Risk Assessment Studies
Report No. 62

Chemical Hazard Evaluation

Glycidyl Esters in Edible Fats and Oils and Infant Formula

July 2020
Centre for Food Safety
Food and Environmental Hygiene Department
The Government of the Hong Kong Special Administrative Region

Correspondence:
Risk Assessment Section
Centre for Food Safety
Food and Environmental Hygiene Department
43/F, Queensway Government Offices,
66 Queensway, Hong Kong.
Email: enquiries@fehd.gov.hk

Contents

EXECUTIVE SUMMARY

This study aims to determine the levels of glycidyl esters (GE) in edible fats and oils, as well as infant formula available in the local market.

2. GE are process contaminants formed mainly during the deodorisation step in the refining of vegetable oils. Exposure to GE can occur through consumption of refined oils and various food products containing refined oils.

3. After ingestion, GE are broken down in the human body to release glycidol. Glycidol is a genotoxic carcinogen being classified as group 2A agent (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC) based on sufficient evidence in experimental animals. It may also cause toxic effects on the nervous, renal and reproductive systems in experimental animals.

Results

4. A total of 207 samples, including 169 samples of edible fats and oils (including palm oil, canola oil, corn oil, peanut oil, soybean oil, sunflower oil, other vegetable oils, butter, lard, shortening, margarine and spread) and 38 samples of infant formula, were tested for GE.

5. For edible fats and oils, the GE levels ranged from 16 to 4 500 µg/kg*, with a mean level of 569 µg/kg. As for infant formula, the GE level detected ranged from 3.1 µg/kg to 53 µg/kg, with a mean level of 10.6 µg/ kg.

* GE levels presented in the Executive Summary refers to upper bound (UB) values, unless otherwise specified.

Conclusions and Recommendations

6. The mean levels of GE for both the edible fats and oils samples and infant formula samples collected in this study were lower than similar study findings of the European Food Safety Authority (EFSA) as well as New Zealand Food Safety (NZFS) and Food Standards Australia New Zealand (FSANZ). In addition, the upper ends of the range of GE level of both the edible fats and oils samples and infant formula samples collected in this study were lower than the two abovementioned studies.

7. Reduction of GE in refined oils and food is possible when traders increase their awareness on the issue and adopt relevant mitigation measures. A decreasing trend of GE level in these foods is expected with the increasing awareness of GE by food safety authorities and industries worldwide by following the recommendations from Codex Alimentarius Commission (Codex) released in 2019. In fact, a signal of decrement on the GE level in food was observed when some locally available follow-up oil samples were re-tested in 2020 and it showed a reduction of more than 40% in most samples as compared with the GE level of the same products collected in late 2018-early 2019.

8. The food trade is recommended to take measures to minimize the levels of GE in their food products by making reference to the relevant Code of Practice adopted by the Codex in accordance to the principle of as low as reasonably achievable.

9. Members of public are advised to maintain a balanced and varied diet in order to avoid excessive exposure to any contaminant from a small range of food

items. In general, maintaining a healthy diet of lower fat content could reduce the overall intake of GE from our food.

10. As breastfeeding is beneficial to the health of both mothers and their babies, mothers are encouraged to feed their babies with breastmilk. However, if infants are not breastfed, they should continue to be fed with infant formula to ensure optimum nutrition.

Risk Assessment Studies –

Glycidyl Esters in Edible Fats and Oils and Infant Formula

OBJECTIVES

This study aims to determine the levels of glycidyl esters (GE) in edible fats and oils, as well as infant formula available in the local market.

BACKGROUND

2. GE are process contaminants which are found in refined fats and oils and in turn also found in foods that contain refined fats and oils. This group of compounds has caught attention from the international scientific community recently when conducting researches on other contaminants in edible oil[1].

Occurrence of Glycidyl Esters

3. The only identified source of GE in food so far is refined vegetable oils. GE are formed during the deodorization step of oil refining, where the diacylglycerol (DAG) reacts with glycidol that are from the decomposition of 3- and 2-MCPD at high temperature, starting from temperature above 200ºC and becoming more significant beyond 230ºC. This is of particular concern in palm oils as they can have relatively high (4 – 12%) DAG content when compared with other types of oil[1,2]. Although GE can also be formed by the dehydration of monoacylglycerol, the levels of monoacylglycerol in vegetable oils are naturally low and reduced

further during deodorization, making them not significant contributors to GE contamination[2].

Sources of GE in Foods

4. In 2016, the European Food Safety Authority (ESFA) summarized the data collected on occurrence of GE in foodstuffs which revealed that the highest level of GE is found in the group "animal and vegetable fats and oil". The mean middle bound (MB) levels for "animal and vegetable fats and oils" was 1176 µg/kg. Among "vegetable fats and oils", "palm oils and fats" were found to contain the highest MB levels at 3955 µg/kg[2].

Toxicity

Kinetics and metabolism

5. Glycidol and GE are efficiently absorbed following ingestion. The glycidol moiety metabolized rapidly by several enzymatic pathways, including gluthathione conjugation and mercapturate formation. The glycidol moiety is predominately excreted in urine[2]. GE are substantially hydrolysed and elicits toxicity as glycidol in the gastrointestinal tract[2].

Toxicity

6. Glycidol was reported to have neurotoxicity, renal toxicity, carcinogenicity, anti-fertility effects, and genotoxicity in animal studies[1,3]. Neurotoxicity was observed after 28 days of treating rats with 200 mg/kg bw/day glycidol. Glycidol also caused renal toxicity in repeated dose studies in rats and mice at doses in the range of 150 – 400 mg/kg bw/day. Two-year carcinogenicity studies in mice (25 and 50 mg/kg bw/day) and rats (37.5 and 75 mg/kg bw/day) showed induction of tumors in multiple organs from both sexes. Male anti-fertility effects were noted in rats and mice, where the lowest observed adverse effect level (LOAEL) was 25 mg/kg bw/day in rats, causing a 36% reduction in epididymal sperm count[1].

7. As for carcinogenicity and genotoxicity, there were evidence from *in vitro* and *in vivo* data that glycidol is genotoxic. No *in vivo* data was available for GE genotoxicity[1,2]. The International Agency for Research on Cancer (IACR) considered that there is sufficient evidence in experimental animals for carcinogenicity of glycidol and classified glycidol as probably carcinogenic to human, (Group 2A)[4].

Regulatory Control

International situation

8. There is no international standards on GE or glycidol in food at present. The Codex Alimentarius Commission (Codex) has not established any standards for GE or glycidol in food. Apart from the European Union (EU) that has established

maximum level for GE in certain foods, no other food safety regulatory authorities have established maximum levels for GE in food[5].

9. As a risk management approach to address the issue, Codex in 2019 has developed 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" (Code of Practice) to provide authorities, producers and manufacturers with guidance to reduce formation of GE in refined oils and food products made with refined oils[6].

SCOPE OF STUDY

10. This study focused on (i) edible fats and oils and (ii) infant formula[†] available in the local market, as these foods are considered to be important sources of GE in the diet for the adult and infant populations, respectively.

METHODOLOGY AND LABORATORY ANALYSIS

Methodology

11. A total of 207 food samples were collected from local retailers and food premises between December 2018 and February 2019 in Hong Kong. The samples included 169 samples of edible fats and oils (including palm oil, canola oil, corn oil, peanut oil, soybean oil, sunflower oil, other vegetable oils, butter, lard, shortening,

[†] infant formula refers to products that are intended to be consumed by infants (i.e. persons aged 12 months old or below) as their sole source of nutrition as a breast milk substitute

margarine and spread) and 38 samples of infant formula. The types of food samples collected are listed in Table 1.

Table 1. Types of food sampled

Type	No. of samples
Edible fats and oils	**169**
Animal fats, margarine and spreads	63
Vegetable oils and fats *	106
Infant formula	**38**
Total	**207**

***** Including palm oil, canola oil, corn oil, peanut oil, soybean oil, sunflower oil, mixed vegetable oils, and other vegetable oils

Laboratory Analysis

12. Laboratory analysis of GE was conducted by the Food Research Laboratory (FRL) of the Centre for Food Safety (CFS). In this project, 207 samples of edible fats and oils and infant formula have been tested for 14 individual GEs (expressed in glycidol) with fatty acid carbon chain from six to twenty.

13. The GE levels in samples were analysed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Stable isotope labelled GE were spiked quantitatively into a measured amount of sample. For infant formula and solid fat samples, fat was extracted by ultra-sonication with ethyl acetate. The edible oil, fat extracted from infant formula or from solid fat samples was dissolved in n-hexane and then cleaned up by passing through an aminopropyl

solid phase extraction cartridge. The GE in eluent were extracted to acetonitrile and then passed through a LipiFitr® cartridge before instrument analysis. The limits of detection (LODs) of the individual GEs were 1 and 0.2 ppb for oils and fats, and infant formula samples, respectively.

Treatment of Analytical Values Below the LOD

14. In this study, data were treated with the lower bound (LB) and upper bound (UB) approach. That is, at the LB, results below the LOD were replaced by zero whilst at the UB, results below the LOD were replaced by the value reported as the LOD. This approach compares the two extreme scenarios, based on the consideration that the true value for results less than LOD may actually be any value between zero and the achieved LOD. The LB scenario assumes that the chemical is absent; therefore, to results reported as <LOD a value of zero is assigned. The UB scenario assumes that the chemical is present at the level of the LOD; thus, to results reported as <LOD a value of the corresponding LOD is assigned.

RESULTS AND DISCUSSION

GE in Edible Fats and Oils

15. All the 169 edible fats and oils samples analysed had GE detected at quantified levels. The results of GE level detected in different types of samples were listed in Table 2.

Table 2. Results of GE in different types of samples collected in the study

Types	No. of samples collected	GE level (µg/ kg) (expressed as glycidol)		
		Mean	Minimum	Maximum
		LB – UB	LB – UB	LB – UB
Animal fats, margarine and spreads	63	116 – 123	2.5 – 16	980 – 980
Vegetable oils and fats *	106	824 – 833	3.4 – 16	4500 – 4500
All edible fats and oils	**169**	**560 - 569**	**2.5 - 16**	**4500- 4500**

* Including canola oil, corn oil, peanut oil, soybean oil, sunflower oil, mixed vegetable oils, and other vegetable oils

16. The study revealed that the mean GE level in all edible fats and oils sampled collected was 569 µg/ kg (UB). Variations in the GE level were also observed among different samples of the same product type. Palm oil was found to contain the highest mean GE level at 4050 µg/kg, which was consistent with the observations and findings in overseas studies.

GE in Infant Formula

17. All 38 infant formula samples analysed had GE detected at quantified levels. The mean GE level in infant formula was 8.8 -10.6 µg/ kg (LB-UB), with the detected level ranging from 3.1 to 53 µg/ kg (UB).

International Comparison

18. According to overseas studies, GE are commonly found in refined edible vegetable fats and oil, and are the most abundant in palm oil. Our study results echo these studies. When compared with the study findings of (i) the European Food Safety Authority (EFSA)[2] and (ii) New Zealand Food Safety (NZFS) and Food

Standards Australia New Zealand (FSANZ) [7], the mean levels of GE for edible fats and oils samples and infant formula samples collected in this study were lower (Table 4). In addition, the upper end of the range of GE level of both the edible fats and oils samples and infant formula samples collected in this study were also lower (Table 5). However, it should be noted that when comparing data with overseas studies, cautions like the differences in time when the studies were carried out, research methodology, sampling strategies, test methods, etc. have to be taken into consideration.

Table 4. Comparison of mean GE level in edible fats and oils and infant formula in EFSA, NZFS/FSANZ and CFS studies

	Mean GE level (µg/ kg) (expressed as glycidol)		
	EFSA (LB-UB)	**NZFS/FSANZ**	**CFS (LB-UB)**
Edible fats and oils	1167 - 1184	758 [a]	560 - 569
Infant formula	80 - 94	26	8.8 - 10.6

[a] Calculation based on individual results presented in the report. Results below the LOD were replaced by the value reported as the LOD.

Table 5. Comparison of range of GE level in edible fats and oils and infant formula in EFSA,NZFS/FSANZ and CFS studies

	Range of GE level (µg/ kg) (expressed as glycidol)		
	EFSA	**NZFS/FSANZ**	**CFS (UB)**
Edible fats and oils	<172 [a] - >6070 [b]	<126 - 7110	16 - 4500
Infant formula	<56 [a] - >220 [b]	<1.7 - 484	3.1 - 53

[a] Maximum value of the range of limit of quantification (LOQ)
[b] 95% percentile (middle bound)

19. Since GE is genotoxic and carcinogenic, the food trade should adopt measures to reduce the GE level in the products as low as reasonably achievable. The Code of Practice adopted by Codex in 2019 has included a number of recommendations including those on manufacturing of refined edible oils, and selection of uses of refined oils in food products made from these oils. These recommendations serve as useful reference to the food trade[6].

20. With the increasing awareness of GE by the food safety authorities and industries worldwide, it is expected that the level of GE in food would be in a decreasing trend by following the Code of Practice from Codex. In fact, a signal of decrement on the GE level in food was observed when some locally available follow-up oil samples were re-tested in 2020 and it showed a reduction of more than 40% in most samples as compared with the GE level of the same products collected in late 2018-early 2019.

Fats and Oils in Diet

21. Fats and oils contribute to an important part of our diet. They are a source of essential fatty acids and energy in terms of human nutrition. They also carry fat-soluble vitamins, i.e. vitamin A, D, E and K and are therefore essential in our diet.

22. Fats and oils are concentrated sources of energy, providing 9 kcal per gram of fat. Excessive fat intake, particularly saturated fats and trans-fats, can be harmful to health. It has been linked to major health problems, including an increased risk of heart disease, obesity, hypertension, diabetes and certain types of

cancers. Therefore, maintaining a balanced diet by consuming a variety of foods and avoiding a diet that is high in sugars, fats and salt are important for people to stay healthy. The WHO recommends 15 - 30% of total energy from total fats obtained from different sources such as meats, dairy products, seeds, nuts, cooking oils and fats. For preventing non-communicable diseases mentioned above, apart from avoiding excessive fat intake, WHO suggested limiting the intake of saturated fats[8,9,10] . Palm oil, which was known to contain high GE level, is also well-known for its high saturated fats content. Therefore, taking into consideration from both nutrition and contaminant perspective, limiting consumption of palm oil helps maintaining a healthy diet. On the other hand, WHO suggested replacing both saturated fats and trans-fats with unsaturated fats, in particular polyunsaturated fats[8,7,10] . In fact, a variety of oils rich in polyunsaturated fats (such as soybean oil, canola oil, corn oil and sunflower oil) were found to have lower GE content as compared with palm oil and are readily available in the local market.

23. GE is one of the contaminants found in refined vegetable oils and in foods that are made with it. To achieve a low level of contaminants in food that is reasonably achievable for protecting public health, there is a number of risk management options that international and national food safety authorities can employ, including issuing guidelines and code of practices, setting maximum limit (ML), providing consumer advisory, etc. GE are process contaminants formed mainly during the deodorisation step in the refining of vegetable oils which can be reduced in accordance to Good Manufacturing Practices (GMPs) in principle. In addressing the issue, Codex has launched a Code of Practice which serves as a useful measure to reduce occurrence levels of GE and therefore exposure. For setting MLs, still there are knowledge gaps for comprehensive risk assessment. These knowledge gaps, as identified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), include relevant biomarkers, method of analysis and occurrence

data[3], etc.. ESFA also suggested more extensive research on chronic carcinogenic effect of glycidol and GE to reduce uncertainty in risk assessment[2]. As a principle, before the decision of setting MLs, scientific knowledge gaps and other issues such as the technological possibilities to control a contamination problem have to be considered [11].

24. The composition of infant formula is tailored, in line with the latest scientific knowledge on nutrient requirements, to infants' specific nutritional needs. Refined vegetable fats have long been used to provide the fat content for these products[12]. The German Federal Institute for Risk Assessment (BfR) opined that despite the fact that higher exposure of formula-fed infants to GE has probably existed for long years, there are no indications that children fed with infant formula might have suffered any adverse health effects due to the intake of GE. Hence, BfR considered that the probability that the current exposure levels of formula-fed infants will result in health impairments is low[12]. While the current data on GE does not warrant any change on the consumption of infant formula, further research on the subject is required to provide comprehensive information for risk assessment.

25. Nevertheless, as revealed in this study that GE content in edible fats and oils and infant formula could vary in large extent even among the same type of product, there are rooms for food manufacturers to reduce the GE level in many products in accordance with the principle of as low as reasonably achievable. The 2016 EFSA report shows that average GE contents in palm fat/oil, as high as 9000 µg/kg in 2010, were halved by manufacturers from 2010 to 2015 [2].

Uncertainties and Limitations

26. Although more accuracy and precision in exposure estimation could be achieved with more samples analysed, compromises had to be made in relation to the use of finite laboratory resources. As GE are process contaminants which can be affected by various factors in the production, the results of this study could represent only a snapshot of the GE levels in foods.

27. As an usual practice, caution should be taken when comparing the results from different studies in view that, apart from the test methods adopted, other factors such as research methodology, sampling strategies, approaches of capturing and handling consumption data, etc. would affect the outcome of the studies.

CONCLUSIONS AND RECOMMENDATIONS

28. The aim of this study is to determine the levels of glycidyl esters (GE) in edible fats and oils, as well as infant formula available in the local market. This provides baseline information for assessing improvement measures to be adopted by the trade. The mean levels of GE for all edible fats and oils samples and infant formula samples collected in this study were lower when compared with the study findings of (i) the European Food Safety Authority (EFSA) and (ii) New Zealand Food Safety (NZFS) and Food Standards Australia New Zealand (FSANZ). In addition, the upper end of the range of GE level of both the edible fats and oils samples and infant formula samples collected in this study were lower.

29. Reduction of GE in food is possible when traders increase their awareness on the issue and adopt relevant mitigation measures. A decreasing trend of GE level in these foods is expected with the increasing awareness of GE by food safety authorities and industries worldwide by following the recommendations in the Codex Code of Practice released in 2019. In fact, a signal of decrement on the GE level in food was observed by testing some locally available follow-up oil samples in 2020 which showed a reduction of more than 40% in most samples as compared with the GE level of the same products collected in late 2018-early 2019.

30. The food trade is recommended to take measures to minimize the level of GE in their food products by making reference to the relevant Code of Practice adopted by Codex in 2019 in accordance to the principle of as low as reasonably achievable.

31. Members of public are advised to maintain a balanced and varied diet in order to avoid excessive exposure to any contaminant from a small range of food items. In general, maintaining a healthy diet of lower fat content could reduce the overall intake of GE from our food.

32. As breastfeeding is beneficial to the health of both mothers and their babies, mothers are encouraged to feed their babies with breastmilk. However, if infants are not breastfed, they should continue to be fed with infant formula to ensure optimum nutrition.

REFERENCES

1. JECFA. Summary report of the eighty-third meeting of JECFA. 2016. Available from: URL: http://www.fao.org/3/a-bq821e.pdf

2. EFSA Panel on Contaminants in the Food Chain (CONTAM). Risk for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. EFSA Journal 2016; 14(5):4426. Available from: URL: https://www.efsa.europa.eu/de/efsajournal/pub/4426

3. JECFA. Glycidyl esters. In: Evaluation of certain contaminants in food. Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 1002. WHO and FAO. 2017. Available from: URL: http://apps.who.int/iris/bitstream/10665/254893/1/9789241210027-eng.pdf?ua=1

4. IARC. IARC Monograph Volume 77 (2000). Glycidol. Available from: URL: http://monographs.iarc.fr/ENG/Monographs/vol77/mono77-19.pdf

5. European Union. Commission Regulation (EU) 2018/290 of 26th February 2018 amending Regulation (EC) No. 1881/2006 as regards maximum levels 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. Available from: URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0290&from=EN

6. Codex Alimentarius Commission. 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. 2019. Available from: URL: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B79-2019%252FCXC_079e.pdf

7. New Zealand Food Safety. Snapshot Survey for 2-MCPD, 3-MCPD, glycidol and their esters in selected vegetable oils and infant formulas in Australia and New Zealand. New Zealand Food Safety Technical Paper No.: 2020/05. Available from: URL: https://www.mpi.govt.nz/dmsdocument/39890-snapshot-

survey-for-2-mcpd-3-mcpd-glycidol-and-their-esters-in-selected-vegetable-oils-and-infant-formulas-in-australia-and-new-zealand-technical-paper

8. Food and Agriculture Organization of the United Nations. Fats and fatty acids in human nutrition: Report of an expert consultation. Rome: FAO; 2010.

9. World Health Organization. Diet, nutrition and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva: WHO; 2003.

10. World Health Organization. Fact Sheets- Healthy Diet. October 2018. Available from: URL: https://www.who.int/news-room/fact-sheets/detail/healthy-diet

11. Codex General Standard for Contaminants and Toxins in Food and Feed. CXS 193-1995. Available from: URL: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf

12. The German Federal Institute for Risk Assessment. Frequently asked questions regarding the contamination of foods with 3-MCPD, 2-MCPD and glycidyl fatty acid esters.2016. Available from: URL: https://www.bfr.bund.de/cm/349/frequently-asked-questions-regarding-the-contamination-of-foods-with-3-mcpd-2-mcpd-and-glycidyl-fatty-acid-esters.pdf