Results of the BfR MEAL Study: In Germany, mercury is mostly contained in fish and seafood while cadmium, lead, and nickel are present in a broad spectrum of foods

Carolin Fechner a,*, Christin Hackethal a, b, Tobias Höpfner a, Jessica Dietrich a, Dorit Bloch a, Oliver Lindtner a, Irmela Sarvan a

a German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Straße 8-10, 10589 Berlin, Germany
b Institute of Nutritional Science (IEW), University of Potsdam, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

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Abstract

The BfR MEAL Study provides representative levels of substances in foods consumed in Germany. Mercury, cadmium, lead, and nickel are contaminants present in foods introduced by environmental and industrial processes. Levels of these elements were investigated in 356 foods. Foods were purchased representatively, prepared as consumed and pooled with similar foods before analysis. Highest mean levels of mercury were determined in fish and seafood, while high levels of cadmium, lead, and nickel were present in cocoa products and legumes, nuts, oilseeds, and spices. The sampling by region, season, and production type showed minor differences in element levels for specific foods, however no tendency over all foods or for some food groups was apparent. The data on mercury, cadmium, lead, and nickel provide a comprehensive basis for chronic dietary exposure assessment of the population in Germany. All levels found were below regulated maximum levels.

1. Introduction

Mercury (Hg), cadmium (Cd), lead (Pb), and nickel (Ni) are contaminants occurring in foods. In the BfR MEAL Study the first data sets are now available and should be shared with the scientific community within this publication. For foods, the EU established maximum levels for mercury (Hg), cadmium (Cd), and lead (Pb) to protect consumers from adverse health effects (EC, 2006). In contrast, currently no maximum levels in the EU legislation or at the Codex Alimentarius level for Ni in food are present, while regulatory limits have been established for drinking water (EC, 1998, EC, 2003).

Hg is released from natural and anthropogenic sources and undergoes transformations between atmosphere, ocean and land (Carocci et al., 2016). The organic compound methylmercury (MeHg) is the predominant form in fish and seafood while elemental and inorganic Hg (iHg) prevail in terrestrial foods (EFSA & CONTAM, 2012; Sarvan, Kolbaum, Pabel, Buhrke, Greiner, & Lindtner, 2021). Ingestion of foods represents one of the major pathways of human Hg exposure. Toxicity for humans varies with the chemical compound of Hg, the dose, and the route of exposure (Bernhoft, 2012). Absorption of iHg salts in the gastrointestinal tract is low, in comparison MeHg species are absorbed to a greater extent in the human body (EFSA & CONTAM, 2012). MeHg exposure in humans causes neurological adverse effects as well as adverse effects on the renal, cardiovascular, reproductive, and immune system (Carocci et al., 2016). The exposure to iHg induces inflammatory reactions in the kidneys and gastrointestinal tract and affects cardiovascular function (Fernandes Azevedo et al., 2012). Additionally, iHg accumulates in the human breast and is secreted in breast milk, which can affect the development of the central nervous, pulmonary and renal system in infants (Counter & Buchanan, 2004).

Cd enters the environment through volcanic emissions, rock weathering and through industrial and agricultural processes such as metallurgy, fossil fuel and waste incineration, and the use of phosphate- and sewage sludge-containing fertilizers (EFSA et al., 2009; IARC & WHO, 2012). High Cd levels are reported in cocoa products, offal, edible mushrooms, oilseeds, crustaceans, algal formulations, seaweed, and shellfish and besides food, cigarette smoke is a source of human Cd.

* Corresponding author.

E-mail addresses: Carolin.Fechner@bfr.bund.de (C. Fechner), Christin.Hackethal@bfr.bund.de (C. Hackethal), Tobias.Hoepfner@bfr.bund.de (T. Hoepfner), Jessica.Dietrich@bfr.bund.de (J. Dietrich), Dorit.Bloch@bfr.bund.de (D. Bloch), Oliver.Lindtner@bfr.bund.de (O. Lindtner), Irmela.Sarvan@bfr.bund.de (I. Sarvan).

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2. Material and methods

2.1. Selection of foods for the food list

The design of the BfR MEAL study was described elsewhere (Sarvan et al., 2017) and followed international recommendations for TDSs (EFSA/FAO/WHO, 2011). Representative consumption data for the population in Germany were used from the National Nutrition Survey II (NVS II; n = 13,926; two 24 h recalls) for female and male participants aged between 14 and 80 years including pregnant woman (Krems et al., 2006). Additionally, a consumption survey for children between 0.5 and six years (VELS, n = 804; food records of three days on two occasions) (Banasiaik, Heseker, Sieke, Sommerfeld, & Vohmann, 2005) was used to establish the MEAL food list. The MEAL food list includes 356 MEAL foods, which were assigned to 19 main food groups according to FoodEx 2 classification. In each of the 19 main food groups, 90 % of the German diet were covered for different age groups and genders (Sarvan et al., 2017). Additionally, rarely consumed foods (<10 %) containing potentially high amounts of substances of interest were included in the MEAL food list.

2.2. Sampling and purchasing of foods

Purchasing and further steps were performed between 2017 and 2019 as described previously (Hackethal, Kopp, Sarvan, Schwerdtle, & Lindtner, 2021; Sarvan et al., 2021). The 209 MEAL foods that were not expected to vary in composition based on region, season, or production type were sampled only in the area of Berlin (see Table S3 to S6, type N). These foods were sampled throughout the year (not in specific seasons) and the pooled samples of these foods could be composed of both organic and conventional subsamples according to market share data. Foods expected to vary in composition based on region, season, or production type (147 MEAL foods) were sampled accordingly. Seventy foods were tested in four regions, 58 foods were tested in two seasons and 105 foods were tested to assess differences between organic and conventional production (see Table S3 to S6, type R, S or P). Subsamples were purchased based on weighted market share data on shopping places (e.g. supermarket, discount store, weekly market), brands, varieties (e.g. type of fruit, flavour) or geographical origins.

For each MEAL food sampled nationally without differentiation, 20 individual food items (subsamples) were collected (EFSA/FAO/WHO, 2011) as ingredients for recipes or ready-to-eat products in Berlin. Pooled samples of MEAL foods sampled by region, season, or production type were composed of at least 15 subsamples (EFSA/FAO/WHO, 2011). Regionally sampled foods were purchased as ingredients for recipes or ready-to-eat products in four different regions of Germany (east, south, west, north). Seasonally sampled foods were purchased at two different times of the year specific for each food. Foods sampled by production type do not only depict differences caused by the production method, but also differences due to the availability in the organic and conventional market segments. Especially industrially processed organic foods typically available on the market could have a different composition than their conventional counterparts. Three pooled samples (edible offal (pork, beef), corn oil/maize-germ oil, boletus/porcini mushroom) are composed of less subsamples due to market availability.

2.3. Preparation of foods

After sampling, MEAL foods were prepared in the study kitchen according to typically used recipes. For ingredients constituting <5 % (w/w) of a recipe (e.g. salt, oil), the top brand according to market share data was purchased nationally regardless of region, season, and production type. Ingredients constituting more than 5 % (w/w) of a recipe were purchased nationally or by region, season or production type according to the sampling plan. Food composition and substance levels depend on preparation and consumption behavior. Market share data and consumer surveys were used to include different consumer behavior in the German population into in pooled samples.

For the preparation of foods, drinking water from the BfR MEAL Study kitchen was used. It was sampled according to a protocol four times after the water was running for 30 min. This sampling was...
repeated once. Additionally, drinking water from 29 different sampling points in Germany was sampled (single samples, analysed individually, not pooled). For preparation of foods, kitchen utensils made of different materials were used to cover a possible influence on the substance levels resulting from typical materials used in Germany.

2.4. Pooling and homogenisation of samples

Pooling and homogenisation was realised using a knife mill (Grindomix GM300, Retsch GmbH, Haan, Germany). For pooled samples which should be analysed for Hg, Cd, Pb, or Ni a polypropylene container and titanium knives were used instead of stainless steel materials to avoid transition of Ni from stainless steel during homogenisation. Where required, ultrapure water (Milli-Q Integral 5, Merck, Darmstadt, Germany) or liquid nitrogen was added to achieve complete homogenisation. Samples were stored in polypropylene vessels at −20 °C until analysis. Due to multisampling, in total, 869 pooled samples were produced for the analysis of Hg, Cd, Pb, and Ni.

2.5. Analysis of Hg, Cd, Pb, and Ni

Analysis of Hg in foods was performed by an accredited contract laboratory using a direct mercury analyzer as described elsewhere (Sarvan et al., 2021), more details are presented in the supplement text S1. The determination of Cd, Pb, and Ni in food samples was conducted by an accredited contract laboratory using inductively coupled plasma mass spectrometry (ICP-MS) as described elsewhere (Hackethal et al., 2021). More details are presented in the supplement Text S1.

2.6. Statistical methods

Results below the limit of detection (LOD) or the limit of quantification (LOQ) were substituted as described by FAO and WHO (FAO & WHO, 2009), to include not quantifiable results in the considerations. For calculating the modified lower bound (mLB), results below LOD were set to zero and results below LOQ were substituted by the LOD. For upper bound (UB) calculations, results below LOD were replaced by LOD and results below LOQ and above LOD were substituted by LOQ. Because mLB and UB did not differ substantially in the present study, results were presented as UB. mlB results are reported in the Tables S3 to S6.

For each pooled sample, substance levels resulting from the analysis were arithmetically averaged to obtain an MLB result and an UB result. For each MEAL food, substance levels for one to ten pooled samples were available to calculate mean levels, standard deviation (SD), median, minimum (min), and maximum (max) as statistical parameters using Microsoft Excel 2016. To obtain mean levels of main food groups, firstly, all pooled samples of each food were arithmetically averaged and secondly, the mean for each main food group was calculated based on MEAL foods. SD determined on main food group level reflect the variability between different MEAL foods in the corresponding group whereas they do not reflect the variation between subsamples or between pooled samples, as subsamples are aggregated to a pooled sample during homogenization.

Further statistical analysis was carried out using IBM SPSS Statistics version 26. Differences between substance levels for all foods sampled in different regions and for all foods sampled by production type were investigated. Differences for seasons were not evaluated, as season 1 and season 2 vary between foods and include different months of the year. Averaged results for each MEAL food per region or production type were used in the test. The Kolmogorov-Smirnov test showed no normal distribution of substance levels for all foods per region or production type. Hence, differences were tested using the Kruskal-Wallis test for the four different regions and the Mann-Whitney-U test for the production type with a significance level of 0.05. As the statistical tests only reveal differences across all MEAL foods sampled by region or production type, possible differences in levels between individual MEAL foods were characterised descriptively.

3. Results and discussion

3.1. Highest levels of Hg, Cd, Pb, and Ni

3.1.1. Mercury

Hg levels in the pooled samples of the present study did not exceed maximum levels established for specific foods (EC, 2006). The main food group with the highest UB Hg levels was fish, seafood, and invertebrates ranging between 0.002 and 0.670 mg/kg (min-max) followed by vegetables and vegetable products but with considerably lower UB Hg levels in this group ranging between 0.001 and 0.623 mg/kg (mix-max) (Table 1). This relation was similarly reflected in the 15 MEAL foods with the highest UB levels, as 14 of these MEAL foods belonged to the main food group fish, seafood, and invertebrates, while one MEAL food belonged to vegetables and vegetable products (Fig. 1A). The MEAL food with the highest measured Hg level was tuna smoked with 0.670 mg/kg followed by various fish species with decreasing levels (Fig. 1A). However, boletus/porcini mushroom as one proxy of the main food group vegetables and vegetable products contained 0.623 mg/kg and was the MEAL food with the second highest UB mean Hg level measured (Fig. 1A). UB Hg levels of MEAL foods other than fish and boletus/porcini mushroom were mostly close to or below the respective LOQ. Exceptions were sushi (UB mean 0.010 mg/kg), containing fish as an Hg source and vegetarian sausage (UB mean 0.018 mg/kg) (Table S3, Figure S7).

Previously published results of the BfR MEAL Study showed MeHg as the predominant form of Hg in fish and seafood, contributing mostly to high total Hg levels (Sarvan et al., 2021). Similar total Hg levels as presented in the current study were found by the German Food Monitoring (GFM) in unprepared foods in the past (BVL, 2005-2018). High Hg levels were detected in fish and boletus/porcini mushrooms in the present study (Fig. 1A). Results of the GFM confirm high levels for tuna filet (n: 60; year: 2006; mean: 0.237 mg/kg), tuna canned in own juice (n: 74; year: 2012; mean: 0.141 mg/kg) as well as plaice (n: 143; year 2013: mean: 0.050 mg/kg) or a mix of fresh wild mushrooms containing bay bolete, boletus/porcini mushroom, and Chanterelle mushroom (n: 76; year 2016; mean: 0.088 mg/kg) (BVL, 2005-2018). In contrast, 7-fold higher UB mean Hg levels are reported for prepared boletus/porcini mushrooms in the present study (0.623 mg/kg), compared to fresh wild mushrooms in the GFM (0.088 mg/kg) (BVL, 2005-2018). This difference might be caused by water loss during food preparation and by a varying composition of the samples as well as sampling year and location. In a Polish study, however, marketed dry boletus/porcini mushroom pieces contained a five-fold higher Hg mean level of 3.039 mg/kg (Orywal et al., 2021) than in the present study. Processing and drying of mushrooms influences the water content and the contaminant levels. A water content of 85.9 g/100 g for prepared boletus/porcini mushrooms was reported by Hartmann et al. (Hartmann, Schmidt, & Sandfuchs, 2014), while the water content in dried boletus/porcini mushrooms was given with 1.4 g/100 g. In comparison, the USDA (2021) indicated a water content for cooked mushrooms of 90.6 g/100 g and for fried mushrooms of 59.9 g/100 g (USDA, 2021). Considering processing factors, the results presented by the GFM and Orywal et al. support the results of the present study (BVL, 2005-2018, Orywal et al., 2021).

In the 2nd French TDS, highest UB mean Hg levels were found in fish (0.134 mg/kg), followed by crustaceans and molluscs (0.016 mg/kg), and chocolate (0.017 mg/kg) (Arnich et al., 2012). Fish was investigated as an aggregated food in the French study while different fish species were examined in the BfR MEAL Study (Fig. 1A). The observed Hg level for fish in the French TDS was higher than for the main food group fish, seafood, and invertebrates (0.100 mg/kg) (Table 1) but in the range of the fish species with highest UB mean Hg levels (0.670 – 0.060 mg/kg)
Table 1
Estimated UB Hg levels in MEAL foods, summarised by main food groups [mg/kg].

| Main food group                          | Pooled samples | MEAL foods | Pooled samples < LOD/LOQ | Mean | SD | Median | Min | Max |
|------------------------------------------|----------------|------------|--------------------------|------|----|--------|-----|-----|
| Grains and grain-based products          | 97             | 40         | 59                       | 0.003| 0.001| 0.003  | 0.001 | 0.006|
| Vegetables and vegetable products        | 152            | 34         | 47                       | 0.020| 0.015| 0.002  | 0.001 | 0.623|
| Starchy roots or tubers and products thereof | 26           | 8          | 65                       | 0.002| 0.001| 0.002  | 0.001 | 0.004|
| Legumes, nuts, oilseeds and spices      | 24             | 20         | 58                       | 0.003| 0.001| 0.003  | 0.002 | 0.007|
| Fruit and fruit products                | 64             | 22         | 63                       | 0.002| 0.001| 0.002  | 0.001 | 0.004|
| Meat and meat products                  | 101            | 35         | 62                       | 0.002| 0.001| 0.002  | 0.001 | 0.007|
| Fish, seafood and invertebrates         | 39             | 30         | 0                        | 0.100| 0.151| 0.045  | 0.002 | 0.670|
| Milk and dairy products                 | 37             | 23         | 62                       | 0.002| 0.000| 0.002  | 0.001 | 0.002|
| Eggs and egg products                   | 10             | 2          | 90                       | 0.001| 0.000| 0.001  | 0.001 | 0.001|
| Sugar, confectionery and water-based sweet desserts | 18         | 15         | 89                       | 0.003| 0.001| 0.003  | 0.002 | 0.006|
| Animal and vegetable fats and oils      | 13             | 8          | 100                      | 0.003| 0.000| 0.003  | 0.003 | 0.003|
| Fruit and vegetable juices and nectars  | 12             | 10         | 17                       | 0.002| 0.000| 0.002  | 0.002 | 0.002|
| Water and water-based beverages         | 41             | 6          | 22                       | 0.001| 0.001| 0.002  | 0.002 | 0.002|
| Coffee, cocoa, tea and infusions        | 12             | 9          | 42                       | 0.002| 0.001| 0.002  | 0.001 | 0.003|
| Alcoholic beverages                     | 11             | 8          | 27                       | 0.002| 0.000| 0.002  | 0.001 | 0.002|
| Food products for infants and toddlers   | 15             | 11         | 40                       | 0.002| 0.001| 0.002  | 0.001 | 0.003|
| Products for non-standard diets and food imitates | 8         | 7          | 13                       | 0.004| 0.006| 0.002  | 0.002 | 0.018|
| Composite dishes                        | 170            | 52         | 85                       | 0.002| 0.002| 0.001  | 0.001 | 0.014|
| Seasoning, sauces and condiments        | 19             | 16         | 74                       | 0.001| 0.001| 0.002  | 0.002 | 0.003|

Left-censored data were analysed using the upper bound (UB) approach, i.e. results below LOD were replaced by the value reported as LOD and results above LOQ by the value reported as LOQ. Different LOQs applied, i.e. 0.002 mg/kg for moist foods, 0.005 mg/kg for dry foods and 0.00005 – 0.0001 mg/kg for drinking water. The percentage of left-censored data for Hg amounts to 90 % (804 pooled samples) in the mLB and 57 % (508 pooled samples) in the UB.

Fig. 1. Highest mean levels [mg/kg] of A) Hg, B) Cd, C) Pb, and D) Ni in 15 out of 356 MEAL foods calculated using the upper bound approach (UB). A total of 869 pooled samples were aggregated to 356 MEAL foods for each substance. To include non-quantifiable results in the considerations the UB was applied and results below LOD were replaced by LOQ. Levels of all pooled samples of the 15 MEAL foods were above LOQ i.e. mLB und UB mean levels are identical. Preparation: MEAL foods are ready prepared for consumption (e.g. addition of liquid and / or further ingredients, heat treatment applied) if not indicated differently here. Deviations from full preparation: Specific MEAL foods contain a mixture of un-prepared and prepared subsamples ready for consumption according to consumer habits, i.e. hazelnuts, nuts, pecans, cocoa powder, cocoa beverage-preparation, millet, spices.
The percentage of left-censored data for Cd amounts to 31% (278 pooled samples) in the mLB and 19% (171 pooled samples) in the UB. The UB mean Hg levels in pooled samples of milk chocolate and dark chocolate were four times lower in the present study (for both 0.004 mg/kg and from 0.002 to 0.113 mg/kg for Pb (min-max) and legumes, nuts, oilsseeds, and spices (ranging from 0.001 to 0.265 mg/kg for Cd and 0.003 to 0.155 mg/kg for Pb (min-max)) and vegetables and vegetable products (ranging from 0.001 to 0.320 mg/kg for Cd and 0.001 to 0.128 mg/kg for Pb (min-max) (Table 2 and 3)).

### 3.1.2. Cadmium and lead

Cd and Pb levels in the pooled samples of the present study did not exceed maximum levels established for specific foods (EC, 2006). The highest UB levels of Cd and Pb were detected in the main food groups coffee, cocoa, tea, and infusions (ranging from 0 to 0.270 mg/kg for Cd and from 0.002 to 0.113 mg/kg for Pb (min-max) and legumes, nuts, oilsseeds, and spices (ranging from 0.001 to 0.265 mg/kg for Cd and 0.003 to 0.155 mg/kg for Pb (min-max)) and vegetables and vegetable products (ranging from 0.001 to 0.320 mg/kg for Cd and 0.001 to 0.128 mg/kg for Pb (min-max) (Table 2 and 3)). The levels in the main food group coffee, cocoa, tea, and infusions are mostly influenced by cocoa powder and cocoa beverage-preparation, as these MEAL foods were analysed unprepared (Table S4 and S5). In contrast, relatively low UB Cd and Pb levels were detected in the main food groups water and water-based beverages, eggs and egg products, milk and dairy products, or fruit and vegetable juices and nectars ranging mostly close to or below the respective LOQ.

For Cd and Pb, six of the same MEAL foods were found among the 15 foods with the highest UB mean levels: boletus/porcini mushroom (0.320 mg/kg and 0.128 mg/kg, respectively), cocoa powder (0.270 mg/kg and 0.113 mg/kg), dark chocolate (0.110 mg/kg and 0.030 mg/kg), mussels (0.084 mg/kg and 0.115 mg/kg), algae (0.120 mg/kg and 0.055 mg/kg) as well as, vegetable crisps (0.075 mg/kg and 0.032 mg/kg) (Fig. 1B and C).

In the GFM, 76 samples of a mix of fresh wild mushrooms containing bay bolete, boletus/porcini mushroom, and Chanterelle mushroom were investigated in 2016. Lower mean levels for Cd (0.046 mg/kg) and Pb (0.026 mg/kg) were detected in the mushroom mix (BVL, 2005-2018).

### Table 2

**Estimated UB Cd levels in MEAL foods, summarised by main food groups [mg/kg].**

| Main food group                      | Pooled samples (n) | MEAL foods (n) | Pooled samples < LOQ/LOQ (%) | Mean    | SD    | Median | Min | Max |
|-------------------------------------|--------------------|----------------|------------------------------|---------|-------|--------|-----|-----|
| Grains and grain-based products     | 97                 | 40             |                              | 0.018   | 0.013 | 0.014  | 0.002 | 0.066 |
| Vegetables and vegetable products   | 152                | 34             |                              | 0.023   | 0.058 | 0.004  | 0.001 | 0.320 |
| Starchy roots or tubers and products thereof | 26          | 8              |                              | 0.022   | 0.020 | 0.013  | 0.007 | 0.072 |
| Legumes, nuts, oilsseeds and spices| 24                 | 20             | 13                           | 0.033   | 0.066 | 0.008  | 0.001 | 0.265 |
| Fruit and fruit products            | 64                 | 22             | 45                           | 0.002   | 0.002 | 0.001  | 0.000 | 0.011 |
| Meat and meat products              | 101                | 35             | 5                            | 0.010   | 0.021 | 0.001  | 0.000 | 0.113 |
| Fish, seafood and invertebrates      | 39                 | 30             | 15                           | 0.017   | 0.039 | 0.004  | 0.000 | 0.205 |
| Milk and dairy products             | 37                 | 23             | 57                           | 0.001   | 0.002 | 0.001  | 0.000 | 0.007 |
| Eggs and egg products               | 10                 | 2              | 100                          | 0.000   | 0.000 | 0.000  | 0.000 | 0.000 |
| Sugar, confectionery and water-based sweet desserts | 18 | 15 | 33 | 0.018 | 0.027 | 0.008 | 0.002 | 0.110 |
| Animal and vegetable fats and oils   | 13                 | 8              | 100                          | 0.002   | 0.000 | 0.000  | 0.002 | 0.002 |
| Fruit and vegetable juices and nectars | 12            | 10             | 67                           | 0.001   | 0.001 | 0.000  | 0.000 | 0.002 |
| Water and water-based beverages     | 41                 | 6              | 93                           | 0.008   | 0.000 | 0.000  | 0.000 | 0.000 |
| Coffee, cocoa, tea and infusions    | 12                 | 9              | 50                           | 0.034   | 0.084 | 0.001  | 0.000 | 0.270 |
| Alcoholic beverages                 | 11                 | 8              | 100                          | 0.001   | 0.001 | 0.000  | 0.000 | 0.002 |
| Food products for infants and toddlers | 15             | 11             | 20                           | 0.008   | 0.006 | 0.008  | 0.000 | 0.021 |
| Products for non-standard diets and food imitates | 8          | 7              | 0                            | 0.011   | 0.008 | 0.010  | 0.001 | 0.026 |
| Composite dishes                    | 170                | 52             | 0                            | 0.010   | 0.008 | 0.008  | 0.001 | 0.050 |
| Seasoning, sauces and condiments    | 19                 | 16             | 11                           | 0.007   | 0.008 | 0.004  | 0.000 | 0.033 |

Left-censored data were analysed using the upper bound (UB) approach, i.e. results below LOD were replaced by the value reported as LOD and results below LOQ and above LOD by the value reported as LOQ. Different LOQs applied, i.e. 0.001 mg/kg for moist foods, 0.005 mg/kg for dry foods and 0.0002 mg/kg for drinking water. The percentage of left-censored data for Cd amounts to 31% (278 pooled samples) in the mLB and 19% (171 pooled samples) in the UB.
Pb levels (0.113 mg/kg) compared to mussels of the present study (0.084 mg/kg and 0.115 mg/kg, respectively) (Fig. 1B and C). The composition of crustaceans and molluscs in the French study and mus- 
s in the BfR MEAL Study were not directly comparable and levels may vary due to the composition of the pooled samples. Contrarily, the Catalan TDS found slightly higher mean Cd (0.220 mg/kg) and Pb levels (0.171 mg/kg) in mussels (Gonzalez et al., 2019) compared to the pre-
 sent study. The different composition of the pooled samples in the two studies could lead to this deviation.

High Cd levels in vegetable crisps and high Pb levels in vegetable and potato crisps might be caused by the reduction of water during the manufacturing process. The water content of potato crisps decreases depending on the frying time (Pedreschi, Hernando, Moyano, 2005). Most pooled samples of potato crisps contained mostly manufacturing process. The water content of potato crisps decreases potato crisps might be caused by the reduction of water during the study which might be caused by a different composition of snacks (Chen, Lam, Chung, Ho, & Moyano, 2005). The composition of crustaceans and molluscs in the French study and mus- 
s in the BfR MEAL Study were not directly comparable and levels may vary due to the composition of the pooled samples. Contrarily, the Catalan TDS found slightly higher mean Cd (0.220 mg/kg) and Pb levels (0.171 mg/kg) in mussels (Gonzalez et al., 2019) compared to the pre-
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High Cd levels in vegetable crisps and high Pb levels in vegetable and potato crisps might be caused by the reduction of water during the manufacturing process. The water content of potato crisps decreases depending on the frying time (Pedreschi, Hernández, Figueroa, & Moyano, 2005). Most pooled samples of potato crisps contained mostly more Cd and Pb than pooled samples of potato products with higher water content e.g. potatoes peeled/unpeeled (Table S4 and S5). Vegetable
crisps consisted of vegetables such as parsnip, beetroot, carrot, and sweet potato, which can also contribute to the Cd and Pb level of the pooled sample. The Hong Kong TDS investigated snack foods as aggregated food group and found a higher mean Cd level (0.120 mg/kg) and a lower mean Pb level (0.007 mg/kg) compared to crisps in the present study which might be caused by a different composition of snacks (Chen, Chan, Lam, Chung, Ho, & Xiao, 2014).

Moreover, high Cd levels were found in sunflower seeds (0.265 mg/kg) and linseeds (0.185 mg/kg) (Fig. 1B), contributing to the high mean level of the main food group legumes, nuts, oils, and spices in the present study (Table 2). This complies with the high mean levels of Cd in products containing other oilsides, such as poppy seed cake and pastry (0.066 mg/kg) (Fig. 1B). In the GFM, similar Cd levels were found in sunflower seeds in 2015 (n: 109; mean: 0.212 mg/kg) and linseeds in 2005 (n: 62; mean: 0.233 mg/kg) (BVL, 2005-2018). In the 2nd French TDS, a lower mean Cd level of 0.014 mg/kg in beef liver (Spungen, 2019) complies with that of the pooled sample spices in the present study.

In the BFR MEAL Study, specific edible offal products showed high UB mean Pb levels, i.e. 0.080 mg/kg for sheep liver and 0.022 mg/kg for bovine liver. Both foods are the main contributors to the UB mean Pb level in the group meat and meat products (0.008 mg/kg). The GFM found Pb levels in sheep liver (n: 94; year: 2016; mean: 0.078 mg/kg) and bovine liver (n: 59; year: 2006; mean: 0.035 mg/kg) (BVL, 2005-2018) comparable to the present study. In the 2nd French TDS, the Pb level in offal was 0.020 mg/kg (Arnick et al., 2012) and comparable to the levels of the MEAL bovine liver. Additionally, the high mean Pb level of 0.014 mg/kg in beef liver (Spungen, 2019) complies with that of bovine liver in the present study.

Recently, the dietary exposure to Cd and Pb through the consumption of selected foods was calculated using data from the BFR MEAL Study. The high consumption of grain based products contributed mostly to the dietary Cd and Pb exposure in adults (Ptok, Berg, Hackethal, Pabel, Lindtner, & Greiner, 2020).

### 3.1.3. Nickel

Highest levels of Ni were determined in the main food group le-
gumes, nuts, oilsides, and spices (ranging from 0.064 to 5.350 mg/kg (min–max)) followed by coffee, cocoa, tea, and infusions (ranging from 0.013 to 11.050 mg/kg (min–max)). In contrast, low UB Ni levels were observed for the main food groups alcoholic beverages, water and water-based beverages, and eggs and egg products (Table 4). The 15 MEAL foods with the highest UB mean Ni levels are nuts and oilsides (5.350 mg/kg in cashew nuts followed by decreasing levels to linseeds with 1.300 mg/kg) as well as cocoa powder (11.050 mg/kg) and products containing cocoa (dark chocolate (2.800 mg/kg); cocoa beverage-preparation, instant (powder) (2.150 mg/kg); nut nougat cream, chocolate beverage (1.450 mg/kg)). (Fig. 1D). These findings were confirmed by results of the GFM, as Ni levels in cocoa powder (n: 87; year: 2012; mean: 10.249 mg/kg) and dark chocolate (n: 128; year: 2012; mean:
4.560 mg/kg) (BVL, 2005-2018) were in the range of the results of the present study. Additionally, results for different nuts and oilseeds available in the GFM were similar to the present study, e.g. walnuts (n: 50; year: 2015; mean: 2.978 mg/kg) and hazelnuts (n: 10; year: 2008; mean: 1.802 mg/kg) as well as sunflower seeds (n: 109; year: 2015; mean: 3.228 mg/kg) and linseeds (n: 56; year: 2018; mean: 1.220 mg/kg) compared to legumes, nuts, oilseeds, and spices (10.19 mg/kg vs. 1.562 mg/kg) (Arnich et al., 2012), compared to the present study. Additionally, results for different nuts and oilseeds in the food group dried fruits, nuts, and seeds (Arnich et al., 2012) mean: 1.802 mg/kg) as well as sunflower seeds (n: 109; year: 2015; mean: 3.228 mg/kg) and linseeds (n: 56; year: 2018; mean: 1.220 mg/kg) compared to the present study.

Table 4 Estimated UB Ni levels in MEAL foods, summarised by main food groups [mg/kg].

| Main food group | Pooled samples | MEAL foods | Pooled samples < LOD/LOQ |
|-----------------|----------------|------------|--------------------------|
|                 | (n)            | (n)        | (%)                      |
| Grains and grain-based products | 97            | 40         | 4                        |
| Vegetables and vegetable products | 152           | 34         | 2                        |
| Starchy roots or tubers and products thereof | 26            | 8          | 0                        |
| Legumes, nuts, oilseeds and spices | 24            | 20         | 0                        |
| Fruit and fruit products | 64            | 22         | 13                       |
| Meat and meat products | 101           | 35         | 4                        |
| Fish, seafood and invertebrates | 39            | 30         | 15                       |
| Milk and dairy products | 37            | 23         | 30                       |
| Eggs and egg products | 10            | 2          | 30                       |
| Sugar, confectionery and water-based sweet desserts | 18            | 15         | 11                       |
| Animal and vegetable fats and oils | 13            | 8          | 62                       |
| Fruit and vegetable juices and nectars | 12            | 10         | 0                        |
| Water and water-based beverages | 41            | 6          | 24                       |
| Coffee, cocoa, tea and infusions | 12            | 9          | 8                        |
| Alcoholic beverages | 11            | 8          | 45                       |
| Food products for infants and toddlers | 15            | 11         | 7                        |
| Products for non-standard diets and food imitates | 8             | 7          | 13                       |
| Composite dishes | 170           | 52         | 0                        |
| Seasoning, sauces and condiments | 19            | 16         | 11                       |

Left-censored data were analysed using the upper bound (UB) approach, i.e. results below LOD were replaced by the value reported as LOD and results below LOQ and above LOD by the value reported as LOQ. Different LOQs applied, i.e. 0.02 mg/kg for moist foods, 0.1 mg/kg for dry foods and 0.001 mg/kg for drinking water. The percentage of left-censored data for Ni amounts to 29 % (257 pooled samples) in the mLB and 8 % (69 pooled samples) in the UB.

3.2. Levels of Hg, Cd, Pb, and Ni in foods by sampling criteria

3.2.1. Regionality and seasonality

Comparing UB mean levels of Hg, Cd, Pb, and Ni across all 70 MEAL foods between the four regions, minor differences for specific MEAL foods can be observed per season, however, no tendency over all foods or for some food groups is apparent. No significant differences in Hg, Cd, Pb, and Ni levels were found across all foods sampled in four different regions (p > 0.05). Small differences between pooled samples of specific MEAL foods could be caused by the sampling design of the study. Due to a representative sampling per region, supplies might differ by brands, geographical origins or food composition. Accordingly, the sampling design led to pooled samples with different subgraphs for each region, reflecting the availability of foods in the regions.

For instance, minor regional differences could be shown for trout. The pooled sample trout of region west showed the lowest UB mean Hg level of 0.014 mg/kg, region east and north showed comparable UB mean Hg levels of 0.022 mg/kg and 0.024 mg/kg and region south had the highest UB mean level of 0.031 mg/kg (Table S3). In order to purchase trout representative for the population in Germany, 15 subsamples were sampled in each region. Products were sampled prepacked in supermarkets and discount stores (6 – 7 subsamples per region) and not prepacked in regional specialised fish shops or at shop counters in supermarkets (8 – 9 subsamples per region). The prepacked trout from all four regions (in total 25 subsamples) mostly originated from Germany (17 subsamples) followed by Italy (3 subsamples), the Netherlands (2 subsamples), and Spain (1 subsample). For the not prepacked trout from all four regions (in total 35 subsamples) the geographical origin was mostly not documented or unknown (33 subsamples) and 2 subsamples originated from Germany. The geographical origin of trout and farming conditions could have an influence on the Hg level, as e.g. farmed trout from Poland contained a higher mean Hg level of 0.055 mg/kg (Szlinder-Richter, Usydus, Malewa-Ciecierwiz, Polak-Juszczak, & Ruczynska, 2011) than trout of the present study. Based on the present data, no connection can be drawn between the sampled regions, varying Hg levels and existing supply chains. Reasons for these differences are connected to the sampling procedure, which was based on market data, i.e. i) some brands of prepacked trout were available throughout Germany with the same origin indications, ii) other brands of prepacked trout were purchased in a specific region only, and iii) not prepacked subsamples purchased in each region cannot be traced to their origin. Levels of Cd, Pb, and Ni in trout were relatively low and mostly close to or below the respective LOQ. Similar effects of minor differences are connected to the sampling procedure, which was based on market data, i.e. i) some brands of prepacked trout were available throughout Germany with the same origin indications, ii) other brands of prepacked trout were purchased in a specific region only, and iii) not prepacked subsamples purchased in each region cannot be traced to their origin. Levels of Cd, Pb, and Ni in trout were relatively low and mostly close to or below the respective LOQ. Similar effects of minor differences are connected to the sampling procedure, which was based on market data, i.e. i) some brands of prepacked trout were available throughout Germany with the same origin indications, ii) other brands of prepacked trout were purchased in a specific region only, and iii) not prepacked subsamples purchased in each region cannot be traced to their origin.
0.037 mg/kg), for UB mean Pb levels in sheep liver (season 1 0.123 mg/kg, season 2 0.037 mg/kg) or for UB mean Ni levels in courgette (season 1 0.040 mg/kg, season 2 0.116 mg/kg) (Table S4 to S6). Hg levels in seasonally sampled foods are mostly close to or below the respective LOQ and differences cannot be evaluated (Table S4). Differences in levels for a MEAL food sampled in two different seasons might be caused by the sampling design of the study. Different supplies (e.g. geographical origins) were sampled for each season as available. Exemplary, courgette showed differences in the geographical origin as in season 1, most of the 15 subsamples available in each of the four regions were from Spain and only one subsample in region west originated from Germany while in season 2, six to 15 subsamples originated from Germany. Different origins and production conditions could lead to lower UB mean Ni levels in courgette in season 1 compared to season 2. The sampling design led to pooled samples with different subsamples for each season, reflecting the availability of foods in the seasons.

3.2.2. Production type

Sampling of overall 105 MEAL foods by production type was proportioned to the respective market share data for conventional and organic production. The market data and the availability of products on the market might vary and should be depicted in the study. Recipe variations of industrial foods and different household recipes for composite dishes were covered in the sampling and influenced the final composition of pooled samples. Comparing UB mean levels between organic and conventionally pool samples, minor differences for specific MEAL foods can be observed per substance, however, no tendency over all foods or for some food groups is apparent. No significant differences of Hg, Cd, Pb, and Ni levels were found between all organically and conventionally sampled foods (p < 0.05). Small differences between pooled samples could be caused by the sampling design of the study. For instance in cereal cracker higher Ni and Cd levels were observed in the organic pool sample (0.430 mg/kg and 0.023 mg/kg, respectively) in comparison to the conventional pooled sample (0.100 mg/kg and 0.002 mg/kg) (Table S4 and S6). Organic products sampled on the market often consisted of combinations of different grains (maize, rice, spelt, millet) and other ingredients (buckwheat, sesame seeds, amaranth, linseeds). Contrary, available conventional products were maize crackers only. As organic cereal crackers contained partly oilseeds and oilseeds showed higher levels in Ni and Cd, also higher levels in organically produced cereal crackers are comprehensible compared to conventional cereal crackers (Fig. 1B, D). Even if pooled samples of organically and conventionally production have a different composition, they reflect products typically available in the organic and the conventional market segment representatively and therefore indicate habits of consumers buying strictly organically or conventionally produced products. Hg and Pb levels were similar for organic and conventional pooled samples (Table S3 and S5). Differences in Ni and Cd levels are therefore not necessarily caused by the organic or conventional production but could be due to a different composition of products on the market.

In rice cracker, higher Ni, and slightly higher Cd, and Pb levels were observed in the organic pool sample (0.855 mg/kg, 0.048 mg/kg, and 0.008 mg/kg, respectively) in comparison to the conventional pooled sample (0.480 mg/kg, 0.038 mg/kg, and 0.006 mg/kg) (Table S4 to S6). Organic rice crackers included eight subsamples coated with milk chocolate or dark chocolate while conventional rice crackers included only six coated subsamples. As cocoa and chocolate are known to have higher levels of Ni, Cd, and Pb (Kruszewski et al., 2015) (Fig. 1B-D), a higher cocoa content could be related to higher Ni, Cd, and Pb levels in organic rice crackers compared to conventional products. The levels of Hg were similar for organic and conventional rice cracker (Table S3).

The cocoa content in flavoured milk might be related to higher UB mean Cd and Ni levels in the two organic pooled samples (Cd: 0.006 mg/kg and 0.013 mg/kg; Ni: 0.130 mg/kg and 0.130 mg/kg) compared to the conventional pooled sample (Cd: 0.001 mg/kg and Ni: 0.091 mg/kg) (Table S4 and S6). This could be related to more subsamples containing cocoa in the organic sampling (28 out of 30) than the conventional pooled subsample (14 out of 20). The levels of Hg and Pb were similar for the organic and the conventional pooled sample (Table S3 and S5).

3.3. Limitations and uncertainties of the study

3.3.1. TDS design

TDSs are a recommended, powerful tool for determining average levels of substances in foods as consumed for exposure assessment (EFSA/FAO/WHO, 2011). Nevertheless, the design of TDSs has to be taken into account to correctly interpret the results. As subsamples are pooled to one sample in a TDS, levels of substances cannot be assigned to specific foods or brands, to different cooking methods or recipes in the case of prepared foods or to geographical origins e.g. for fruit and vegetables. While the variability of substance levels cannot be described, the advantage of a TDS is providing representative data for estimating the average exposure of the entire population (EFSA/FAO/WHO, 2011). Substance levels for 356 different MEAL foods are available on a relatively low aggregation level of pooled samples. MLB and UB approaches are used and provide an uncertainty range in which the actual substance level is located (Table S4 to S6).

3.3.2. MEAL food list

The MEAL food list contains more than 90% of foods typically consumed by the population in Germany based on consumption data from 2002 (VELS) and 2006 (NVS II) covering age groups between 0.5 to < 5 years (VELS) and 14 to 80 years (NVS II). The German National Nutrition Monitoring (NEMONIT) conducted a follow-up study of the NVS II from 2008 to 2012/2013 and found no general deviations of the food consumption behavior by the population in Germany (Gose, Krems, Heuer, & Hoffmann, 2016). Nevertheless, latest food trends might not be covered by the present study, as new representative consumption data would be required.

Consumption habits of 5 to 13-year-old children were not explicitly considered to compile the food list. Food consumption is based on 24 h recalls of two days (NVS II) and six days food records (VELS) and rarely consumed foods might be underrepresented (Sarvan et al., 2017). Foods consumed by non-German-speaking individuals and people following a vegetarian or vegan diet might be underrepresented in the present study due to the underlying consumption data.

3.3.3. Regionality

For 70 out of 356 MEAL foods regional differences in Hg, Cd, Pb, and Ni levels were expected and sampling was conducted in four German regions separately. Drinking water (tap water) was sampled at 29 different sampling points of Germany. Due to logistics, for food preparation drinking water from the BfR MEAL Study kitchen was used so that possible regional differences in substance levels of tap water were not reflected in the substance levels of the prepared foods. However, levels of Hg, Cd, Pb, and Ni in regional drinking water were mostly close to or below the respective LOQ comparable to levels found in drinking water from the BfR MEAL Study kitchen (Table S3 to S6).

3.3.4. Type of production

For 105 out of 356 MEAL foods differences in substance levels were expected between organically and conventionally production and sampling was conducted by production type. Representative market data were used to sample organic and conventional subsamples representing shopping behaviour in Germany. Based on market data, market variation is sometimes limited to only few products (e.g. brands). Consequently, in these cases identical individual products were sampled several times to depict the market situation and to make sure that each pooled sample consisted of 15 subsamples. While organic products were purchased in the Berlin area, conventional products were also sampled
regionally for some foods, as these sampling criteria were combined for some MEAL foods. The market situation has to be taken into account when interpreting differences in substance levels of organic and conventional pooled samples of MEAL foods.

4. Conclusion

Levels of Hg, Cd, Pb, and Ni in foods determined in the BfR MEAL Study represent a comprehensive dataset as a basis for a refined chronic dietary exposure assessment for the population in Germany. This dataset can be used to evaluate substance levels detected in other research projects and studies. More than 90% of foods consumed in Germany were included in the study as 356 foods were analysed. High UB Hg levels were found in the food group fish, seafood, and invertebrates, the highest UB mean in tuna, smoked with 0.670 mg/kg. In contrast, high UB levels of Cd, Pb, and Ni were present in the food groups legumes, nuts, oilseeds, and spices as well as in coffee, cocoa, tea, and infusions. Especially the food cocoa powder showed high UB mean levels of Cd (0.270 mg/kg), Pb (0.113 mg/kg), and Ni (11.050 mg/kg), and high levels were also found in cocoa containing products. Levels of Hg, Cd, Pb, and Ni determined in the MEAL foods can be attributed to environmental sources, as these substances are environmental contaminants. Some foods were sampled by region, season, and production type and subsequently prepared as consumed resulting in a total of 869 pooled samples. The sampling by region, season and production type showed minor differences in substance levels for specific foods, but no tendency over all foods or for some food groups was apparent. No significant differences between substance levels in all foods sampled regionally or by production type were identified. This extensive data set for levels of Hg, Cd, Pb, and Ni in prepared foods sampled representatively for the population in Germany will lead to reduced uncertainties in exposure assessment and to improved consumer protection. The present study reports levels of Hg, Cd, Pb, and Ni in foods relevant for the consumption in Germany but future re-evaluation is required to generate knowledge about changes in the food composition and the consumption behaviour.

CRediT authorship contribution statement

Carolin Fechner: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. Christin Hackethal: Investigation, Methodology, Validation, Writing – review & editing. Tobias Hopfner: Validation, Writing – review & editing. Jessica Dietrich: Validation, Writing – review & editing. Dorit Bloch: Validation, Writing – review & editing. Oliver Lindner: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. Irmeta Sarvan: Conceptualization, Project administration, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The co-author Christin Hackethal started working for the contract laboratory Institut Kirchhoff Berlin GmbH (Merieux NutriSciences) after the results of the BfR MEAL Study were validated.

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Appendix A. Supplementary data

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