slight departure from the approach used in the recent assessment (JECFA, 2016), it is considered appropriate as it aligns with the dose-response modelling practices currently recommended by WHO and in use by JECFA.

There is evidence to suggest that xenobiotic-induced TVM are a male F344 rat-specific phenomenon that may be of no relevance for human health risk assessment. Rather than selecting the most conservative value from the rodent carcinogenicity study irrespective of its relevance to humans, consideration was

given to setting aside those tumour types that are likely to be rodent-specific and then selecting the next most sensitive site from which to derive a reference point. However, glycidol produces not only TVM but is also a multi-site genotoxic carcinogen and, as Boobis *et al.* (2008) have noted, due to species- and tissue-specific variation in metabolic activation and detoxification, there is often only poor site concordance for genotoxic carcinogens. Therefore, excluding TVM for lack of human relevance may not be appropriate in the case of a multisite genotoxic carcinogen vis à vis a substance that only induces TVM.

Glycidol is an alkylating agent that forms adducts with DNA, and tumour formation is presumed to be the apical expression of this genotoxicity. Adduct formation can be measured in human or animal studies, although typically hemoglobin (protein) adducts are used as a surrogate for DNA adduct formation in target tissues on account of being more accessible and less transient. Aasa, Granath and Törnqvist (2019) estimated internal doses of glycidol in mice and rats following short-term oral administration, as determined by hemoglobin adduct measurements. These values were compared with tumour incidence data from the NTP (1990) chronic bioassay and based on a multiplicative risk model, good agreement between predicted and experimental responses was observed. When this model was extrapolated to humans, the daily intake associated with a 1 in 10^5 cancer risk was estimated to be 0.40 µg/d, or 0.0067 µg/kg bw/day for a 60 kg individual. Quantitative cancer risk assessment based on adduct formation, however, is not yet well established and considerable uncertainties remain. Generally speaking, DNA adducts are considered important biomarkers of exposure but not necessarily effect, as not all DNA adducts result in mutation and not all mutations are in critical genes for carcinogenesis. There is also evidence that at least certain alkylators exhibit thresholds for mutagenicity and exposure may only be of toxicological significance when DNA repair mechanisms become saturated (Jenkins *et al.*, 2005; Doak *et al.*, 2007; Gocke and Müller, 2009; Thomas *et al.*, 2013; Guérard *et al.*, 2015; Guth *et al.*, 2023). DNA repair is also known to be species- and tissue-specific and thus the same alkylator may exhibit distinct dose-response characteristics depending on the species and/or site.

Finally, exposure to GEs from LNS/RUTF is limited to a defined duration and life stage, and consideration was given to characterizing the risk based on a less-than-lifetime, non-cancer endpoint, such as those observed in the NTP (1990) 13-week range finding study. In the case of subchronic exposure, reproductive toxicity in male rats was observed to be the most sensitive endpoint for glycidol exposure. However, exposure to GEs from other sources is chronic in nature and both JECFA and EFSA determined that carcinogenicity is the most sensitive endpoint for this substance. Assessing the non-cancer risk of glycidol exposure specific to LNS/RUTF intake would ignore the potential contribution of these products to the total combined exposure (i.e. the LADD) and may underestimate the incremental lifetime cancer risk. Therefore, the use of a short-term, non-cancer endpoint was not considered to be sufficiently conservative. However, it should be noted that the use of carcinogenicity as the critical effect to characterize risk is also protective of short-term, non-cancer effects.

Assuming the mean JECFA LB background exposure scenario and an intake assumption of 36 g/kg bw/day for 12 months, the GEs concentration (in glycidol equivalents) that would correspond to a 1 in 10^5 increase in ILCR was estimated to be 164 µg/kg LNS/RUTF (164 ppb). This value is considered highly conservative as it is based on the daily intake of RUTF specifically, as this product represents the greatest potential for exposure but is intended for use over shorter durations (four to eight weeks). Despite this conservatism, based on the data currently available for various LNS and RUTF products, nearly one third (31 of 97 products, or ~32 percent) currently have GEs concentrations below this threshold, and therefore a reduction to this level is considered technologically feasible, at least under certain conditions of manufacture and/or using certain raw materials. Further investigation would be required to identify the specific factors (e.g. type or source of oil, purity, processing method, geographic region, etc.) that differentiate those products with low levels of GEs contamination from those with higher concentrations.

The levels proposed herein for the concentrations of 3-MCPD and its esters and GEs in LNS/RUTF products exceed the maximum limits established for certain foodstuffs in the European Union. However, based on quantitative risk assessment, exposure at these levels via LNS/RUTF represents a low level of concern in which the risks associated with these process-induced contaminants are clearly outweighed by the benefits of having access to therapeutic foods in children suffering from or predisposed to acute malnutrition. Whereas dietary exposure to 3-MCPD and its esters and GEs is chronic in nature, LNS is intended to be consumed over a period of two to three months and RUTF for a period of four to eight weeks. Assuming a 70 year life expectancy, this corresponds to just 0.1 – 0.2 percent of the total lifespan. In more extreme circumstances where exposure could be continuous for up to one year, exposure to these substances via LNS/RUTF would occur for 1.4 percent of a 70 year lifespan. However, the most sensitive endpoints for characterizing the risk of these substances are derived from studies of chronic exposure, and the LADD approach was used to account for the potential contribution of LNS/RUTF products to the overall lifetime risk. These limits should be regarded as provisional and while considered to represent a low level of concern relative to the therapeutic benefits, manufacturers of LNS/RUTF should nevertheless strive for process improvements to limit exposure to these contaminants to the extent feasible.

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CHAPTER 13
UNCERTAINTIES

Several key uncertainties were identified in this assessment and are presented in Table 8 below.

TABLE 8 SOURCES OF UNCERTAINTY IN THE RISK ASSESSMENT OF 3-MCPD AND GLYCIDYL FATTY ACID ESTERS IN LIPID-BASED NUTRIENT SUPPLEMENTS/READY-TO-USE THERAPEUTIC FOOD

SOURCES OF UNCERTAINTY	DIRECTION
There is insufficient information on the potential for interaction between 3-MCPD and GEs, particularly with respect to non-cancer endpoints.	+/-
Studies suggest the potential for species/strain sensitivity for both 3-MCPD and glycidol and uncertainties remain with respect to the human relevance of reference points derived from rodent studies.	+
The small number of dose levels ($n = 2$) in the chronic bioassay of glycidol results in relatively wide BMD confidence intervals.	+
Background dietary exposure is unaccounted for in the key study of glycidol.	+
Risk characterization of GEs relied on JECFA (2016) background exposure estimates derived from countries where LNS/RUTF is not typically consumed and their relevance to background exposure among populations where LNS/RUTF is consumed is unknown.	+/-
The data relied upon by JECFA to derive background exposure estimates are somewhat dated and ongoing advances in methods to mitigate exposure to process-induced contaminants from infant formula in particular have likely reduced current dietary exposure levels.	+
There is uncertainty regarding the use of dose averaging to amortize doses of glycidol received over a relatively brief period, particularly for a substance with a relatively short biological half-life. The principle of dose averaging is based on Haber's rule and the assumption that toxicity is related to the total combined exposure. While the basic concept is routinely applied in cancer risk assessment for genotoxic carcinogens, there is uncertainty as to the extent to which average exposure calculated by dose averaging reflects the relevant measure of exposure in toxicological terms.	-
While the available evidence does not suggest that infants and children are notably more susceptible to 3-MCPD or GE toxicity relative to older children and adults, the potential influence of malnutrition on detoxification mechanisms has not been adequately characterized.	-

Note: "+" = uncertainty with potential to cause over-estimation of risk; "-" = uncertainty with potential to cause underestimation of risk; "+/-" = unknown potential to cause over or under estimation of risk.

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ANNEX 1
3-MCPD DOSE-RESPONSE MODEL AVERAGING USING TOXICR

TABLE A1.1 **ENDPOINT: MALE RENAL TUBULE HYPERPLASIA**

DOSE	NUMBER	EFFECT
0	1	50
2.0	11	50
8.3	21	50
29.5	36	50
Reference	Cho, W.S., Han, B.S., Nam, K.T., Park, K., Choi, M., Kim, S.H., Jeong, J. & Jang, D.D. 2008. Carcinogenicity study of 3-monochloropropane-1,2-diol in Sprague-Dawley rats. *Food and Chemical Toxicology*, 46, 3172–3177. https://doi.org/10.1016/j.fct.2008.07.003	
Data type	Dichotomous (Quantal)	
BMR	0.1	
Number of bootstrap samples	25 000	

MODELS

Dichotomous response models assume that the response has a probability $\pi(x)$ of occurring at some dose x, where $\pi(x)$ is a parametric function of dose that needs to be estimated. The following nine models, with corresponding parameter prior, are used in the model average:

PROBIT

$$\pi(x) = \Phi(a + b \times x)$$

With $a \sim N(0,1)$, and $b \sim N(0,1)$.

LOG-PROBIT

$$\pi(x) = a + (1 - a)\,\Phi(c + d \times x)$$

With $\text{logit}[a] \sim N(0,2)$, $c \sim N(0,1)$, and $\log[d] \sim N(\log[2], 0.5)$

LOGISTIC

$$\pi(x) = \frac{1}{1 + \exp(-[a + b \times x])}$$

With $a \sim N(0,1)$ and $\log[b] \sim \text{N}(0.1,1)$.

LOG-LOGISTIC

$$\pi(x) = \frac{1 - a}{1 + \exp(-[c + d \times x])}$$

With $\text{logit}[a] \sim N(0,2)$, $c \sim N(0,1)$, and $\log[d] \sim N(\log[2], 0.5)$.

HILL

$$\pi(x) = a + \frac{b \times (1 - a)}{1 + \exp(-[c + d \times x])}$$

With $\text{logit}[a] \sim N(-1,2)$, $\text{logit}\,[b] \sim N(4,2)$, $c \sim N(0,0.25)$, and $\log[d] \sim N(\log[2], 0.5)$

WEIBULL

$$\pi(x) = a + (1 - a)\,(1 - \exp[-b \times x^{d}])$$

With $\text{logit}[a] \sim N(0,2)$, $b \sim N(0,1.5)$, and $\log[d] \sim N(log[2], 0.5)$

GAMMA

$$\pi(x) = a + (1 - a)\int_{0}^{b \times x} t^{d-1} \exp(-t)\,\text{dt}$$

With $\text{logit}[a] \sim N(0,2)$, $\log[b] \sim N(0,1)$, and $\log[d] \sim N(log[2], 0.424)$

QUANTAL-LINEAR

$$\pi(x) = a + (1 - a)(1 - \exp[-b \times x])$$

With $\text{logit}[a] \sim N(0,2)$ and $\log[b] \sim N(0.15,1)$

MULTISTAGE

$$\pi(x) = a + (1 - a)(1 - exp[-b_1 \times x - b_2 \times x^2])$$

With $\text{logit}[a] \sim N(0,2)$, $\log[b_1] \sim N(0,0.5)$ and $\log[b_2] \sim N(0,1.0)$.

PRIORS

The default analysis in ToxicR uses prior information detailed in the manuscript of Wheeler *et al.* (2020). These priors were developed for general dichotomous dose-response data and showed good performance in simulation studies.

BENCHMARK DOSE ESTIMATES

TABLE A1.2 SUMMARY OF SINGLE MA BMD - INDIVIDUAL MODEL BMDs

Model	BMD	(BMDL,	BMDU)	Pr(M|Data)
Quantal-Linear	2.29	(1.76,	3.10)	0.505
Log-Logistic	1.14	(0.42,	2.54)	0.168
Weibull	0.93	(0.29,	2.28)	0.122
Hill	1.17	(0.45,	2.47)	0.091
Multistage	2.65	(1.96,	3.99)	0.051
Gamma	1.25	(0.44,	2.90)	0.042
Log-Probit	1.42	(0.62,	2.99)	0.018
Probit	5.41	(4.50,	6.57)	0.002
Logistic	5.68	(4.64,	6.99)	0.001

Model average BMD: 2.01 (0.48, 3.04) 90.0% CI

FIGURE A1.1. **BMD ESTIMATE BY EACH MODEL (SORTED BY POSTERIOR PROBABILITY) FOR RENAL TUBULE HYPERPLASIA IN MALE RATS**

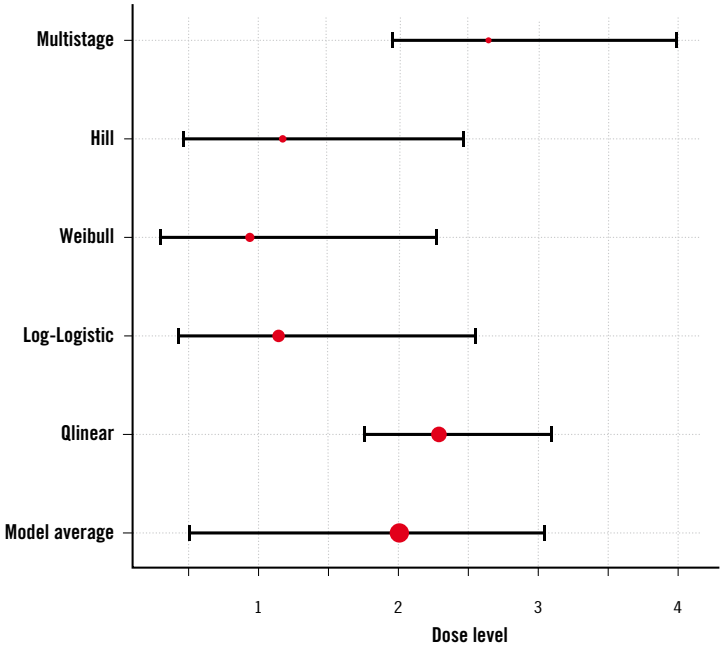
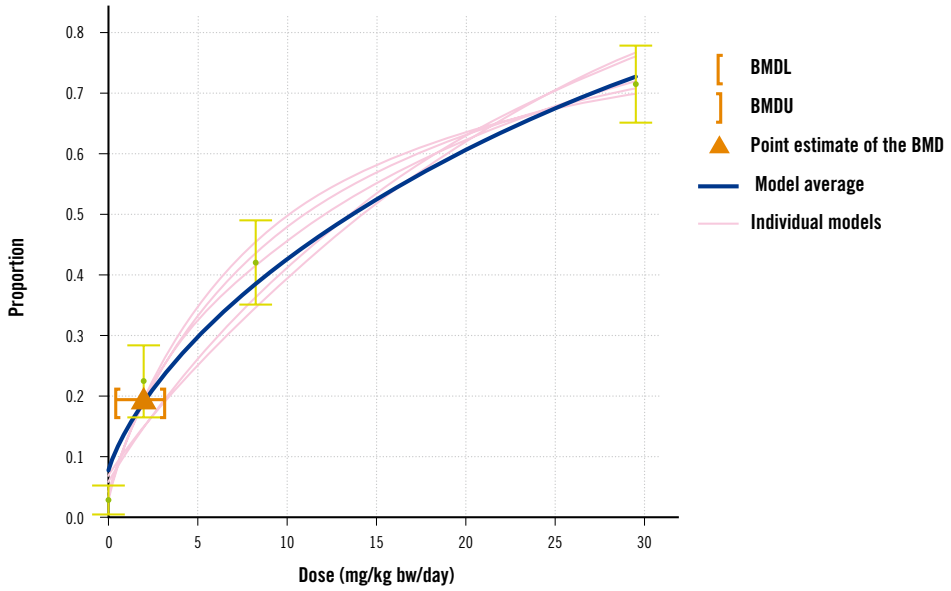


FIGURE A1.2. **BMD WITH BAYESIAN MODEL AVERAGING FOR 3-MCPD (RENAL TUBULE HYPERPLASIA IN MALE RATS): DICHOTOMOUS MA, FIT TYPE: LAPLACE**



ToxicR output – Bayesian model average BMD of relative kidney weight in F344 male rats (Toyoda *et al.*, 2017)

SUMMARY

TABLE A1.3 BENCHMARK DOSE ESTIMATES FOR 3-MCPD

STRAIN	$BMDL_{10}$	BMD	$BMDU_{10}$
F344	0.70	2.13	6.26

Units are mg/kg bw/day. BMD is defined as the dose associated with a one standard deviation change from the control dose mean. $BMDL_{10}$ represents the one-sided 95 percent CI of the BMD.

F344 RATS – RELATIVE KIDNEY WEIGHT (Toyoda *et al.*, 2017)

TABLE A1.4 SUMMARY OF DATA MODELLED – SUBCHRONIC TOXICITY STUDY OF 3-MCPD USING AN OBESE RAT MODEL

DOSE	RESPONSE	N	SD
0	0.59	10	0.02
0.7	0.59	10	0.03
2.2	0.62	10	0.04
6.7	0.66	10	0.04
21.3	0.74	10	0.04
54	0.96	10	0.04

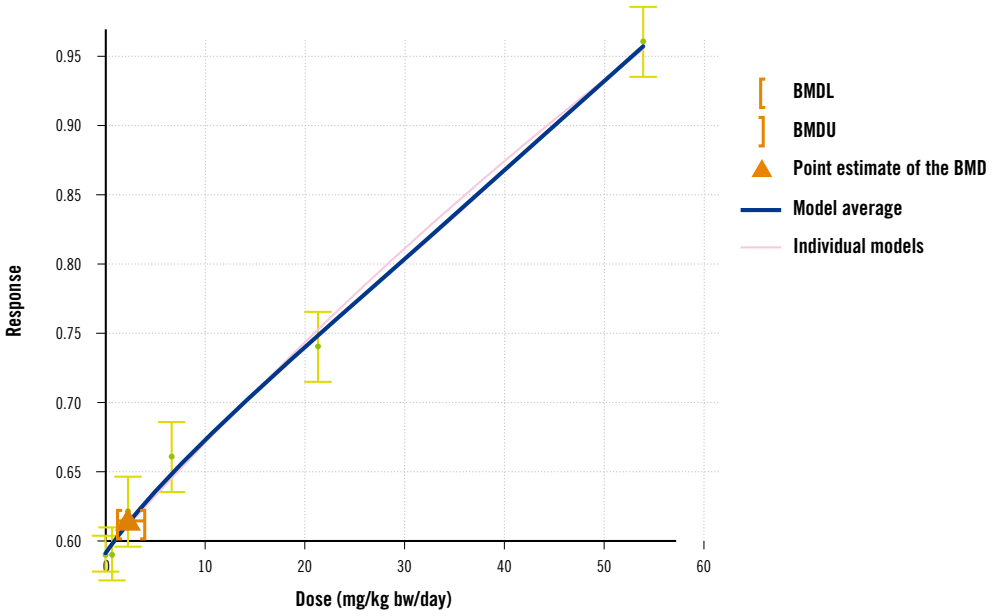
Source: based on data from **Toyoda, T., Cho, Y.-M., Akagi, J., Mizuta, Y., Matsushita, K., Nishikawa, A., Imaida, K. & Ogawa, K.** 2017. Altered susceptibility of an obese rat model to 13-week subchronic toxicity induced by 3 monochloropropane-1,2 diol. *Journal of Toxicological Sciences*, 42(1), 1–11.

TABLE A1.5 SUMMARY OF SINGLE MA BMD - INDIVIDUAL MODEL BMDs (RELATIVE KIDNEY WEIGHT IN RATS)

Model	BMD	(BMDL,	BMDU)	Pr(M|Data)
Exponential-3 distribution: Log-Normal	2.20	(1.35,	3.57)	0.858
Exponential-5 distribution: Log-Normal	3.33	(1.90,	4.76)	0.142
Hill distribution: Normal	5.62	(3.67,	8.42)	0.000
Hill distribution: Normal-NCV	NA	(NA,	NA)	0.000
Exponential-3 distribution: Normal	3.53	(2.14,	5.71)	0.000
Exponential-3 distribution: Normal-NCV	2.00	(0.63,	6.06)	0.000
Exponential-5 distribution: Normal	4.62	(2.97,	7.09)	0.000
Exponential-5 distribution: Normal-NCV	2.16	(0.78,	2.21)	0.000
Power distribution: Normal	4.14	(2.63,	6.38)	0.000
Power distribution: Normal-NCV	2.13	(0.70,	6.26)	0.000

Model average BMD: 2.13 (0.70, 6.26) 90.0% CI

FIGURE A1.3. **CONTINUOUS MA FITTING**



TOXICR OUTPUT – BAYESIAN MODEL AVERAGE BMD OF CURVILINEAR SPERM VELOCITY WEIGHT IN SD MALE RATS

TABLE A1.6 **SUMMARY OF DATA MODELLED**

DOSE (mg/kg bw/day)	VCL (lm/s)	SD (lm/s)	N
0	449.9	27.8	12
1	441.3	39.8	12
3	402.0	20.4	13
10	309.2	73.2	13

Notes: bw: body weight. VCL: curvilinear velocity. SD: standard deviation.
Source: based on data from **Ban, Y., Asanabe, U., Inagaki, S., Sasaki, M., Nakatsuka, T. & Matsumoto, H.** 1999. Effects of alpha-chlorohydrin on rat sperm motions in relation to male reproductive functions. *Journal of Toxicological Sciences*, 24, 407–413.

TABLE A1.7 SUMMARY OF SINGLE MA BMD - INDIVIDUAL MODEL BMDs (CURVILINEAR SPREM VELOCITY IN RATS)

Model	BMD	(BMDL,	BMDU)	Pr(M|Data)
Exponential-5 distribution: Normal	6.52	(2.51,	25.34)	0.335
Hill distribution: Normal	2.46	(0.96,	NaN)	0.297
Power distribution: Normal	7.82	(3.51,	16.65)	0.221
Exponential-3 distribution: Normal	7.32	(3.05,	18.29)	0.100
Exponential-5 distribution: Normal-NCV	9.62	(3.67,	NaN)	0.026
Hill distribution: Normal-NCV	3.32	(1.06,	NaN)	0.016
Power distribution: Normal-NCV	10.51	(5.16,	26.81)	0.003
Exponential-3 distribution: Normal-NCV	10.38	(4.62,	27.43)	0.002
Exponential-3 distribution: Log-Normal	NA	(NA,	NA)	0.000
Exponential-5 distribution: Log-Normal	NA	(NA,	NA)	0.000

Model average BMD: 5.92 (1.38, 141.45) 90.0% CI

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ANNEX 2
GLYCIDOL DOSE-RESPONSE MODEL AVERAGING USING TOXICR

TABLE A2.1 ENDPOINT: TUNICA VAGINALIS/PERITONEUM – MESOTHELIOMA IN MALE F344 RATS

DOSE	NUMBER	EFFECT
0	49	3
26.8	50	34
53.6	47	39
Reference	NTP (National Toxicology Program). 1990. *National Toxicology Program, Toxicology and carcinogenesis studies of glycidol (CAS No. 556-52-5) in F344/N rats and B6C3F1 mice (gavage studies).* Technical Report Series No. 374. National Institutes of Health Publication No. 90-2829. Research Triangle Park, NC. https://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr374.pdf	
Data type	Dichotomous (Quantal)	
BMR	0.1	
Number of bootstrap samples	25 000	

Models are as described in Annex 1.

TABLE A2.2 SUMMARY OF SINGLE MA BMD - INDIVIDUAL MODEL BMDs (TVM IN RATS)

Model	BMD	(BMDL,	BMDU)	Pr(M|Data)
Quantal-Linear	3.09	(2.47,	3.96)	0.537
Multistage	3.75	(2.80,	6.07)	0.153
og-Logistic	3.08	(0.31,	8.40)	0.093
Hill	3.70	(0.33,	10.15)	0.067
Weibull	1.99	(0.18,	6.19)	0.061
Gamma	3.18	(0.76,	7.11)	0.043
Log-Probit	5.45	(1.27,	10.92)	0.040
Logistic	7.75	(6.21,	9.74)	0.003
Probit	7.65	(6.27,	9.48)	0.003

Model average BMD: 2.01 (0.48, 3.04) 90.0% CI

FIGURE A2.1. BMD ESTIMATE BY EACH MODEL (SORTED BY POSTERIOR PROBABILITY) FOR TUNICA VAGINALIS/PERITONEUM – MESOTHELIOMA IN MALE RATS

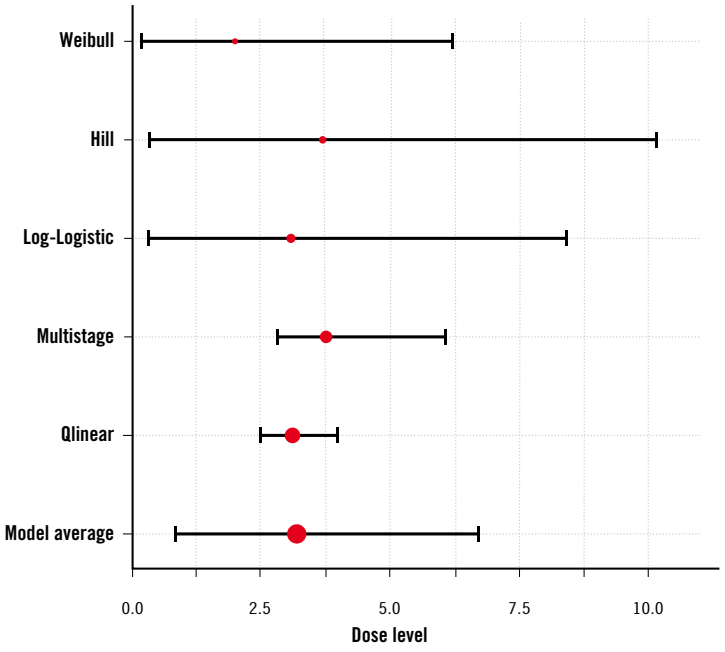
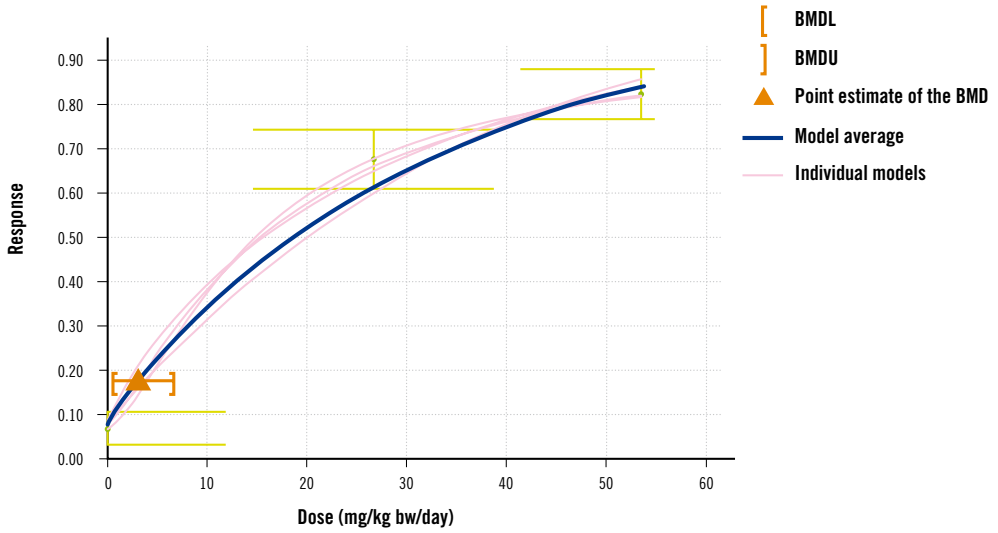


FIGURE A2.2. BMD WITH BAYESIAN MODEL AVERAGING FOR GE (TUNICA VAGINALIS/PERITONEUM – MESOTHELIOMA IN MALE RATS): DICHOTOMOUS MA, FIT TYPE: LAPLACE

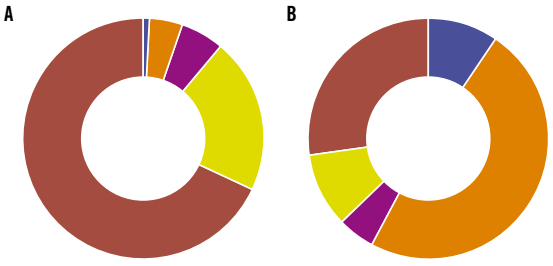


ANNEX 3
LIFETIME AVERAGE DAILY DOSE APPROACH FOR 3-MCPD

The LADD estimation assigned an intake of 3.2 µg/kg bw/day for the age period of 0–1 years (10 kg) and 1–5 years (18.5 kg), 2.6 µg/kg bw/day for 6–10 (30 kg) and 11–19 years (60 kg) and 1.3 µg/kg bw/day for 20–70 years (70 kg).

EFS: mean exposure to 3-MCPD was 0.5–1.5 µg/kg bw/day 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/day across dietary surveys in these age groups. Infant formula alone exposures were estimated at 2.4 (mean) and 3.2 µg/kg bw/day (95th percentile). In adolescents and adult population groups (adults, elderly, very elderly), the mean exposure to 3-MCPD ranged from 0.2–0.7 µg/kg bw/day and the high exposure (P95) ranged from 0.3–1.3 µg/kg bw/day. All intakes are mean middle bound.

FIGURE A3.1. **PERCENTAGE AGE CONTRIBUTION TO TOTAL INTAKE**



AGE CATEGORY (years)		BACKGROUND EXPOSURE (µg/kg bw/day)	% LIFETIME
	0–1	3.2	1.4
	1–5	3.2	7.1
	6–10	2.6	7.1
	11–19	2.6	12.8
	20–70	1.3	71.4

Note: A. Background exposure; B. Exposure using x̄ 3-MCPD 588 µg/kg for 0–5 years.

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ANNEX 4
ALTERNATE EXPOSURE SCENARIOS (3-MCPD)

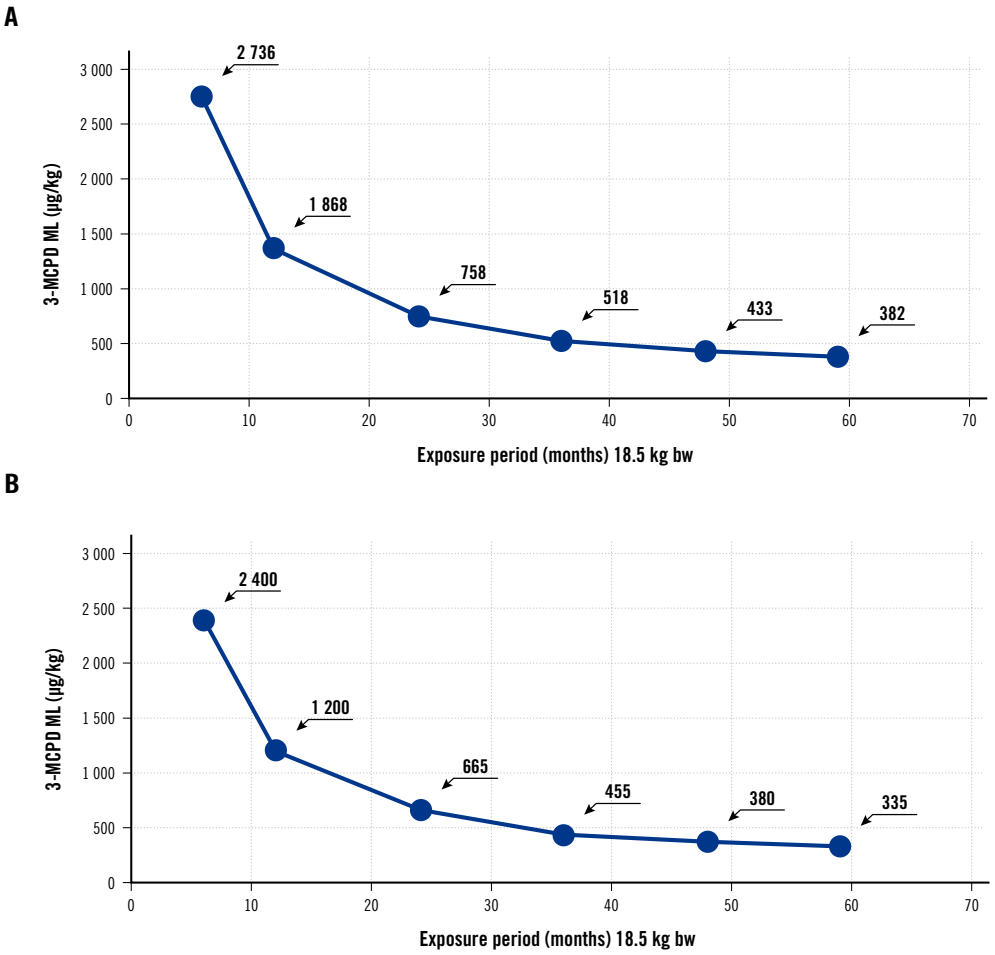
Current exposures using the LADD approach considers consumption of RUTF at 42 g/kg bw/day using the following body weights: 0–6 months: 5 kg; 6–12 months: 10 kg; 1–5 years: 18.5 kg. At this intake, the most conservative scenario (consuming RUTF for 59 months) would tolerate a 3-MCPD concentration of 335 µg/kg (ppb) in product as consumed. Decreasing the exposure period would increase the tolerated concentration of 3-MCPD accordingly. For example, if exposure to RUTF at 42 g/kg bw/day was only by infants for 12 months, the tolerated 3-MCPD concentration would increase to 1 200 µg/kg.

1. Decreasing RUTF intake from 42 g/kg bw/day to 36 g/kg bw/day and maintaining the same body weights (bw) would result in an increase in the current 3-MCPD tolerances by approximately 14 percent. Therefore, the worst-case 3-MCPD tolerance would increase to 382 µg/kg (continual consumption of RUTF at 36 g/kg bw/day for 59 months); for intake of RUTF for only one year by infants 1–5 years of age, the tolerance would increase to 1 368 µg/kg. For up to 36 months of RUTF intake at 36 g/kg bw/day, the maximum tolerated 3-MCPD concentration would increase to 518 µg/kg. Decreasing the duration of exposure to only six months by the same age category would increase the 3-MCPD tolerance to 2 736 µg/kg. (Figure A4.1).

   Decreasing the bw from 18.5 kg to either 5 kg or 12 kg for infants 6–59 months of age would also result in increasing the tolerances for 3-MCPD. For example, at 36 g/kg bw/day and an 18.5 kg bw, infants would be consuming a maximum of 666 g RUTF/day. At 12 kg bw, RUTF intake would decrease to 432 g/day or an approximate 35 percent decrease compared to the amount consumed by an 18.5 kg bw infant. Accordingly, exposure to 3-MCPD could then increase by approximately 35 percent using the LADD approach. Therefore, for infants 6–59 months of age, the worst-case scenario (consuming RUTF for 59 months) maximum 3-MCPD concentration would increase to 515 µg/kg; consumption of RUTF by infants 1–5 years of age for only one year would again increase the 3-MCPD tolerance to 1 846 µg/kg. If a 5 kg bw was applied to infants 6–59 months of age, RUTF intake

on a daily basis would decrease to 180 g/day or approximately 73 percent from the previous 18.5 kg bw. As such, the worst-case scenario 3-MCPD tolerance would increase to 660 µg/kg; for consumption of RUTF for only 12 months at 36 g/kg bw and a 5 kg bw; the 3-MCPD tolerance would increase to greater than 2 000 µg/kg (Figure A4.2). In comparison, the maximum concentration of 3-MCPD reported in LNS/RUTF was 1 420 µg/kg.

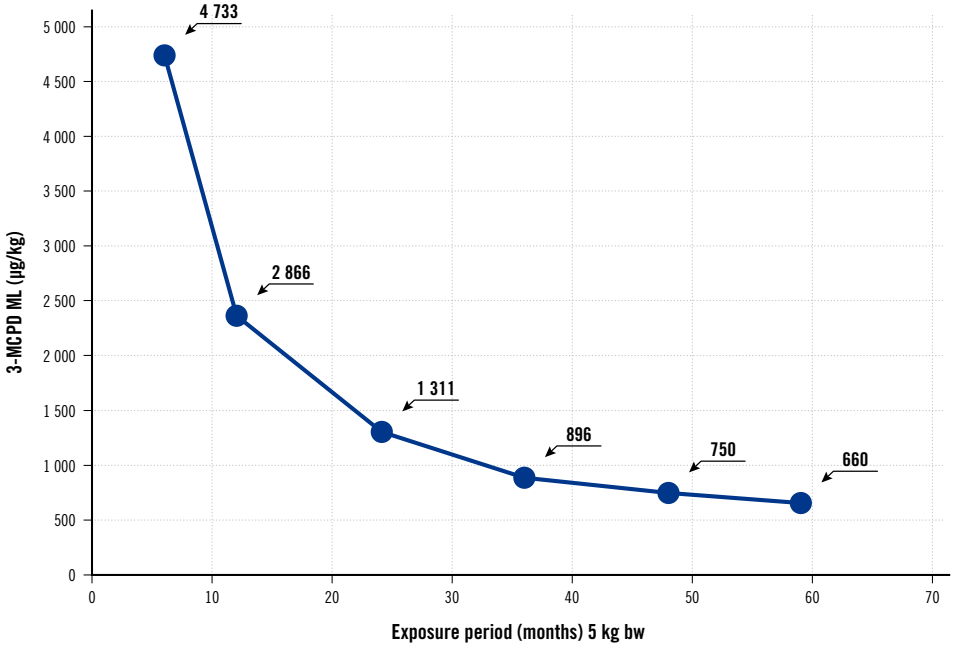
FIGURE A4.1 **HYPOTHETICAL 3-MCPD TOLERANCE VS DAILY EXPOSURE: 36 G/KG BW/DAY (A) AND 42 G/KG BW/DAY (B)**



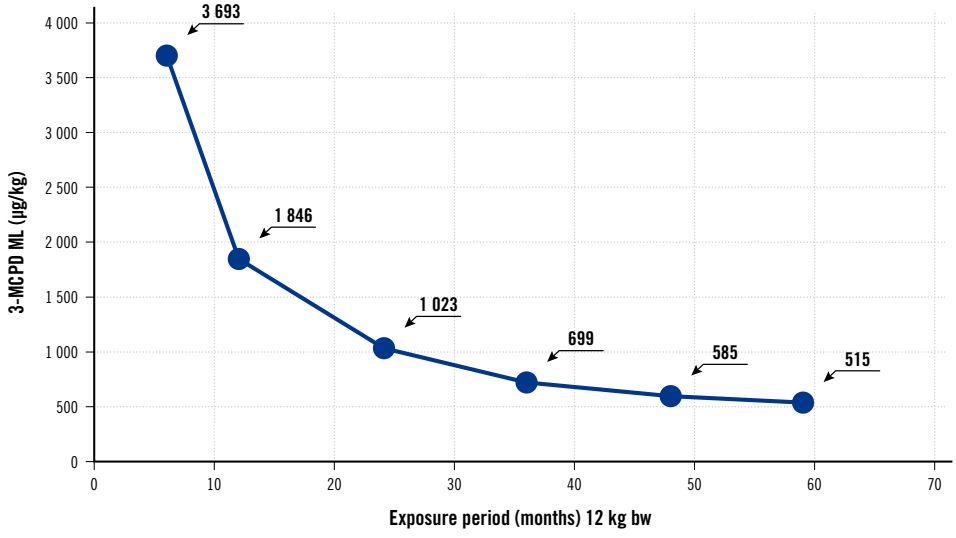
ML: maximum level.

FIGURE A4.2 HYPOTHETICAL 3-MCPD TOLERANCE VS BODY WEIGHT: 5 KG BW/DAY (A) AND 12 KG BW/DAY (B)

A



B



ML: maximum level.

2. RUSFs are designed for use in supplementary feeding programmes (addition to the daily diet) and are, therefore, typically consumed in smaller daily portions compared to RUTF. While the dose of RUTF is adjusted, depending on the body weight of the infant/child, the usual daily dose of RUSF is a single 100 g packet, regardless of body weight.

   Applying default body weights of 5 or 12 kg to infants/children 6–59 months of age, combined with a single daily 100 g serving, would result in lower daily intakes on a per kg bw compared to those described in the first scenario. For example, a 5 kg child would be consuming RUSF at 20 g/kg bw while a 12 kg child would be consuming RUSF at 8.3 g/kg bw. Daily intake, compared to a 5 kg child consuming RUTF at 36 g/kg bw, would decrease by approximately 45 percent. As such, any 3-MCPD ML could increase by the same fraction. For a 12 kg child, daily intake would decrease by 77 percent compared to the same bw child consuming RUTF at 36 g/kg bw/day. In both cases, a 3-MCPD tolerance for the worst case scenario (continual consumption of RUSF at 100 g/day for 59 months) would increase to 957 µg/kg (5 kg bw) or 912 µg/kg (12 kg bw). Decreasing the exposure periods to less than 59 months would result in greater hypothetical tolerances for 3-MCPD. For example, for 36 months of exposure, the 3-MCPD ML would increase from 699 µg/kg (12 kg bw, 36 g/kg bw/day) to 1 237 µg/kg while for the same duration of exposure for a 5 kg child, the 3-MCPD ML would increase to 1 300 µg/kg. Even shorter exposure periods would result in hypothetical 3-MCPD MLs greater than the maximum concentration seen to date for RUTF/LNS products.

3. RUSFs are also intended for use by pregnant/lactating women but for shorter exposure periods (6–18 months). When 0.5–1.5 packets of RUSF (75–150 g) are consumed daily for exposure periods of 6–18 months, intake on a per kg bw basis would range from 1.3–2.6 g/kg bw (57.1 kg pre-pregnancy weight for sub-Saharan African women [Gebremedhin and Bekele, 2021]). Intake of RUSF on a per kg bw would be expected to decline during pregnancy due to gestational weight gain. As such, any 3-MCPD tolerances developed for RUTF and applied to RUSF would be considered protective.

DISCUSSION

The alternate exposure scenarios for consumption of RUTF/RUSF provide different body weights, different daily intakes and different intake durations than previously described. Any variable(s) that result in lower daily intakes of RUTF/RUSF, either based on body weights or serving sizes, would result in lower daily exposures to 3-MCPD. While the hypothetical tolerances listed for 3-MCPD would result in a LADD that does not exceed a total dose associated with the more conservative HBGV of 2.4 µg/kg bw/day (Option 4), any increase in tolerance would result in an increase in exposure to 3-MCPD. While chronic exposure to 3-MCPD is associated with kidney-related toxicity, there is limited toxicological data that specifically relates to short-term exposure by pre-pubertal animals. Testicular effects

have been induced by 3-MCPD in experimental animals at lower doses for shorter time periods; for example, doses of 1–3 mg/kg bw/day for up to 30 days have been shown to decrease fertility. However, in a study where exposure to 3-MCPD happened during development (El Ramy *et al.*, 2006), testicular morphology, testosterone production and gene expression was described as being comparable to control animals in both fetal animals and neonates after dosing with up to 25 mg/kg bw/day 3-MCPD.

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ANNEX 5
EXAMPLE CALCULATION OF INCREMENTAL LIFETIME CANCER RISK

The following is an example of how the change in the ILCR attributable to GEs exposure via LNS/RUTF is calculated. In this example, a child aged 1 to 2 years consumes LNS/RUTF as the sole source nutrition for six months, assuming a daily intake of 42 g/kg bw/day[10] LNS/RUTF with a glycidol-equivalent concentration in product of 420 µg/kg (the mean concentration of the 97 products for which data were available). It is further assumed that background exposure corresponds to the JECFA mean lower bound estimate (JECFA, 2015, Table 23) and the revised JECFA $BMDL_{10}$ of 0.83 mg/kg bw/day is used as the reference point.

TABLE A5.1 **EXAMPLE PARAMETERS USED IN THE ILCR CALCULATION**

PARAMETER	VALUE
Background exposure (µg/kg bw/day)	0.1
Duration lifestage (y)	1
Duration LNS/RUTF (fraction of lifestage)	0.5
Duration background exposure (fraction of lifestage)	0.5
Quantity LNS/RUTF (g/kg bw/day)	42
Glycidol content LNS/RUTF (µg/kg)	420
Lifestage exposure (µg/kg bw/day)	8.87
Total lifestage exposure (µg/kg bw)	3238
$LADD_i$ (mg/kg bw/day)	1.27E-04
$BMDL_{10}$ (mg/kg bw/day)	0.83
CSF $[mg/kg/day]^{-1}$	0.12
Lifestage ILCR	1.53E-05
Lifetime ILCR	2.12E-05
Δ ILCR	1 in 66 249

[10] The value of 42 g/kg bw/day is based on a dose recommendation of 135 to 220 kcal/kg bw/day for the treatment of acute malnutrition. RUTF provides 520 to 550 kcal per 100 g, and therefore, assuming a dose of 220 kcal/kg bw/day and an energy content of 520 kcal/100 g, the indicated dose of RUTF is equivalent to 42 g/kg bw/day. However, in June 2023, the WHO guidelines were updated and the recommended dose was amended to 150 to 185 kcal/kg bw/day, which equates to a daily consumption of 36 g/kg bw/day and is used elsewhere in this report.

1. Lifestage exposure = exposure due to background + exposure due to LNS/RUTF

   ∴ Lifestage exposure = (0.1 µg/kg bw/day * 0.5 (fraction of lifestage exposed to background)) + (42 g/kg bw/day ÷ 1 000 (g/kg to kg/kg) * 420 µg glycidol/kg * 0.5 (fraction of lifestage exposed to LNS/RUTF)) = 8.87 µg/kg bw/day

2. Total lifestage exposure = Lifestage exposure * duration lifestage (y) * 365 d/y

   Total lifestage exposure = 8.87 µg/kg bw/day * 1 y * 365 d/y = 3 238 µg/kg bw

3. LADDi = dose received during life stage averaged over a lifetime (mg/kg bw/day)

   ∴ LADDi = 3 238 µg/kg bw (total lifestage exposure) ÷ (365 d * 70 y) ÷ 1 000 (µg/mg) = 1.27E-04 mg/kg bw/day

4. Cancer slope factor (CSF) is equivalent to ILCR per unit of dose and is derived from the benchmark dose model (see also Annex 2).

   CSF = 0.1 (Benchmark response) ÷ 0.83 mg/kg bw/day (revised JECFA BMDL10) = 0.12 [mg/kg/d]-1

5. Lifestage ILCR = CSF * LADDi

   ∴ Lifestage ILCR = 0.12 [mg/kg/d]-1 * 1.27E-04 mg/kg bw/day = 1.53E-05

6. Lifetime ILCR = $\sum_i$(LADDi*CSF)

TABLE A5.2 **ESTIMATING LIFETIME ILCR AFTER EXPOSURE TO GE VIA LNS/RUTF FOR SIX MONTHS AT AGE 1 TO <2 YEARS**

LIFESTAGE (age)	DURATION LIFESTAGE (y)	DURATION LNS/RUTF EXPOSURE (fraction of lifestage)	LIFESTAGE BACKGROUND EXPOSURE (µg/kg bw/day)	LIFESTAGE ILCR
Infants (6 to <12 months)	0.5	0	0.1	8.61E-08
Infants (1 to <2 years)	1	0.5	0.1	1.53E-05
Children (2 to <18 years)	16	0	0.2	2.81E-06
Adults (18 to 70 years)	52	0	0.1	3.036E-06
LIFETIME ILCR				**2.12E-05**

7. Δ ILCR is the change in ILCR attributable to GE exposure via LNS/RUTF consumption relative to the counterfactual condition in which LNS/RUTF is not consumed. In order to calculate Δ ILCR, the lifetime ILCR of an individual who has never been exposed to LNS/RUTF is subtracted from the lifetime ILCR calculated for the individual who did have exposure via LNS/RUTF at a particular lifestage (in this example, for six months between the ages of 12 and 24 months).

   Using the JECFA mean lower bound background exposure estimate, the lifetime ILCR attributed to GE exposure for an individual who has never consumed LNS/RUTF is 6.10×10^{-06} (calculated in the same manner as above with the parameter "Quantity LNS/RUTF" set to zero for all ages).

$\therefore \Delta$ ILCR = $(2.12 \times 10^{-05}) - (6.10 \times 10^{-06}) = 1.51 \times 10^{-05}$

The reciprocal of this value corresponds to a "1 in x" change in ILCR attributable to GE exposure from LNS/RUTF exposure alone:

Δ ILCR = $1 \div 1.51 \times 10^{-05}$ = 1 in 66 249 or ~ 1 in 65 000 increase in ILCR.

As the increase in cancer risk is greater than 1 in 10^5 (1 in 100 000), this particular exposure scenario would be of concern.

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FOOD SAFETY IN THE CONTEXT OF LIMITED FOOD AVAILABILITY
RISK ASSESSMENT OF 3-MCPD AND FATTY ACID ESTERS IN NUTRIENT SUPPLEMENTS AND THERAPEUTIC FOOD

Lipid-based nutrient supplements (LNS) and ready-to-use therapeutic food (RUTF) are fortified foods used to prevent and/or treat malnutrition in children. They are often produced locally in regions experiencing food insecurity and include edible oils obtained from oleaginous seeds or fruits that must be refined to remove undesirable substances and ensure adequate shelf-life for the product. However, the formation of the heat-induced contaminants 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters and glycidyl fatty acid esters (GEs) may occur during the refining process of edible oils, with the highest levels typically observed in refined palm oil which is used extensively in the manufacture of LNS/RUTF products. 3-MCPD and its fatty acid esters are present in many other foodstuffs and most of the total lifetime exposure is attributed to foods other than LNS/RUTF. As these substances or their metabolites have been shown to elicit toxicity effects in experimental animals, their presence in foods is of concern. While the only Codex standard developed for 3-MCPD is for liquid condiments containing acid hydrolyzed vegetable proteins, no Codex standards are available for GEs. This publication provides an overview of risk assessments for 3-MCPD and GEs previously performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA) and other authorities, based on chronic exposure.

In contrast to other dietary sources containing these compounds, the use of LNS/RUTF is intended to be of finite duration and confined to a specific life stage. Therefore, the aim of this report was to provide an assessment to characterize the risk of less-than-lifetime exposure to 3-MCPD (including 3-MCPD fatty acid esters) and GEs via LNS/RUTF in the context of limited food availability. The thresholds identified herein for concentrations of 3-MCPD and glycidol equivalents in LNS/RUTF products are considered to represent a level of exposure that is of low concern for human health.

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ISBN 978-92-5-138777-1 ISSN 2415-1173
9 789251 387771
CD0761EN/1/05.24