Food and Chemical Toxicology 106 (2017) 36–46

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Comparative analysis of transcriptomic responses to repeated-dose exposure to 2-MCPD and 3-MCPD in rat kidney, liver and testis

Thorsten Buhrke [1], Katharina Schultrich [1], Albert Braeuning*, Alfonso Lampen

German Federal Institute for Risk Assessment, Dept. Food Safety, Max-Dohrn-Str. 8-10, 10589, Berlin, Germany

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form
12 May 2017
Accepted 14 May 2017
Available online 15 May 2017

Keywords:
3-Chloro-1,2-propanediol
2-Chloro-1,3-propanediol
Toxicogenomics
Food contaminant
Oral exposure
Nephrotoxicity

ABSTRACT

3-Chloro-1,2-propanediol (3-MCPD) and its isomer 2-chloro-1,3-propanediol (2-MCPD) are heat-induced food contaminants present in oil- and fat-containing foodstuff. Kidney and testes are among the main target organs of 3-MCPD. Almost no data on 2-MCPD toxicity are available. Here, transcriptomic responses following repeated-dose exposure of rats to non-toxic doses of 10 mg/kg body weight per day 2-MCPD or 3-MCPD for 28 days were characterized by microarray analysis of kidney, liver, and testes. 3-MCPD exerted more pronounced effects than 2-MCPD in all organs. The limited overlap between the datasets indicates that 2-MCPD and 3-MCPD do not share the same molecular mechanisms of toxicity. By combining transcriptomic data with datasets on proteomic regulation by 3-MCPD, a comprehensive view on 3-MCPD-induced regulation of glucose utilization and oxidative stress response was developed. Bioinformatic analyses revealed that Nrf2 (nuclear factor (erythroid-derived 2)-like 2) signaling is likely to be involved in mediating the oxidative stress response to 3-MCPD. In summary, this study for the first time presents data on alterations in global gene expression by two important food contaminants, 2-MCPD and 3-MCPD. Data demonstrate profound differences between the effects of the two compounds and substantially broaden our knowledge on molecular details of 3-MCPD-induced disturbance of glucose utilization and redox balance.

1. Introduction

Fatty acid esters of 3-chloro-1,2-propanediol (3-monochloropropanediol, 3-MCPD) and of 2-chloro-1,3-propanediol (2-monochloropropanediol, 2-MCPD) are formed during the refinement of vegetable oils and fats and are therefore present in numerous oil- and fat-containing foods such as infant formula, bakery products, and cereals; see e.g EFSA (2016) or Bakhiya et al. (2011) for an overview of MCPD occurrence in foodstuff. After oral uptake, the esters are hydrolyzed in the gastrointestinal tract by the action of unspecific esterases, thereby liberating free 3-MCPD and 2-MCPD which are then easily resorbed by the body (Abraham et al., 2013; Buhrke et al., 2011, 2015; Kaze et al., 2016). Due to efficient 3-MCPD ester hydrolysis in the gastrointestinal tract, the toxicological profiles of 3-MCPD and 3-MCPD fatty acid esters share a high degree of similarity.

Animal studies have revealed that kidney and testes are primary target organs for 3-MCPD toxicity (EFSA, 2016; IARC, 2012; Lynch et al., 1998). 3-MCPD has been classified as a possible human carcinogen (category 2B) by the International Agency for Research on Cancer (IARC) based on a proposed non-genotoxic mechanism of action for carcinogenicity (Grosse et al., 2011). Slightly varying safety values for 3-MCPD have been derived by different regulatory bodies; e.g. a tolerable daily intake of 0.8 μg/kg body weight per day 3-MCPD has been proposed by the European Food Safety Agency (EFSA) recently (EFSA, 2016).

At the molecular level, the mechanisms by which 3-MCPD induces adverse effects in these organs are still under debate. Modes of action potentially implicated in 3-MCPD toxicity in these organs include an inhibition of glucose utilization (Jones and Porter, 1995; Mohri et al., 1975) as well as cellular damage induced by oxidative stress (Sawada et al., 2016; Skamarauskas et al., 2007; Steiner et al., 2013). The proteomic responses to subchronic exposure of rats to 3-MCPD and its dipalmitate have been extensively characterized for kidney, liver and testis (Braeuning et al., 2015; Sawada et al., 2015, 2016). Evaluation of the proteomic data was in line with the assumption that 3-MCPD treatment resulted in oxidative stress in kidney and testes, even if a role of Nrf2, a key transcription factor

* Corresponding author.
E-mail address: Albert.Braeuning@bfr.bund.de (A. Braeuning).
[1] These authors contributed equally and should both be considered first author.

http://dx.doi.org/10.1016/j.fct.2017.05.028
0278-6915/

for cellular defense against oxidative stress (Li and Kong, 2009), could not be concluded with certainty (Sawada et al., 2016). Moreover, proteins involved in glucose metabolism were affected by exposure to 3-MCPD in rat kidney and testes (Sawada et al., 2015, 2016).

While the toxicological properties of 3-MCPD are rather well characterized, toxicological data for 2-MCPD are very sparse, especially with regard to molecular mechanisms of toxicity. Data gaps yet preclude a conclusive risk assessment for 2-MCPD (Andres et al., 2013; EFSA, 2016). Unpublished histopathological data allowing for the identification of 2-MCPD target organs are mentioned in a recent report of the EFSA on 2-MCPD (EFSA, 2016). According to this paper, yet unpublished studies have detected effects of 2-MCPD in rats especially in striated muscle and kidney, while testicular damage has not been reported (EFSA, 2016). As the only available study reporting molecular details of 2-MCPD toxicity, a recent comparative proteomic analysis of alterations in the hearts of rats following subchronic oral exposure to 3-MCPD and 2-MCPD is available (Schultrich et al., 2017). The heart had been recently identified as another target organ of 3-MCPD and 2-MCPD toxicity (EFSA, 2016; Lee et al., 2015). The proteomic study revealed considerable differences between the proteomic responses to the two compounds in cardiac tissue, suggesting that 2-MCPD exerts its effects at least in parts via molecular mechanisms different to those affected by its structural isomer 3-MCPD (Schultrich et al., 2017).

To gain deeper insight into the molecular mechanisms of 3-MCPD and 2-MCPD toxicity, tissue samples from the organs kidney, liver, and testis samples from the proteomic study by Schultrich et al. (2017) were used for RNA extraction in order to perform genome-wide gene expression analyses in known target organs of 3-MCPD and 2-MCPD. The aim of the study was to characterize the global 2-MCPD- or 3-MCPD-induced transcriptomic alterations in relevant target organs following subchronic exposure, and to combine these new transcriptomic data with the information obtained from the previous proteomic projects (Braeuning et al., 2015; Sawada et al., 2015, 2016) in order to obtain a more comprehensive view on the 3-MCPD and 2-MCPD-induced molecular effects at the tissue level. Treatment with low doses of the two compounds was specifically chosen to allow for the detection of specific molecular effects of the toxins already observable at doses not exerting overt toxicity at the histopathological or clinical levels, because the use of tissue samples from heavily intoxicated animals might have resulted in the detection of unspecific signals of cell death and toxicity rather than in the identification of early, more specific changes related more directly to the molecular modes of action of the compounds.

2. Materials and methods

2.1. Chemicals

3 -Chloro-1,2-propanediol (3-MCPD) was purchased from Sigma Aldrich (Deisenhofen, Germany), and 2-chloro-1,3-propanediol (2-MCPD) was purchased from Toronto Research Chemicals (Ontario, Canada).

2.2. Animal experiment

The organ samples used in the present study were derived from a 28-days oral toxicity study with 3-MCPD and 2-MCPD (each dosed at 10 mg/kg body weight per day) in Wistar rats that was performed at the Fraunhofer Institute for Toxicology and Experimental Medicine (Hanover, Germany) following approval of the experimental protocol by an Ethics Commission for Animal Protection. The detailed study protocol has been published recently (Schultrich et al., 2017). Please note that the experiment is not identical to a previous animal study with 3-MCPD (Braeuning et al., 2015; Sawada et al., 2015, 2016), from which the successfully used dosing regimen with 10 mg/kg body weight per day was adopted. In order to allow for direct comparability, identical dosing for 2-MCPD was chosen.

2.3. Histopathology

Histopathological analyses were carried out on hematoxylin/eosin-stained slices according to standard methods. For more details please refer to Sawada et al. (2016).

2.4. RNA extraction and RNA integrity

From each experimental group, five animals were randomly selected for RNA extraction. Total RNA was extracted from approximately 50-60 mg frozen rat tissue sample by using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's recommendations. The concentration of RNA was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and RNA integrity was assessed by using the Agilent RNA 6000 Nano LabChip kit together with the Agilent 2100 Bioanalyzer according to the manufacturer's protocol (Agilent Technologies, Palo Alto, CA, USA). All samples for the microarray analysis had a RNA Integrity Number (RIN), a measure of degradation, of 8 or higher (RIN; 1 = totally degraded, 10 = intact).

2.5. Affymetrix gene array analysis and bioinformatic evaluation of data

The microarray experiment was conducted by Eurofins Genomics GmbH (Ebersberg, Germany) by using Affymetrix GeneChip Rat Transcriptome 1.0 arrays (Affymetrix, Santa Clara, CA, USA). 100 ng of total RNA was used for labeling and hybridization according to the GeneChip Whole Transcript (WT) Sense Target Labeling Assay Manual (Affymetrix) by using all materials and reagents for target labeling, cDNA synthesis and amplification, cDNA cleanup, fragmentation and terminal labeling, hybridization, stain and wash as provided by Affymetrix and as described in the assay manual. Samples obtained from the five animals of each treatment group represented independent biological replicates. Data were normalized by GeneChip Robust Multiarray Averaging (GC-RMA). A |fold change| of >1.5 and a p-value <0.01 (unpaired One-Way Between Subject ANOVA) were chosen as cut-off criteria for further analysis (see 3.2). For more details please refer to the Transcriptome Analysis Console (TAC) 2.0 software (Affymetrix) user guide.

Lists of differentially expressed genes between treatment groups compared to the vehicle control group were generated using the TAC 2.0 software. The resulting datasets of differentially expressed genes were further processed by using the software Ingenuity Pathway Analysis (IPA; spring release March 2017; Qiagen) which eliminated all unmapped probe sets and which furthermore only allowed the probe set with the highest fold change for further analysis in the case that a single gene was represented by more than one significantly regulated probe set on the microarray. The mapped data set was furthermore evaluated by the IPA-based “Expression Analysis” tool whereby only experimentally observed relationships were considered. No further filters such as organs- or organism-specific criteria were applied. An additional gene ontology analysis was performed using the “Protein Analysis Through Evolutionary Relationship” (PANTHER) classification system (Version 11.1). This comprehensive tool allows the