Risiken erkennen – Gesundheit schützen

Infant formula and follow-up formula may contain harmful 3-MCPD fatty acid esters

BfR Opinion No. 047/2007, 11 December 2007

Free 3-monochloropropane-1,2-diol (3-MCPD) has been identified as a contaminant for a long time in various foods like liquid seasoning or bakery goods heated to high temperatures. This substance is formed when fat-containing and salt-containing foods are processed at high temperatures during production. In animal experiments 3-MCPD has led to an increase in the cell count (hyperplasia) in renal tubules and, at higher levels, it triggered benign tumours. No genotoxic effect was observed. It can, therefore, be assumed that the tumours observed in the long-term animal study (mainly benign) only occur above a threshold value. There are no findings from human studies. The clinical picture of hyperplasia of the renal tubules in humans has not been described. The latest studies by the official food control authorities identified high levels of 3-MCPD fatty acid esters for the first time in refined edible fats like margarine and oil and in fat-containing foods including infant formula and follow-up formula. BfR has evaluated the data submitted by the food control authorities. It has come to the conclusion that - based on the scientific knowledge currently available - babies in particular may ingest amounts of 3-MCPD esters from infant formula and follow-up formula for which, in the worst case scenario, the margin of safety to the effects observed in animal experiments is deemed to be too small. BfR, therefore, believes there is a need for action to reduce the levels but does not see any acute health risk.

3-MCPD esters are compounds of 3-MCPD and various fatty acids which are formed at high temperatures under hydration following a reaction between fats and chloride ions. These studies by the food control authorities in Baden-Württemberg showed that all refined vegetable oils and fats contain considerable amounts of 3-MCPD fatty acid esters. Only oil that had not undergone any heat treatment (e.g. native olive oil) did not contain the substance. The substance is formed at high temperatures, probably during the deodorisation of edible fats and oils, the last stage in refinement, during which undesirable odorous and taste-bearing substances are removed. Infant formula and follow-up formula consisting of dried powder contain vegetable and, in some cases, animal oils. They supply infants with essential fatty acids. Since the admixed oils are tasteless, they have almost always been refined.

No toxicological data are available on 3-MCPD esters. Hence in its health assessment BfR draws on the risk assessment results for 3-MCPD. In this context the Institute assumes that toxicologically relevant 3-MCPD is released from 3-MCPD fatty acid esters during the digestion process. For this substance a tolerable daily intake (TDI) has been established of 2 micrograms per kilogram body weight. Although the TDI value is not normally applied to infants in the first months of life, BfR has used this as an alternative in its risk assessment. The assessment shows that infants with normal consumption of infant formula and follow-up formula containing the proven levels exceed the TDI 20-fold to 30-fold if 100% of the 3-MCPD is released from the esters.

BfR took the margin of exposure (MOE) as a further comparative value for risk assessment. The MOE indicates the margin (calculated as a quotient) between the dose at which first changes in the kidneys became visible in animal experiments and the dose which infants ingest from food. In the worst case scenarios (high concentrations of 3-MCPD), the MOE of 44 for infant formula and 28 for follow-up formula is small.

BfR believes there is a need for action to reduce the levels of 3-MCPD esters in edible fats and fat-containing foods as there is no alternative to infant formula and follow-up formula for babies who are not breastfed, aside from breast milk from other mothers. BfR advises mothers who are unable to breastfeed their babies to continue feeding their infants the commercially available products. Cow milk or the milk of other domestic animals is not a viable alternative as it does not contain some essential nutrients which infants need for their development.

BfR bases this recommendation on the fact that the risk assessment of 3-MCPD fatty acid esters left a number of questions unanswered which must be taken into account in a risk-benefit assessment. It is still not clear what impact 3-MCPD has on humans. Hence no definitive statements can be made about the sensitivity of humans compared with that of experimental animals. The clinical picture of hyperplasia in the renal tubules described in animal experiments has not been described in humans. As it is not just individual products or products of individual manufacturers that are affected by the problems surrounding 3-MCPD fatty acid esters, alternative techniques must, in principle, be developed for the production of refined fats and oils.

1 Subject of the assessment

The Ministry for Nutrition and Rural Areas Baden-Württemberg has provided data-based information on the occurrence and distribution of 3-monochloropropane-1,2-diol fatty acid esters (3-MCPD esters) in edible fats and fat-containing food including infant formula. Based on the results of these studies BfR conducted a risk assessment at short notice. In this opinion it restricts itself to the most important aspects.

2 Results

Based on the latest data on the incidence and distribution of 3-monochloropropane-1,2-diol fatty acid esters in edible fats and fat-containing foods including infant formula and follow-up formula, it can be assumed that the TDI value of 2 µg/kg body weight for 3-MCPD is exceeded in adults with a high consumption of refined vegetable fats. This applies in particular to infants who ingest formula and follow-up formula (to the extent that the TDI concept is also applied to infants for whom it normally does not apply in the first months of life).

3 Reasons

3.1 Risk assessment

In its opinion dated 19 May 2003 BfR assessed the occurrence of 3-MPCD in bread (BfR 2003a). In its opinion dated 9 July 2003 it focussed more particularly on the threat to children (BfR 2003b). The assessments of the Joint FAO/WHO Expert Committee on Food Additives (WHO 2002, 2007) and the then Scientific Committee on Food (SCF, 2001) of the European Commission are based, amongst other things, on these reports and also on this opinion.

3.1.1 Possible source of danger

3-MCPD esters are compounds of 3-MCPD and various fatty acids. It can be assumed that chloropropanols may be released from 3-MCPD fatty acid esters during digestion (Robert *et al.* 2004, Zelinkova *et al.* 2006, Stadler *et al.* 2007). The findings so far are based on model experiments with lipases. However, there are no robust findings on the hydrolysability of 3-MCPD esters in the human digestive tract. So far it was only possible to speculate about the

scale of the possible release of 3-MCPD fatty acid esters during digestion. BfR agrees with the Chemical and Veterinary Examination Office (CVUA) Stuttgart that it should be assumed, unless corresponding studies prove the contrary, that free and, by extension, toxicologically relevant 3-MCPD is almost fully released from 3-MCPD esters through lipases in the human digestive tract.

3.1.2 Hazard potential

In its opinion dated 9 July 2003 BfR characterised the hazard potential of 3-MCPD in the following way:

"In short-term and long-term studies on the oral toxicity of 3-MCPD in rats and mice, the kidneys proved to be the target organs. In the long-term study in Fischer-344 rats which is of importance for further assessment, elevated relative kidney weights were observed at all tested doses. Furthermore, an elevated incidence of tubular hyperplasias of the kidneys was found at all doses and in both genders which was not, however, statistically significant at the lowest dose (1.1 mg/kg body weight/day).

The results of most of the *in vitro* bacterial mutagenicity tests were positive although negative findings were observed in conjunction with the admixture of S9 mix (exogenous metabolic activation system from mammalian tissue). *In vitro* mutagenicity studies involving mammalian cells were also positive; however the concentrations used were comparatively high (0.1 - 9 mg/ml), which means that their relevance is questionable.

In vivo genotoxicity studies (a micronucleus test in mouse bone marrow and a UDS test in rats) were negative. Hence, it can be assumed that 3-MCPD is not genotoxic *in vivo*.

A total of four long-term studies on the toxicity and carcinogenicity of 3-MCPD are available. Three of them (two mice studies and one rat study) do not point to any carcinogenicity; however they do not comply with modern quality requirements. In the fourth study with Fischer-344 rats, 3-MCPD increased the incidence of benign tumours in some organs. The tumours only occurred at doses which were higher than doses which had already led to hyperplasias of the renal tubules.

Based on the data of relevance for the assessment, the above-mentioned international bodies came to the conclusion that renal hyperplasia should be taken as the most sensitive endpoint of the more recent long-term study for the establishment of a tolerable daily intake. The fact that renal hyperplasias even occurred at the lowest dose of 1.1 mg/kg body weight/day, which were not however statistically significant, was taken into account by applying a higher margin of safety of 500 and a ‘provisional maximum tolerable daily intake’ (PMTDI) was established of 2 micrograms 3-MCPD/kg body weight.”

According to the Joint FAO/WHO Expert Committee on Food Additives JECFA (WHO 2002) hyperplasias occurred in a dose-dependent manner in the kidneys of male rats (3/50, 6/50, 15/50 and 34/50 in the control group and at 1.1, 5.2 and 28 mg/kg body weight). In the case of the female animals 2/50, 4/50, 20/50 and 31/50 animals were affected (control group, 1.4, 7.0 and 35 mg/kg body weight) (Sunahara *et al.* 1993, according to WHO 2002). The unpublished study report is not available.

The genotoxic potential of 3-MCPD was recently examined in a study by El Ramy *et al.* (2007) *in vivo* using the Comet Assay. It examined, amongst other things, the kidneys and testicles as the target organs in male rats. The negative findings confirm the opinion that a threshold mechanism can be used in the case of the tumours observed in rats in the long-term study.

No toxicological data are available on 3-MCPD esters.

3.1.3. Exposure

The maximum level of 3-MCPD described by CVUA Stuttgart in the fat content in the dried powder of infant formula and follow-up formula (10 samples) is 4169 µg/kg, calculated as

free 3-MCPD. The mean is 2568 and the minimum value is 1210 µg/kg. This maximum value was found in infant formula. The examination of 20 additional samples confirmed this finding. However, a maximum value of 8467 µg/kg was determined in follow-up formula with a fat content of 18.5% in the dried powder.

In the case of infant formula 15 g dried powder were topped up with water to 100 ml. The dried powder has approximately 25% fat content; this corresponds to around 3.75 g fat per 100 ml ready-to-drink milk. The maximum content of 4.169 µg 3-MCPD/g fat results in 0.156 µg 3-MCPD/ml milk. During the first months of life infants are given approximately 150 to 160 ml milk per kg body weight. Assuming an amount of 160 ml this leads to 3-MCPD intake of 25 µg/kg body weight (Table 1). The median 3-MCPD level in infant formula and follow-up formula (based on 10 samples) is 2568 µg/kg fat portion in dried powder. This leads to an intake of 15 µg/kg body weight. The minimum 3-MCPD content level in infant formula and follow-up formula is 1210 µg/kg fat content in dried powder. This leads to 3-MCPD intake of 7 µg/kg body weight.

Table 1: Intake of 3-MCPD from infant milk (based on 10 samples examined)

	3-MCPD in the fat content of dried powder µg/g	Dried powder g/100 ml	Fat (25% fat content in the dried powder) g/ml milk	3-MCPD in ready-to-drink milk µg/ml	Amount consumed ml/kg body weight	3-MCPD intake µg/kg body weight	TDI x-fold	MoE
Maximum	4.169	15	0.0375	0.156	160	25.0	12.5	44
Median	2.568	15	0.0375	0.096	160	15.4	7.7	71
Minimum	1.210	15	0.0375	0.045	160	7.3	3.6	152

In the case of vegetable fats exposure was estimated using the consumption amounts in the nutrition survey of the Robert Koch Institute (2002).

Table 2: Consumption of vegetable fats (according to the RKI nutrition survey 2002)

		Amount of product consumed g	Amount of fat consumed (80% fat content) g
Men	75th percentile	25	20
	maximum	100	80
Women	75th percentile	10	8
	maximum	40	32

Men have a higher consumption than women. That's why exposure for men is estimated (Table 3).

Table 3: Intake of 3-MCPD from vegetable fats by men

Consumption (fat content)	3-MCPD level in the fat content µg/g	Intake µg	Intake (at 60 kg body weight) µg/kg body weight	TDI x-fold
20	3.101 (median)	62.0	1.0	0.5
20	7.356 (maximum)	147.2	2.5	1.25
80	3.101 (median)	248.1	4.1	2
80	7.356 (maximum)	588.48	9.8	5

3.1.4 Risk characterisation

Assuming that esters are fully hydrolysed, infants who are given formula with the maximum level of 4196 µg 3-MCPD/kg fat content, the highest level established so far, may have a 3-MCPD intake of 25 µg/kg body weight.

ADIs and TDIs do not apply to infants in the first months of life (WHO 1978). However, if one applies the TDI concept to infants then the 3-MCPD intake of 25 µg/kg body weight corresponds to a 12.5-fold exceeding of the TDI (Table 1). The median of 2568 µg/kg fat content can lead to a 7.7-fold and the minimum value of 1210 µg/kg fat proportion to a 3.6-fold exceeding of the TDI. The margin (quotient) between the lowest dose, which led to tubular hyperplasias of the kidney in rats in the long-term study (the lowest dose examined) and the amounts ingested by babies from infant formula is in these cases 44, 71 and 152. This quotient is described as the margin of exposure (MoE).

During the examination of 20 additional samples of follow-up formula a maximum level of 8467 µg/kg fat content was found. This can lead to a 20-fold exceeding of the TDI which corresponds to a margin of exposure of 28.

In the case of men the worst case scenario of daily consumption of 100 g vegetable fat (maximum serving) with the maximum level identified for margarine of 7356 µg/kg fat content can lead to a 5-fold exceeding of the TDI; this corresponds to a margin of exposure of 110. The 75th percentile of consumption of vegetable fats is approximately 25 g for men. In conjunction with the maximum level of 3-MCPD this can lead to a 2.5-fold exceeding of the TDI. The median 3-MCPD level of 3101 µg/kg fat content only just reaches the TDI in conjunction with consumption of 25 g vegetable fat per day. As 3-MCPD can be ingested from other sources, it can be assumed that even this exposure scenario leads to an exceeding of the TDI. However all these estimates are based on the assumption that the esters are fully hydrolysed during digestion, but this has not been clarified up to now.

At the present time no clear answer can be given to the question about the level of risk arising from the occurrence of 3-MCPD esters in edible fats and fat-containing foods including infant formula and follow-up formula. No toxicological data are available on 3-MCPD esters. Only inadequate knowledge is available about absorption. It can be assumed that 3-MCPD may be released from 3-MCPD fatty acid esters during digestion. No robust findings are available. The results on the hazard potential of 3-MCPD themselves stem from animal experiments; no findings are available from human studies. Hence no statements can be made either about the sensitivity of humans compared to that of the animals examined in the animal experiments. The margin (quotient) between the lowest dose which led to tubular hyperplasias of the kidneys in rats in a long-term study (the lowest examined dose) and the amounts ingested by infants from infant formula and follow-up formula) may be very small (margin of exposure of 44 for infant formula and 28 for follow-up formula) in the worst case scenario (with the maximum levels of 3-MCPD esters found in infant formula and follow-up formula). Hence, there is a need from the risk assessment angle for immediate action to reduce the levels of 3-MCPD esters in edible fats and fat-containing foods, particularly in the ones used to manufacture infant food (formula and follow-up formula). Regarding the possible risk for infants it should be borne in mind that up to now a clinical picture based on tubular hyperplasias of the kidneys has not been reported in humans.

4 Action framework/measures

In co-operation with the Federal Research Agency for Nutrition and Food, the Institute for

Lipid Research, CVUA Stuttgart examined the possible causes for the formation of 3-MCPD esters. Almost the entire amount of 3-MCPD esters is formed during the deodorisation of edible fats and edible oils. From the risk assessment angle there is a need for immediate examination of the causes and a search for alternative techniques for the manufacture of refined fats with a view to reducing the levels of 3-MCPD esters in infant milk as there is no alternative for infants who cannot be breastfed, aside from human milk from other mothers.

5 References

BfR (2003a) 3-Monochlorpropandiol (3-MCPD) in Brot. Updated BfR opinion, 19 May 2003. http://www.bfr.bund.de/cm/208/3_monochlorpropandiol_in_brot.pdf

BfR (2003b) Ergänzende toxikologische Bewertung von 3-MCPD unter besonderer Berücksichtigung der Gefährdung von Kindern. BfR opinion, 9 July 2003. http://www.bfr.bund.de/cm/208/ergaenzende_toxikologische_bewertung_von_3___mcpd_unter_besonderer_beruecksichtigung_der_gefaehrdung_von_kindern.pdf

El Ramy R, Ould Elhkim M, Lezmi S, Poul JM (2007) Evaluation of the genotoxic potential of 3-monochloropropane-1,2-diol (3-MCPD) and its metabolites, glycidol and beta-chlorolactic acid, using the single cell gel/comet assay. Food Chem Toxicol. 2007 Jan;45(1):41-48.

RKI (2002) "Ernährungssurvey" in: Beiträge zur Gesundheitsberichterstattung des Bundes. Was essen wir heute? Robert Koch-Institut.

Robert MC, Oberson JM, Stadler RH.
Model studies on the formation of monochloropropanediols in the presence of lipase. J Agric Food Chem. 2004 Aug 11; 52(16):5102-8.

Scientific Committee on Food (2001): Opinion on 3-Monochloro-Propane-1,2-Diol (3-MCPD) updating the SCF opinion of 1994 adopted on 30 May 2001. http://ec.europa.eu/food/fs/sc/scf/out91_en.pdf

Stadler et al (2007) The formation of 3-monochloropropane-1,2-diol (3-MCPD) in food and potential measures of control. In: DFG/SKLM, Thermal processing of food: potential health benefits and risks. Wiley-VCH, 141-154.

WHO (2002): 3-Chloro-1, 2-Propandiol, WHO Food Add. Ser. 48, pp 401-432, WHO, Geneva
http://www.inchem.org/documents/jecfa/jecmono/v48je18.htm

WHO (2007): Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Sixty-seventh meeting, Rome, 20–29 June 2006. WHO technical report series no. 940, pp 45-53.
http://whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf

Zelinková Z, Svejkovská B, Velísek J, Dolezal M.
Fatty acid esters of 3-chloropropane-1,2-diol in edible oils.
Food Addit Contam. 2006 Dec;23(12):1290-8.