Molecular Nutrition & Food Research
www.mnf-journal.com

Urinary Excretion of 2/3-Monochloropropanediol (2/3-MCPD) and 2,3-Dihydroxypropylmercapturic Acid (DHPMA) after a Single High dose of Fatty Acid Esters of 2/3-MCPD and Glycidol: A Controlled Exposure Study in Humans

Klaus Abraham, Jan Hielscher, Jan Kuhlmann, and Bernhard H. Monien*

Scope: 2- and 3-monochloropropanediol (2/3-MCPD) and glycidol are absorbed in the intestine after lipase-catalyzed hydrolysis of their fatty acid esters.

Methods and results: In an exposure study with 12 non-smoking participants, the complete urinary excretion of the metabolite 2,3-dihydroxypropylmercapturic acid (DHPMA) and of 2/3-MCPD is measured on four consecutive days before and after consumption of 50 g glycidyl ester-rich palm fat or 12 g 2/3-MCPD ester-rich hazelnut oil. After controlled exposure, urinary excretion rates of 2/3-MCPD per hour strongly increase, followed by a decrease with average half-lives of 5.8 h (2-MCPD) and 3.6 h (3-MCPD). After consumption of hazelnut oil, mean excretion rates are 14.3% (2-MCPD) and 3.7% (3-MCPD) of the study doses. The latter rate is significantly higher (4.6%) after consumption of palm fat, indicating partial conversion (about 5%) of glycidol to 3-MCPD under the acidic conditions in the stomach. The average daily "background" exposure is estimated to be 0.12 and 0.32 µg per kg body weight (BW) for 2-MCPD and 3-MCPD, respectively. The relatively high and constant urinary excretion of DHPMA does not reflect the controlled exposure.

Conclusion: Urinary excretion of 2- and 3-MCPD is suitable as biomarker for the external exposure to the respective fatty acid esters.

1. Introduction

Heat treatment of vegetable oils and fats during the deodorization process leads to partial conversion of acylglycerols into fatty acid esters of glycidol and of the chlorinated propanediols, 2-monochloropropane-1,3-diol (2-MCPD) and 3-monochloropropane-1,2-diol (3-MCPD).[1] Toxicokinetic studies in rats receiving 3-MCPD dipalmitate,[2,3] glycidyl palmitate[4] or glycidyl linoleate[5] indicated that the fatty acid esters are hydrolyzed efficiently in the gastrointestinal tract to release 2/3-MCPD or glycidol which are more or less completely absorbed.

In repeated dose studies in rats, non-neoplastic toxic effects of 3-MCPD manifested in kidney (tubular hyperplasia), testes (atrophy and arteritis), and mammary glands (hyperplasia).[6,7] In addition, male infertility was observed in various experimental animals including primates.[8] By comparison, nephrotoxicity and myopathy are the most prominent effects observed in rats following sub-chronic treatment with 2-MCPD.[9] In two-year bioassays, moderate carcinogenic effects of 3-MCPD were observed without evidence for direct genotoxicity. The compound increased the incidences of adenomas and carcinomas of the Leydig cells and of the mammary gland in male rats, and of renal tubule adenomas and carcinomas in male and female rats.[6,7] In contrast, glycidol is a genotoxic carcinogen that increased the incidence of tumors in multiple tissues in rats, with most prominent effects in tunica vaginalis and peritoneum of male animals.[10] There are no data on the carcinogenicity of 2- and 3-MCPD or glycidol in humans.[11] The International Agency for Research on Cancer (IARC) has classified 3-MCPD as *possibly carcinogenic to humans* (group 2B)[12] and glycidol as *probably carcinogenic to humans* (group 2A).[13]

Hazard quantification for 3-MCPD was based on the tubular hyperplasia as the most sensitive endpoint observed in long-term studies with rats. The EFSA CONTAM Panel has derived a

Dr. K. Abraham, J. Hielscher, Dr. B. H. Monien
German Federal Institute for Risk Assessment
Department Food Safety
Max-Dohrn-Str. 8-10, Berlin 10589, Germany
E-mail: klaus.abraham@bfr.bund.de
Dr. J. Kuhlmann
SGS Germany GmbH
Weidenbaumsweg 137, Hamburg 21035, Germany

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202000735

DOI: 10.1002/mnfr.202000735

tolerable daily intake for 3-MCPD of 2.0 µg per kg body weight (BW).[14] Due to the genotoxicity of glycidol, hazard quantification is not possible for this compound. Instead, based on the T25 (dose inducing a 25% increase of the tumor incidence) of 10.2 mg per kg BW per day for the occurrence of peritoneal mesothelioma in male rats as a reference point, the margin of exposure (MoE) can be calculated for prioritization of risk management measures. This leads to the conclusion that the daily exposure may be of low concern only if it does not exceed 0.408 µg per kg BW (MoE = 25 000).[11,15]

Human exposure to 2/3-MCPD and glycidol is greatly determined by the levels of their fatty acid esters in edible oils and fats. Highest mean contents can be found in vegetable fats and oils (2-MCPD: 341 µg kg^{-1}, 3-MCPD: 1034 µg kg^{-1}, glycidol: 1176 µg kg^{-1}, mean middle bound level), with highest contaminations in palm oil/fat (2-MCPD: 1565 µg kg^{-1}, 3-MCPD: 2912 µg kg^{-1}, glycidol: 3955 µg kg^{-1}, mean middle bound level).[11] Other important sources of human exposure include "potato crisps", "hot surface cooked pastries" or "cookies", but also downstream food products prepared with refined vegetable oils, for example, infant formula.[11] Domestic cooking procedures may further increase the occurrence of the contaminants in the diet.[16,17]

Median and high (95th percentile) dietary exposure of European adults was estimated to be 0.3 and 0.7 µg per kg BW per day, respectively, in case of 3-MCPD, and 0.1 and 0.3 µg per kg BW per day, respectively, in case of 2-MCPD.[11] The corresponding median and high exposure to glycidol of European adults were estimated to be 0.2 and 0.5 µg per kg BW per day, respectively.[11] These estimations have a relatively high degree of uncertainty, as many processed foods may contain these fatty acid esters, and domestic cooking may further increase exposure.

Therefore, the actual exposure to fatty acid esters of 2/3-MCPD and glycidol may be better assessed using biomarkers of exposure, for example, specific urinary metabolites. The amount excreted in the urine within 24 h may allow approximating the daily external exposure to the parent compound if the basic pharmacokinetic parameters are known (reverse dosimetry).[18] Previous studies on urinary excretion related to the "background" exposure to fatty acid esters of 3-MCPD and glycidol considered 3-MCPD (median 2.52 µg L^{-1}) and 2,3-dihydroxypropylmercapturic acid (DHPMA, a mercapturic acid of glycidol and 3-MCPD, median 296 µg per g creatinine) in 255 adult samples of spot urine.[19] Whereas DHPMA was shown to be a metabolite in rat urine after administration of high doses of glycidol[4] and of 3-MCPD,[2] doubts regarding its suitability as urinary biomarker for the "background" exposure in humans already arise from the high amounts excreted (corresponding to a median excretion of 139 µg glycidol per day, if 1.5 g creatinine are excreted per day) as well as from the high correlation of DHPMA and creatinine levels in spot urine (n = 94),[20] indicating an endogenous origin of DHPMA "background" levels.[20,21]

To identify possible urinary biomarkers of exposure to 2/3-MCPD and glycidol in humans, a study with a defined high exposure (distinctly above "background" exposure) and 24 h collection of urine is necessary in terms of quantitative interpretations. We therefore, conducted a controlled exposure study with 12 participants who ate a single portion of hazelnut oil and of palm fat in another week, both containing relatively high amounts of fatty esters of 2/3-MCPD and glycidol, respectively. Urine samples were collected quantitatively for four days of each exposure week in order to answer the following questions: 1) Is it possible to quantify 2/3-MCPD and DHPMA in urine samples before and after exposure, and do these amounts reflect the oral exposure to 2/3-MCPD and glycidol (proof of principle as biomarkers of exposure)? 2) Is it possible to estimate the "background" exposure by comparing the urinary excretion during the days of usual nutrition with that after the defined single high dose? 3) Do we get indications that glycidol is converted to 3-MCPD under the acidic conditions of the stomach as suggested by Jones et al.[22] Furthermore, the study allowed to identify metabolites of 2/3-MCPD; these results are reported in a separate paper.[23]

2. Experimental Section

2.1. Participants

The study was conducted between October 2017 and January 2018 at the Department of Food Safety of the German Federal Institute for Risk Assessment (BfR). The study group consisted of 12 healthy non-smokers of European origin (six non-pregnant females and six males) from the scientific staff of the BfR, aware of the importance to avoid losses of urine and missing the right point of time for the scheduled urinations. They had a median age of 30 years (range 27–51), a median height of 1.77 m (range 1.61–1.90), a median body mass of 64 kg (range 59–114), and a median body mass index of 22.2 kg m^{-2} (range 18.3–31.6). Mean body mass (71.3 kg) was 63.8 and 78.8 kg in women and men, respectively. Individual data are provided in Table S1, Supporting Information. Ten of the participants were omnivores, and two (females) were vegetarians. All participants got a detailed oral consultation about the rationale of the study and gave informed consent in writing. The study protocol was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (No. EA4/130/17).

2.2. Study Foods

During a study investing the contents of ester bound 2/3-MCPD and glycidol in food items from the German market,[24] a glycidyl ester-rich palm fat ("TIP Pflanzenfett", 100% palm fat according to the label, best-by date: 20.11.2016 at room temperature, frozen at −20 °C after purchase) has been identified as the one with the highest level of bound glycidol (8.7 mg kg^{-1}; bound 2-MCPD: 1.5 mg kg^{-1}, bound 3-MCPD: 3.0 mg kg^{-1}). Likewise, an MCPD ester-rich hazelnut oil (Culinaria "Reines Haselnussöl" best-by date 21.04.2018) has been identified as the one with the highest level of bound MCPD (2-MCPD: 24.2 mg kg^{-1}, 3-MCPD: 54.5 mg kg^{-1}, bound glycidol: 0.8 mg kg^{-1}). Food analyses were performed by SGS Germany (Hamburg, Germany), using a technique for the indirect determination of ester bound glycidol in oil matrices. The method is based on an alkaline-catalyzed release of glycidol, followed by a transformation to monobromopropanediol according to a modification of the official method Cd 29b-13 ("3-in-1 method") of the American Oil Chemists' Society (AOCS).[25]

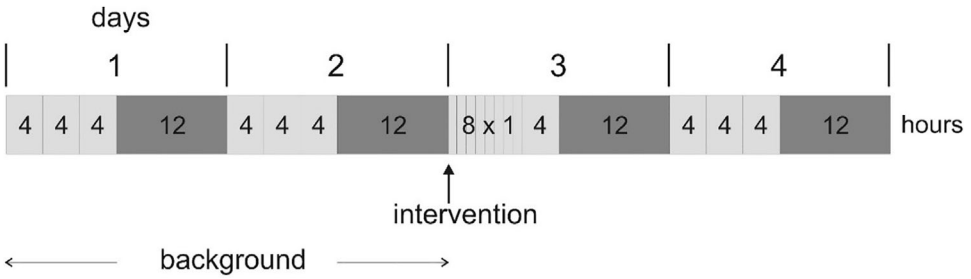

Figure 1. Time schedule for the collection of urine samples during the study on the controlled exposure with palm fat and hazelnut oil.

2.3. Study Design and Conduct

The study period consisted of 2 weeks, one with a high exposure to glycidyl esters (palm fat consumption), and one with a high exposure to the MCPD esters (consumption of hazelnut oil). In each week, the study started after emptying of the urinary bladder on Monday at 09:00. The complete urine produced was henceforth collected quantitatively for 96 h until Friday 09:00 in daily fractions of 4 h (9:00 to 13:00, 13:00 to 17:00, 17:00 to 21:00) and of 12 h overnight (21:00 to 09:00), with the exception of Wednesday when the urine was collected hourly from 09:00 to 17:00. All 528 urine fractions were collected in plastic containers. After weighing to determine each amount of urine, aliquots of 50 mL were frozen at −80 °C until analysis. The time intervals are displayed in **Figure 1**. Creatinine content in all urine samples was analyzed in a medical laboratory (Medizinisches Versorgungszentrum Labor 28 GmbH, Berlin, Germany).

On Wednesday, the participants came to the BfR without having breakfast at home. At 09:00, they ate a meal of 50 g palm fat in one of the weeks, and of 12 g hazelnut oil in the other week. Both were served with two slices (109 g) of bread (Lieken "Urkorn Bauernmild" which did not contain detectable amounts of bound glycidol and 2/3-MCPD, limit of detection: 10 µg kg^{-1}). On Wednesday only, the participants were asked to drink adequately during the daytime to ensure the hourly emptying of the urinary bladder. Otherwise, the participants were asked to keep their nutritional habits as constant as possible during the two study weeks.

The meals eaten as breakfast on Wednesday morning contained a single dose of 75 µg bound 2-MCPD, 150 µg bound 3-MCPD and 435 µg bound glycidol in case of consumption of 50 g palm fat, and of 290 µg bound 2-MCPD, 654 µg bound 3-MCPD and 9.6 µg bound glycidol in case of consumption of 12 g hazelnut oil. These doses were chosen to be the same on a molar basis (5.9 µmol) for bound glycidol in case of palm fat and bound 3-MCPD in case of hazelnut oil. The means of the highest doses eaten with one of the study meals on a body weight basis were 6.3 µg bound glycidol per kg BW and 9.5 µg bound 3-MCPD per kg BW (individual doses of bound glycidol and 2/3-MCPD are summarized in Table S1, Supporting Information).

2.4. Chemicals, Solvents, and Standard Compounds

Phenylboronic acid (≥97%), diethyl ether (analytical grade), ethyl acetate (analytical grade), tert-butyl methyl ether (tBME, analytical grade), iso-hexane (analytical grade), iso-octane (analytical grade) and methanol (analytical grade) were purchased from Merck KGaA (Darmstadt, Germany). Sodium bromide (Ph. Eur.), sodium sulfate (anhydrous, granulated, for organic trace analysis), acetic acid and formic acid were from Merck KGaA. The sodium sulfate was dried overnight in a muffle furnace at approx. 200 °C before use. (±)-3-Chloro-1,2-propanediol (3-MCPD) was from Fisher Scientific (Schwerte, Germany) and 2-chloro-1,3-propanediol (2-MCPD) from Santa Cruz Biotechnology (Heidelberg, Germany). (±)-3-Chloro-1,2-propanediol-d_5 (3-MCPD-d_5) was obtained from Sigma-Aldrich (Steinheim, Germany). The standard of *N*-acetyl-S-(2,3-dihydroxypropyl)-L-cysteine (DHPMA) and the corresponding internal standard [$^{13}C_2$]DHPMA were provided by Prof. Göen (Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany).

2.5. Extraction and Derivatization of 2- and 3-MCPD

2- and 3-MCPD were analyzed after extraction of urine samples and derivatization of the diols using phenylboronic acid.[26] Samples were thawed overnight at 2–8 °C and homogenized by vigorous mixing. Aliquots of 4 mL were spiked with 40 µL of 3-MCPD-d_5 (1 mg L^{-1}) in methanol. The matrix was depleted by a single extraction with 3.5 mL of tBME/iso-hexane (1:4). The analytes 2- and 3-MCPD were extracted three times using 2.5 mL of diethyl ether/ethyl acetate (9:1) followed by a 2 min centrifugation (3500 rpm) for each extraction. The upper phases were taken carefully, combined, and dried over sodium sulfate. The combined organic extracts were mixed with 50 µL of a solution of phenylboronic acid (2 mg mL^{-1}) in diethyl ether/ethyl acetate (9:1) and concentrated in a moderate nitrogen flow to a volume of about 1 mL. Solutions were transferred to 8 mL screw-top containers with 200 mg sodium sulfate and the solutions were concentrated gently to dryness. Residuals were taken up in 200 µL of iso-octane, transferred into a micro-insert and stored in the freezer prior to gas-chromatography-coupled mass-spectrometry (GC-MS) analysis. Analyses were carried out in duplicate.

2.6. GC-MS Analysis

Dioxaborolane derivatives of 2- and 3-MCPD were analyzed by an accredited laboratory (SGS Germany GmbH, Hamburg, Germany) using a gas chromatograph 7890B system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Programmable

Temperature Vaporizer (PTV) injection device. The following conditions were applied: injection volume 2-4 µL (pulsed splitless injection), PTV temperature program: 80 °C (isothermal for 0.1 min), increase with 2 °C s^{-1} to 140 °C (isothermal for 6 min), increase with 10 °C s^{-1} to 320°C (isothermal for 10 min). PTV purge gas flow: 50 mL helium per min at 0.5 min to 1 min (septum purge 3 mL helium per min). Samples were separated on a Rxi-17 (Resteck GmbH, Bad Homburg, Germany) with a stationary phase of 50% diphenyl/50% dimethylpolysiloxane (30 m x 0.25 mm, 0.25 mm film thickness) and the pre-column HP-5ms (Agilent Technologies) with a stationary phase of 5% phenyl/95% dimethylpolysiloxane (2.4 m × 0.32 mm, 0.25 mm film thickness). Helium was used as carrier gas at a constant flow rate of 1.4 mL min^{-1}. The oven temperature started at 80 °C (isothermal for 2 min) followed by a gradient of 5.4 °C min^{-1} up to 150 °C (isothermal for 4 min). Then, the temperature increased from 20 °C min^{-1} to 280 °C (isothermal for 5 min).

Analytes were ionized by electron impact and detected using the single quadrupole mass spectrometer 5977B (Agilent Technologies) operated in the selected ion monitoring (SIM) mode. Mass-to-charge ratios (m/z) of the detected phenylboronic derivatives of the analytes were m/z = 146, 147, 196, 198 (3-MCPD), m/z = 149, 150, 201, 203 (3-MCPD-d_5), m/z = 196, 198 (2-MCPD). 3-MCPD was quantified from the respective signal of the 3-MCPD-d_5 phenyl boronic acid derivative using the ratio of the traces 147/150. 3-MCPD-d_5 (m/z = 150) was also applied for the quantification of 2-MCPD (m/z = 196) with the help of a specific correction factor (2.47). Every eighth sample was a quality control sample (human urine with defined amounts of 2- and 3-MPCD). Ratios of the response factors, for example, m/z = 150 versus 147 (3-MCPD-d_5 vs 3-MCPD), were checked for constancy. Limits of detection (LOD) and quantification (LOQ) were 0.1 and 0.25 µg L^{-1} for 3-MCPD, respectively, and 0.12 and 0.3 µg L^{-1} for 2-MCPD, respectively. Other validation parameters are summarized in Table S2, Supporting Information. The analytes' molecular structures are depicted in Figure S1, Supporting Information. Figure S2, Supporting Information, shows exemplary chromatograms of the dioxaborolane conjugates in the extracts of two urine samples of one participant before and 3 h after controlled exposure to hazelnut oil.

2.7. Analysis of Urinary DHPMA by UHPLC-MS/MS

The technique for the quantification of DHPMA by LC-MS/MS (liquid-chromatography-coupled MS/MS) was adapted from Andreoli et al.[19] In brief, thawed urine samples were homogenized by vigorous mixing. Aliquots of 90 µL urine were diluted with 400 µL 0.2% acetic acid in water and 10 µL [$^{13}C_2$]DHPMA solution (5 µg mL^{-1} in water) was added. For the analysis of DHPMA, we used an ultra-high performance liquid chromatography (UHPLC) system I-Class (Waters, Eschborn, Germany) connected to a QTRAP 6500 (Sciex, Darmstadt, Germany). Samples (5 µL) were injected on an HSS T3 column (2.1 × 100 mm, Waters) and the analyte was eluted with a gradient consisting of water + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B) as mobile phases at a flow rate of 0.4 mL min^{-1}. The gradient was: 0-1 min (98% solvent A), 1-3 min (98–80% solvent A), 3.01-4 min (5% solvent A), 4.01-5 min (98% solvent A). DHPMA was determined after electrospray ionization in the negative mode using the characteristic constant neutral loss of 129 Da for the detection of mercapturic acids (m/z = 236 → 107). Simultaneously, the transition of the internal standard [$^{13}C_2$]DHPMA (m/z = 238 → 107) was monitored for quantification.[27] Instrument control and data analysis were done with Analyst 1.6.0 (Sciex). LOD and LOQ were 2.8 and 11.1 µg L^{-1}, respectively. Other validation parameters are summarized in Table S2, Supporting Information. Exemplary multiple reaction monitoring chromatographic results of DHPMA in a human urine sample are presented together with those of DHPMA in horse and goat urine in Figure S3, Supporting Information.

2.8. Statistical Evaluation

Standard methods were applied for statistical evaluation using Excel (Microsoft Office 2010) and SPSS (IBM Version 21). If necessary, the name of the method used is given in the following chapters. In general, correlation tests were performed as Pearson correlation. Values of 2/3-MCPD below the LOQ were considered as half the value of the LOQ.

2.9. Ethical Approval

The study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (No. EA4/130/17) and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants at the study entry. The study was registered in the German Clinical Trials Register (DRKS, No. 00 013219).

3. Results

3.1. Daily Urine Production and Creatinine Excretion

For the 96 study days (8 days in 12 participants each), daily urine production varied between 0.73 and 5.87 L d^{-1} (mean ± SD: 2.63 ± 1.15 L d^{-1}), with a daily creatinine excretion of between 1.00 and 2.50 g d^{-1} (mean ± SD: 1.60 ± 0.36 g d^{-1}). According to John et al.,[28] the daily creatinine index (ratio of observed and expected creatinine production, based on body weight and sex) is useful for ruling out incomplete 24 h urine specimens (= index <0.7). The values calculated varied between 0.77 and 1.29 (mean ± SD: 1.00 ± 0.13, n = 96), indicating completeness of all urine collections.

3.2. Urinary Excretion of 2- and 3-MCPD

Of all 192 "background" samples from days 1 and 2, 32.3 and 36.5% were below the LOQ in case of 2-MCPD and 3-MCPD, respectively. On day 3 (administration of the study fat/oil in the morning), none of the 240 samples were below the LOQ, whereas on day 4, 6.3 and 21.9% of the 96 samples were below the LOQ in case of 2-MCPD and 3-MCPD, respectively. For the following calculations, values below the LOQ were considered as half the value of the LOQ.

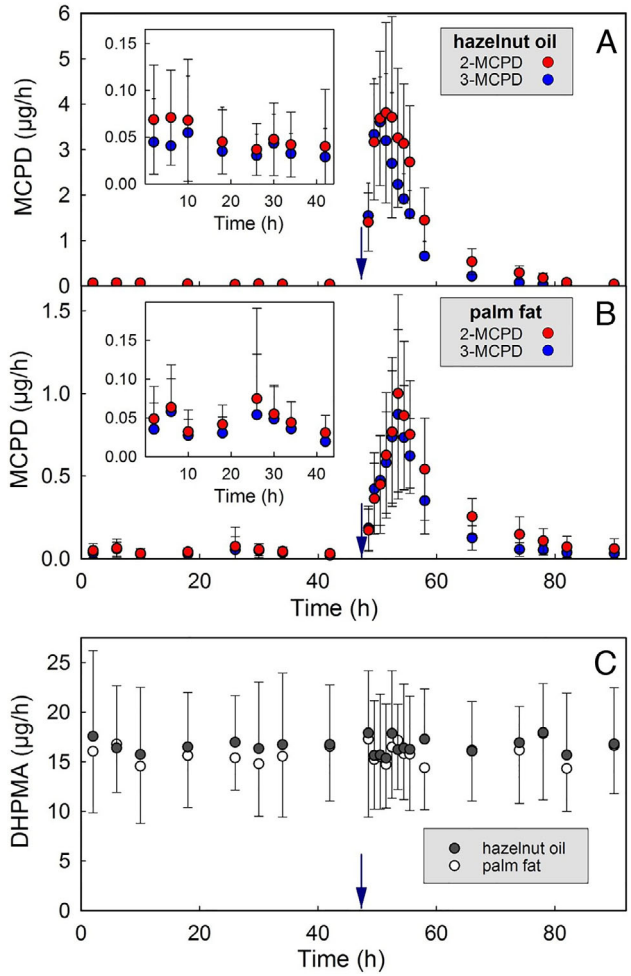

Figure 2. Mean excretion rates of 2-MCPD and 3-MCPD per hour before and after the consumption of A) 12 g hazelnut oil or B) of 50 g palm fat. Enlarged sections (insets): excretion on the two days before the controlled exposure. C) Mean excretion rates of DHPMA per hour after controlled exposure to hazelnut (dark dots) and palm fat (bright dots). Error bars depict standard deviations (n = 12). Blue arrow: intervention (controlled exposure).

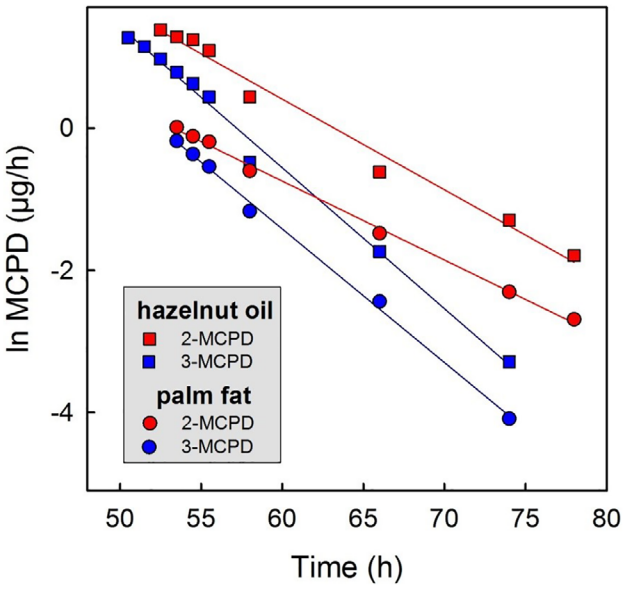

Figure 3. Decrease of the excretion of 2-MCPD and 3-MCPD per hour starting from the maximum values observed after the exposure to hazelnut oil or palm fat. Half-lives calculated: 6.2 and 5.4 h for 2-MCPD, and 3.7 and 3.5 h for 3-MCPD following the consumption of palm fat and hazelnut oil, respectively.

Hourly excretion rates (mean ± SD of 12 individual averages) calculated form the daily urine samples for the four "background" days (day 1 and 2, two days each) were 0.057 ± 0.016 µg h^{-1} (range 0.035–0.076) and 0.039 ± 0.010 µg h^{-1} (range 0.026–0.060) for 2-MCPD and 3-MCPD, respectively (see also inserts in **Figure 2**A,B, displayed for the four daily collection periods). After consumption of the study fat/oil on day 3, excretion rates measured per hour distinctly increased (Figure 2A,B). In case of hazelnut oil, maximum excretion rates were 4.92 ± 1.32 µg h^{-1} (range 2.61–6.91) for 2-MCPD, and 4.11 ± 1.14 µg h^{-1} (mean ± SD, range 2.26–5.80) for 3-MCPD. The mean values were observed in the urine collected between 3 and 4 h after consumption (2-MCPD) and between 2 and 3 h after consumption (3-MCPD). In case of palm fat, maximum excretion rates of 1.23 ± 0.46 µg h^{-1} (range 0.70–2.24) for 2-MCPD and of 0.98 ± 0.45 µg h^{-1} (range 0.51–1.87) for 3-MCPD were observed in the urine collected between 5 and 6 h after consumption. In **Figure 3**, the decreasing values of the urinary excretion rates per hour are displayed on a logarithmic scale over time, allowing the calculations of half-lives of 6.2 and 5.4 h for 2-MCPD and of 3.7 and 3.5 h for 3-MCPD following consumption of palm fat and hazelnut oil, respectively.

Total daily urinary excretion on the four study days each before and after consumption of palm fat and hazelnut oil is compiled in **Table 1** (2-MCPD) and **Table 2** (3-MCPD). In case of 2-MCPD, the average daily excretion of the four "background" days was between 1.05 and 1.42 µg. On application days 3, the average daily excretion was much higher, amounting to 11.5 µg 2-MCPD in case of palm fat and 40.8 µg 2-MCPD in case of hazelnut oil. On the following days 4, the average daily excretion values of 2-MCPD (2.28 and 3.37 µg) were above the range of means observed on days 1 and 2, demonstrating that the complete urinary excretion of 2-MCPD requires more than 24 h. In case of 3-MCPD, the average daily excretion of the four "background" days was between 0.77 and 0.98 µg. On application days 3, the average daily excretion was much higher, amounting to 7.54 µg 3-MCPD in case of palm fat and 25.4 µg 3-MCPD in case of hazelnut oil. On the following days 4, the average daily excretion of 3-MCPD was within the range observed on days 1 and 2, indicating a fast urinary excretion within less than 24 h.

For the calculation of the urinary amounts excreted of the 2/3-MCPD dose eaten with the study fat/oil, excretion on days 3 and 4 was subtracted by the "background" excretion on days 1 and 2. This results in a percentage of the ingested dose (mean ± SD) of 15.0 ± 5.2% and 14.3 ± 3.1% of the ingested dose excreted as 2-MCPD in case of palm fat and hazelnut oil, respectively (Table 1), and of 4.58 ± 1.54%and 3.73 ± 0.95% excreted as 3-MCPD in case of palm fat and hazelnut oil, respectively (Table 2). The inter-individual variation of the excretion rates was relatively

Table 1. Urinary excretion of 2-MCPD in each participant on the 8 days of the study and calculated additional excretion following intake of 290 µg 2-MCPD (hazelnut oil) or 75 µg 3-MCPD (palm fat).

Participant	Excretion of 2-MCPD					
	Day 1	Day 2	Day 3	Day 4	Days 3 + 4 − (1 + 2)	
	[µg]	[µg]	[µg]	[µg]	[µg]	[% of dose]
Hazelnut oil						
A	0.5	1.7	40.6	3.3	41.6	14.3
B	2.4	0.5	55.9	2.4	55.5	19.1
C	2.0	0.4	30.9	4.1	32.6	11.2
D	1.6	0.3	39.2	1.8	39.0	13.4
E	1.2	0.7	38.1	1.6	37.8	13.0
F	2.0	1.2	52.5	4.0	53.3	18.3
G	0.4	0.3	42.4	4.4	46.1	15.9
H	2.1	0.7	41.4	2.8	41.4	14.2
I	2.3	3.3	42.6	4.0	41.1	14.1
J	0.9	0.8	37.0	5.1	40.5	13.9
K	1.1	1.8	23.8	1.0	22.0	7.6
L	0.7	0.8	44.7	5.9	49.1	16.9
Mean ± SD	1.42 ± 0.73	1.05 ± 0.87	40.76 ± 8.51	3.37 ± 1.49	41.66 ± 9.02	14.3 ± 3.1
Palm fat						
A	1.2	0.4	9.6	2.1	10.1	13.5
B	2.5	1.0	16.7	1.9	15.1	20.1
C	1.0	1.0	8.5	2.0	8.5	11.3
D	0.6	1.7	11.7	1.8	11.2	14.9
E	0.4	0.5	11.4	1.2	11.7	15.6
F	1.0	0.8	15.7	2.6	16.5	22.1
G	1.7	0.5	8.7	1.6	8.1	10.7
H	0.5	0.7	8.5	1.9	9.2	12.3
I	0.8	1.1	13.3	6.2	17.6	23.5
J	1.4	3.4	10.2	2.3	7.6	10.1
K	1.6	1.4	7.6	0.4	4.9	6.6
L	1.0	4.3	16.2	3.2	14.1	18.9
Mean ± SD	1.16 ± 0.59	1.40 ± 1.23	11.51 ± 3.26	2.28 ± 1.42	11.23 ± 3.92	15.0 ± 5.2

Table 2. Urinary excretion of 3-MCPD in each participant on the 8 days of the study and calculated additional excretion following intake of 654 µg 3-MCPD (hazelnut oil) or 150 µg 3-MCPD (palm fat).

Participant	Excretion of 3-MCPD					
	Day 1	Day 2	Day 3	Day 4	Days 3 + 4 − (1 + 2)	
	[µg]	[µg]	[µg]	[µg]	[µg]	[% of dose]
Hazelnut oil						
A	0.4	1.2	23.5	0.9	22.8	3.5
B	1.4	0.4	32.8	0.7	31.7	4.8
C	1.0	0.2	15.9	1.0	15.7	2.4
D	0.6	0.3	25.2	0.5	24.8	3.8
E	1.5	0.5	28.7	0.4	27.0	4.1
F	2.6	1.2	35.0	0.6	31.9	4.9
G	0.3	0.4	30.3	1.7	31.3	4.8
H	1.8	0.5	26.4	0.9	25.0	3.8
I	0.6	1.5	22.3	0.5	20.7	3.2
J	0.6	0.9	25.2	0.9	24.6	3.8
K	0.7	1.6	13.3	0.3	11.4	1.7
L	0.4	0.5	25.7	0.8	25.6	3.9
Mean ± SD	0.98 ± 0.70	0.77± 0.49	25.36 ± 6.26	0.77 ± 0.37	24.38 ± 6.22	3.73 ± 0.95
Palm fat						
A	0.9	0.4	6.4	1.0	6.0	4.0
B	1.7	0.4	11.5	0.5	9.8	6.6
C	0.5	0.4	5.0	0.7	4.8	3.2
D	0.7	1.2	6.9	0.5	5.5	3.7
E	0.3	0.4	8.5	0.7	8.4	5.6
F	1.6	0.7	11.8	1.4	11.0	7.3
G	1.2	0.4	7.2	0.6	6.1	4.1
H	0.3	0.6	6.6	0.8	6.4	4.3
I	0.5	0.5	6.9	2.8	8.8	5.8
J	0.7	2.0	7.7	1.2	6.2	4.1
K	1.2	1.0	4.1	0.6	2.5	1.7
L	0.6	1.5	8.0	1.0	7.0	4.6
Mean ± SD	0.85 ± 0.47	0.79 ± 0.52	7.54 ± 2.26	0.97 ± 0.64	6.87 ± 2.32	4.58 ± 1.54

high (see Tables 1 and 2), with variation coefficients for 2-MCPD and 3-MCPD in case of hazelnut oil of 21.7% and 25.5%, respectively, and even higher coefficients in case of palm fat: 34.9% and 33.7%, respectively. A negative association was observed between the average individual excretion rates (for palm fat and hazelnut oil) and body weight, with R^2 = 40% each for 2-MCPD and 3-MCPD (**Figure 4**A). Using linear regression analysis with factors possibly influencing the excretion rates of 2- and 3-MCPD (age, sex, urine production on day 3, body weight) as independent variables, a significant impact on these rates was observed for body weight only. Excretion rates in case of consumption of palm fat and hazelnut oil correlated with R^2 = 49% for 2-MCPD and R^2 = 57% for 3-MCPD (Figure 4B). Average individual excretion rates (for palm fat and hazelnut oil) of 2-MCPD and 3-MCPD correlated relatively strongly (R^2 = 78%, Figure 4C).

3.3. Estimation of 2/3-MCPD "Background" Exposure

Data also allowed estimating the external "background" exposure to 2- and 3-MCPD bound as fatty acid esters, assuming the occurrence of the two compounds in urine to result from food consumption only, and assuming the same bioavailability as for the meal with hazelnut oil (with respect to intestinal hydrolysis and absorption). On the four "background" days 1 and 2, the average daily excretion of 2-MCPD and 3-MCPD was 1.26 and 0.85 µg d^{-1}, respectively. Using the mean excretion rates of 14.3% and 3.7%, respectively, after consumption of hazelnut oil, a mean external "background" exposure of 8.8 µg 2-MCPD per day and 22.0 µg 3-MCPD can be calculated (30- and 33-fold lower than the additional study dose in case of hazelnut oil, respectively). Using the average body weight of 71.3 kg, the average daily "background" exposure was estimated

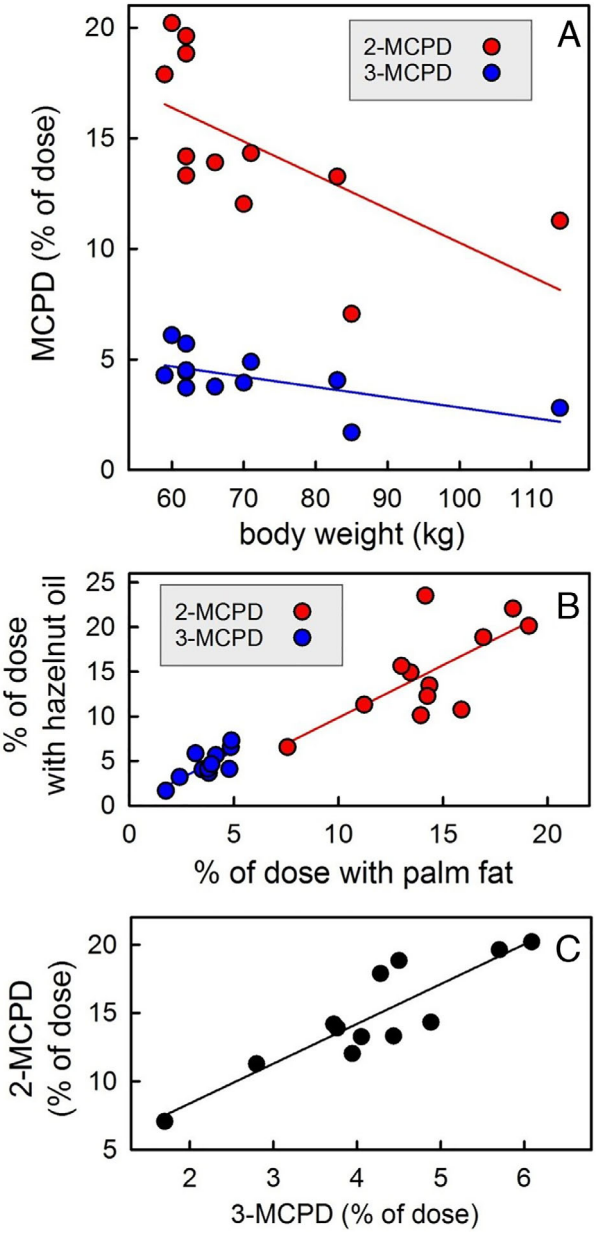

Figure 4. A) Correlation between body weight and urinary excretion rates (% of doses) of 2-MCPD and 3-MCPD (for each compound, individual means are calculated for the intake of hazelnut oil and palm fat, R^2 = 40% each, n = 12). B) Correlation of the excretion rates between intake of hazelnut oil and palm fat for 2-MCPD (R^2 = 49%) and 3-MCPD (R^2 = 57%, n = 12). C) Correlation between the excretion rates of 2-MCPD and 3-MCPD (individual means of the excretion rates calculated as for (A), R^2 = 78%, n = 12).

to be 0.12 and 0.32 µg per kg BW for 2-MCPD and 3-MCPD, respectively.

3.4. Urinary Excretion of DHPMA

DHPMA was quantifiable in all 528 urine samples (mean ± SD: 179 ± 166 µg L^{-1}, median 121 µg L^{-1}, range 15.6–1139 µg L^{-1}).

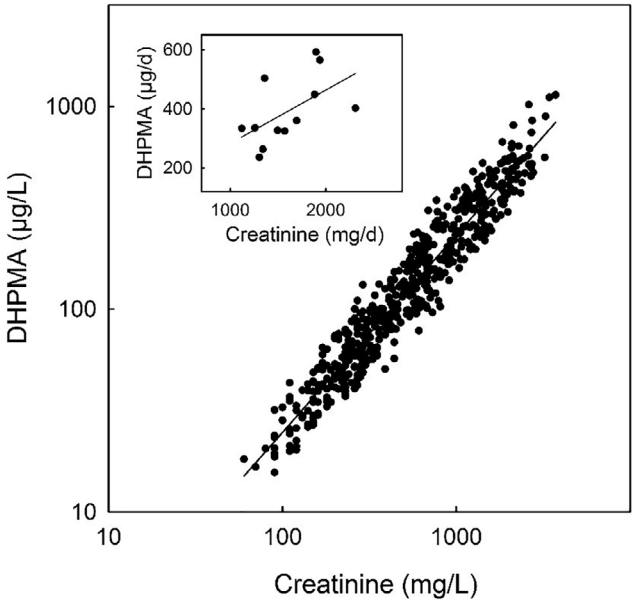

Figure 5. Correlation between concentrations of DHPMA and creatinine determined in all urine samples (n = 528) collected during the study (R^2 = 92%). The inset shows the correlation between mean daily excretion amounts of DHPMA and creatinine for each participant (R^2 = 31%, n = 12).

Daily excretion of DHPMA did not increase following the fat/oil consumption on day 3. The mean daily excretion on days 1, 2, 3, and 4 was 377, 382, 380, 393 µg d^{-1} in case of palm fat, and 397, 401, 393, and 404 µg d^{-1} in case of hazelnut oil, respectively, with standard deviations between 106 and 142 µg d^{-1}. The average hourly excretion rate per period of urine collection in the four study days is displayed in Figure 2C.

Daily excretion of DHPMA varied between 163 and 697 µg d^{-1} (mean ± SD: 391 ± 116 µg d^{-1}, n = 96, that is, 8 study days in 12 participants each). On average (individual mean of 8 study days), the daily DHPMA excretion in the 12 subjects was observed to be between 235 and 592 µg d^{-1} (between 3.53 and 8.12 µg per kg BW per day), without a significant difference between men (mean ± SD: 438 ± 125 µg d^{-1}, 5.69 ± 1.53 µg per kg BW per day) and women (mean ± SD: 343 ± 87 µg d^{-1}, 5.40 ± 1.47 µg per kg BW per day). The average intra-individual variation of the daily DHPMA excretion was small compared to the inter-individual variation (variation coefficient 9.7 vs 29.2%, respectively). Based on the excretion of creatinine, average DHPMA excretion in individuals (of 8 days each) was between 174 and 370 µg per g creatinine (mean ± SD: 247 ± 61 µg per g creatinine, median 228 µg per g creatinine, n = 12). As displayed in **Figure 5**, a high correlation of DHPMA and creatinine concentrations was observed for the 528 urine samples (R^2 = 92%, mainly resulting from the differently concentrated urine samples). For the average daily excretion of DHPMA and creatinine of individuals, the correlation was much weaker (R^2 = 31%, n = 12, see insert in Figure 5). Regarding a (positive) correlation with the body weight, that of the average daily excretion of creatinine was much higher (R^2 = 72%) compared to that of the average daily DHPMA excretion (R^2 = 17%).

4. Discussion

4.1. Urinary Excretion of 2- and 3-MCPD

Following the relatively high exposure in the morning of day 3, hourly measured urinary excretion rates of 2- and 3-MCPD reflecting the concentrations of the compounds in the blood showed an absorption faster in case of hazelnut oil compared to palm fat (Figure 2A,B). This is most likely due to the lower amount of fat in the former (12 g) compared to the latter (50 g), requiring a delayed transportation from stomach to small intestine to allow effective fat absorption. Indeed, the percentage of the 2-MCPD dose ingested found in urine was comparable for hazelnut oil (mean 14.3%) and palm fat (mean 15.0%), indicating a comparable rate of (fat) absorption. The absolute bioavailability of fatty acid esters of 2/3-MCPD and glycidol in humans, however, is unknown, but is thought to be more or less complete, as fat absorption in healthy people is more or less complete and data in rats also have demonstrated high rates of absorption of these esters.[2–5]

Following absorption, 2- and 3-MCPD revealed renal excretion with first-order kinetics and average half-lives of 5.8 and 3.6 h, respectively, obviously reflecting a faster metabolism of 3-MCPD in comparison to 2-MCPD. Accordingly, the total amounts excreted within two days after application were considerably higher for 2-MCPD (nearly 15% of the dose ingested on average) compared to 3-MCPD (3.73% on average after consumption of hazelnut oil). After consumption of palm fat, the amount of 3-MCPD excreted was significantly higher (4.58% on average, $p = 0.015$, t-test for paired values). As already suggested by Jones et al.,[22] this may be due to partial conversion of glycidol to 3-MCPD under the acidic conditions of the stomach, as the dose of glycidol in palm fat was distinctly higher as compared to the dose of 3-MCPD (5.9 vs 1.4 µmol), while the relation was vice versa for hazelnut oil (0.13 vs 5.9 µmol). The average additional 3-MCPD excretion after consumption of palm fat amounted to 0.86% of the 3-MCPD dose (4.58% minus 3.73%), and corresponded to a mean additional 3-MCPD dose of 34.4 µg (0.311 µmol) using the excretion rate of 3-MCPD (mean: 3.73% of the dose) after consumption of hazelnut oil (thereby neglecting the relatively small dose of glycidol in hazelnut oil). This additional dose of 3-MCPD corresponded to a mean dose of 23.1 µg glycidol (0.311 µmol) indicating partial conversion of glycidol to 3-MCPD (5.3% of the glycidol dose eaten with the palm fat).

The daily urinary "background" excretion (days 1 and 2) was used to estimate the external "background" exposure of 2-MCPD and 3-MCPD by using the average excretion rates of the compounds after consumption of hazelnut oil, based on 48 observation days in the 12 participants. Mean estimates of 0.12 and 0.32 µg per kg BW for 2-MCPD and 3-MCPD, respectively, were found to be very close to the median middle bound external doses estimated for European adults of 0.1 and 0.3 µg per kg BW, respectively.[11] However, this is expected to be by chance, as we have investigated 12 participants only, and occurrence of 2/3-MCPD in food, as well as food consumption habits, are different in different European countries.

For individuals, such a calculation of the external dose from 24 h urinary excretion data reflects the exposure of a single day only. In addition, the result is associated with a higher degree of uncertainty, as the variation coefficients were relatively high (up to 35% in case of palm fat, primarily indicating inter-individual variation). Furthermore, the correlation of the excretion rates (consumption of palm fat vs hazelnut oil, Figure 4B) was relatively low for 2-MCPD (R^2 = 49%) and 3-MCPD (R^2 = 57%), indicating a distinct intra-individual variation. This may be due to (unexplained) week-to-week variation, as the correlation of the average individual excretion rates (for palm fat and hazelnut oil) of 2-MCPD and 3-MCPD was much stronger (R^2 = 78%, Figure 4C), despite the possible influence of different pathways responsible for the metabolism of each compound. Metabolism rates of 2-MCPD and 3-MCPD (roughly calculated as ingested minus excreted dose) seemed to increase with body weight. This may reflect a distribution phenomenon, but is unlikely to reflect limited metabolic capacity, as mean values of these metabolism rates were not found to be lower in case of the about fourfold higher doses of 2- and 3-MCPD eaten with hazelnut oil as compared to palm fat.

4.2. Urinary Excretion of DHPMA

We observed a relatively constant and high daily excretion of DHPMA. This was already observed by Eckert et al.[20] who found a median concentration of 217 µg L^{-1} (206 µg per g creatinine) in spot urine of 54 non-smokers comparable to that of 209 µg L^{-1} (217 µg per g creatinine) in 40 smokers. While the values based on creatinine excretion are very close to our result (median 228 µg per g creatinine), we observed lower DHPMA concentrations based on the urine volume (median 121 µg L^{-1}), likely due to the relatively high fluid consumption of our participants resulting in a relatively high daily urine excretion (2.63 L on average). Based on the excretion of creatinine, Andreoli et al. observed a higher median DHPMA concentration of 296 µg per g creatinine in spot urine (n = 255).[19]

The high correlation of DHPMA and creatinine concentrations (Figure 5) was likewise observed in the investigation by Eckert et al. who already discussed the possibility of an endogenous origin of DHPMA "background" levels in urine resulting from an unknown chemical compound serving as a precursor for DHPMA.[20] This hypothesis is supported by our results showing the relatively constant and high daily excretion of DHPMA with relatively low intra-individual variation (as compared to the inter-individual variation), not depending on common external exposures to glycidol: smoking or consumption of a palm fat with high concentrations of glycidyl fatty esters (exposures which are detectable as increased hemoglobin adduct levels).[15,29] The hypothesis of a genetic cause determining the body's metabolism is further supported by additional DHPMA measurements of spot urine samples from different agricultural livestock from BfR's experimental farm which revealed huge interspecies variations (measurements in individual animals) between horses (35, 38 µg L^{-1}), young pigs (12, 38, 42, 62 µg L^{-1}), and cows (37, 85, 176 µg L^{-1}), rats (33, 39, 53, 55, 58, 58, 140, 205 µg L^{-1}), sheep (1252, 2481, 2802, 2885), and goats (7558, 7600, 7978, 13 211 µg L^{-1}).

The urinary excretion of DHPMA did not increase following the fat/oil consumption on day 3. Considering the daily mean

DHPMA excretion of 391 ± 116 µg d^{-1} (1.65 ± 0.49 µmol d^{-1}), an increase of the DHPMA excretion corresponding to half the SD (58 µg d^{-1} = 0.24 µmol d^{-1}) would require a conversion to DHPMA at a rate of about 4.1% of the dose of glycidol or 3-MCPD (5.9 µmol each) on day 3 in case of the consumption of palm fat and hazelnut oil, respectively (assuming 100% intestinal hydrolysis and bioavailability as glycidol and 3-MCPD, respectively). The rate of these compounds converted to DHPMA in humans is yet unknown. After administration of glycidol and glycidyl palmitate in rats, the mean 24 h-recovery as DHPMA was 13.2% and 12.8% of the dose, respectively.[4] The rats had received a single glycidol dose of 50 mg per kg BW which is much higher than the average dose of 6.3 µg per kg BW eaten in our study in case of palm fat. The same holds true for 3-MCPD in rats revealing a mean conversion rate of up to 12.5% (in male animals) after application of the highest dose of 29.5 mg per kg BW.[2] Therefore, DHPMA is obviously suitable as biomarker of exposure in case of high doses of the two compounds in rats (relevant proportion converted to DHPMA which is excreted at levels far above the "background" levels), but not in humans even in case of a single dose distinctly (more than about 30-fold) above the current mean "background" exposure to glycidol or 3-MCPD.

Indeed, higher doses of 3-MCPD are required to observe a detectable increase in DHPMA excretion: in a self-dosing investigation, the medical head of the study (K.A., BW 81 kg) ingested isolated 10 mg 3-MCPD on Wednesday (day 2) and 5 mg 2-MCPD on Wednesday one week later (dissolved in 1% aqueous ethanol, taken up together with 100 mL water). The investigation was carried out with the primary aim to identify metabolites of 2/3-MCPD and was carried by the project's physician in his own responsibility, not requiring an ethical vote. The selection of the doses was based on no-effect-levels observed in rats and the use of an uncertainty factor of 100, and therefore followed rules for the derivation of an acute reference dose (which is not yet established by a scientific committee). Urine was collected completely in fractions from day 1 (before exposure) to day 3, with time intervals of 0–2, 2–4, 4–6, 6–8, 8–12, and 12–24 h after exposure in the morning on day 2. After exposure to 2-MCPD, DHPMA excretion on days 1, 2, and 3 were 326, 349, 303 µg d^{-1}, respectively, and 337, 519, and 372 µg d^{-1} after exposure to 3-MCPD on day 2, respectively. The excess DHPMA excretion (after ingestion of 3-MCPD vs 2-MCPD) on days 2 and 3 was 239 µg, corresponding to 111 µg 3-MCPD. Therefore, about 1.1% of the dose of 3-MCPD was excreted in the urine as DHPMA in this human subject. After subtraction of the average excretion rate (13.7 µg h^{-1}) observed for the 3 days with exposure to 2-MCPD on day 2, the DHPMA excretion rates per sampling period (µg h^{-1}) distinctly increased after exposure to 3-MCPD, with an excretion rate roughly doubled after 8–12 h and normalized about 34 h after the exposure (**Figure 6**). The half-life was about 7 h. These results indicated for the first time that 3-MCPD is metabolized to DHPMA also in humans, however, at a low rate of about 1% only, not allowing its use as biomarker for the current "background" exposure to 3-MCPD fatty acid esters.

Finally, it is of note that DHPMA measured by UHPLC-MS/MS in urine samples of glycidol-treated rats was reported to be accompanied by a minor peak; the signal was assigned to the putative metabolite iso-DHPMA, the mercapturic acid resulting from glutathione conjugation at the 2-carbon of glycidol.[30] This peak was not detected in the urine samples of our participants several hours after eating the palm fat.

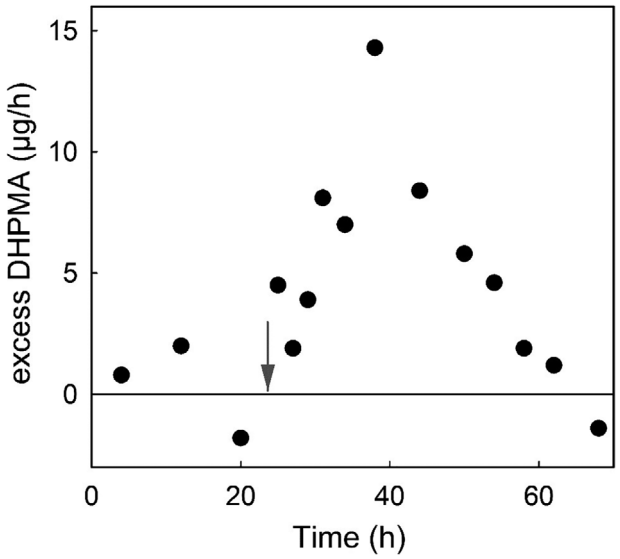

Figure 6. Excess of the DHPMA excretion rates over 3 days after exposure to 3-MCPD on day 2 (arrow). Excess rates are calculated by subtraction of the average excretion rate of 13.7 µg h^{-1} observed for the 3 days with exposure to 2-MCPD on day 2 (n = 1).

4.3. Conclusions

Urinary excretion of 2-MCPD and 3-MCPD was found to represent the external exposure of 2/3-MCPD and its fatty acid esters in a linear fashion (proof of principle). Therefore, 2-MCPD and 3-MCPD determined in 24-h urine apparently are reliable biomarkers for the assessment of the external exposure in populations and can, for example, be used to monitor the ongoing decrease of external exposure over time following industrial mitigation strategies for the reduction of 2- and 3-MCPD fatty acid esters.[31] For quantification of low "background" exposures to 2-MCPD and 3-MCPD, an improvement of the sensitivity of the analytical method applied would be important. In view of the inter- and intra-individual variability observed in our study, assessments of individual 2/3-MCPD exposures appear to be less reliable and represent single day exposure only.

In contrast, urinary DHPMA levels in humans do not reflect the external exposure to 3-MCPD or glycidol in the "background" range, as the relatively high and constant levels observed likely reflect endogenous formation rather than external exposure.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors would like to thank all participants, especially for their compliance regarding collecting urine samples complete and on time. Furthermore, the authors thank Corinna Genrich, Christel Rozycki, and Petra Zocher for their excellent technical assistance at the BfR. The authors

gratefully acknowledge the donation of [$^{13}C_2$]DHPMA by Prof. Dr. Göen at the University of Erlangen-Nürnberg and the support of Dr. Banneke, who provided the urine samples collected at BfR's experimental farm.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

The investigation was conceptualized by K.A. and B.H.M. K.A. was the medical head of the study and responsible for the application for ethical approval and for the information of the participants. J.H. (supervised by K.A.) was responsible for the study conduct (recruitment, organization, study meal on Wednesday, processing of urinary samples). The analytical work was performed by J.H. (DHPMA) and J.K. (2- and 3-MCPD), done in close collaboration with B.H.M. Results were evaluated and interpreted by K.A. and B.H.M. All authors contributed to the preparation of the manuscript.

Data Availability Statement

Data are available on request due to privacy/ethical restrictions.

Keywords

2/3-MCPD, biomarker of exposure, fatty acid esters, glycidol, urinary excretion

Received: July 24, 2020
Revised: October 8, 2020
Published online: January 25, 2021

[1] N. Bakhiya, K. Abraham, R. Gürtler, K. E. Appel, A. Lampen, *Mol. Nutr. Food Res.* **2011**, *55*, 509.
[2] E. Barocelli, A. Corradi, A. Mutti, P. G. Petronini, *EFSA Supporting Publ.* **2011**, *8*, 131.
[3] K. Abraham, K. E. Appel, E. Berger-Preiss, E. Apel, S. Gerling, H. Mielke, O. Creutzenberg, A. Lampen, *Arch. Toxicol.* **2013**, *87*, 649.
[4] K. E. Appel, K. Abraham, E. Berger-Preiss, T. Hansen, E. Apel, S. Schuchardt, C. Vogt, N. Bakhiya, O. Creutzenberg, A. Lampen, *Arch. Toxicol.* **2013**, *87*, 1649.
[5] K. Wakabayashi, Y. Kurata, T. Harada, Y. Tamaki, N. Nishiyama, T. Kasamatsu, *J. Toxicol. Sci.* **2012**, *37*, 691.
[6] W. S. Cho, B. S. Han, K. T. Nam, K. Park, M. Choi, S. H. Kim, J. Jeong, D. D. Jang, *Food Chem. Toxicol.* **2008**, *46*, 3172.
[7] G. Sunahara, I. Perrin, M. Marchessini, *Carcinogenicity study on 3-monochloropropane-1,2-diol (3-MCPD) administered in drinking water to Fischer 344 rats. Report RE-SR93003 submitted to WHO*. 1993, Nestec Ltd., Research and Development: Lausanne (Switzerland).
[8] A. R. Jones, *Aust. J. Biol. Sci.* **1983**, *36*, 333.
[9] I. Perrin, M. Marchesini, G. Sunahara, *Repeated dose oral toxicity 28 day gavage in Sprague Dawley rats of 2-chloropropan-1,3 diol (2-MCPD). Unpublished report No. RE-SR94026.* 1994, Nestec Ltd, Research & Development: Lausanne (Switzerland).
[10] National Toxicology Program, *Natl. Toxicol. Program Tech. Rep. Ser.* **1990**, *374*, 1.
[11] EFSA Panel on Contaminants in the Food Chain, *EFSA J.* **2016**, *14*, 4426.
[12] IARC, *3-Monochloro-1,2-propanediol, in Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water*, International Agency for Research on Cancer, Lyon, France **2013**, p. 349.
[13] IARC (International Agency for Research on Cancer). *Some Industrial Chemicals. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, vol. 77. International Agency for Research on Cancer, Lyon, France **2000**. pp. 469–486.
[14] EFSA Panel on Contaminants in the Food Chain, *EFSA J* **2018**, *16*, 5083.
[15] K. Abraham, J. Hielscher, T. Kaufholz, H. Mielke, A. Lampen, B. Monien, *Arch. Toxicol.* **2019**, *93*, 331.
[16] C. Crews, P. Brereton, A. Davies, *Food Addit. Contam.* **2001**, *18*, 271.
[17] R. Inagaki, C. Hirai, Y. Shimamura, S. Masuda, *J. Food Process. Technol.* **2016**, *7*, 557.
[18] H. J. Clewell, Y. M. Tan, J. L. Campbell, M. E. Andersen, *Toxicol. Appl. Pharmacol.* **2008**, *231*, 122.
[19] R. Andreoli, M. Cirlini, A. Mutti, *Anal. Bioanal. Chem.* **2015**, *407*, 4823.
[20] E. Eckert, K. Schmid, B. Schaller, K. Hiddemann-Koca, H. Drexler, T. Goen, *Int. J. Hyg. Environ. Health* **2011**, *214*, 196.
[21] I. Rietjens, P. Dussort, H. Gunther, P. Hanlon, H. Honda, A. Mally, S. O'Hagan, G. Scholz, A. Seidel, J. Swenberg, J. Teeguarden, G. Eisenbrand, *Arch. Toxicol.* **2018**, *92*, 15.
[22] A. R. Jones, R. W. O'Brien, *Xenobiotica* **1980**, *10*, 365.
[23] N. Bergau, Z. Zhao, K. Abraham, B. H. Monien, *Mol. Nutr. Food Res.* **2021**.
[24] J. Kuhlmann, *Investigation into the presence of 3-MCPD esters and related compounds in foods*. 2017, German Federal Office for Agriculture and Food for the Federal Ministry of Food and Agriculture: Bonn (Germany).
[25] J. Kuhlmann, *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 382.
[26] L. E. Rodman, R. D. Ross, *J. Chromatogr. A* **1986**, *369*, 97.
[27] E. Eckert, H. Drexler, T. Goen, *J. Chromatogr. B* **2010**, *878*, 2506.
[28] K. A. John, M. E. Cogswell, N. R. Campbell, C. A. Nowson, B. Legetic, A. J. Hennis, S. M. Patel, *J. Clin. Hypertens* **2016**, *18*, 456.
[29] H. H. Landin, S. Osterman-Golkar, V. Zorcec, M. Törnqvist, *Anal. Biochem.* **1996**, *240*, 1.
[30] W. Jia, D. Wu, X. Chen, L. Mao, H. Miao, D. Chen, Y. Ren, Y. Zhang, *Talanta* **2019**, *204*, 329.
[31] S. B. Oey, H. J. van der Fels-Klerx, V. Fogliano, S. P. J. van Leeuwen, *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 349.