Exposure to lead from intake of coffee

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1. **Foreword**

The List of Undesirable Substances (LOUS) was established by the Danish Environmental Protection Agency (EPA) as a guide for enterprises. It addresses chemical substances of concern, based on their hazardous properties and the volumes used in Denmark. The latest version of LOUS from 2009 includes 40 chemical substances or groups of substances [DEPA 2010].

During the period 2012-2015, all substances listed on LOUS will be surveyed and further need for risk management measures will be evaluated. In certain cases, implementation projects will be launched to achieve the goals laid down in the strategies for these substances.

The present project "Exposure to lead from intake of coffee" was initiated as a LOUS follow-up project by the Danish EPA. The objective of this study was to estimate the exposure to lead from coffee in the Danish population. The investigation was conducted by chemical analyses of coffee beans as well as brewed coffee. The consumption data was obtained from the Danish food database, which is hosted by the DTU FOOD.

The project was carried out from September 2014 to April 2015 by the National Food Institute at the Technical University of Denmark (DTU Food). Participants from DTU Food were senior advisor Max Hansen, senior scientist Jens Jørgen Sloth and scientist Rie Romme Rasmussen. The quality assurance was performed by Folmer Eriksen.
2. Sammenfatning og konklusion

I DTU Fødevareinstituttets seneste overvågningsrapport over udvalgte kemiske forureninger i fødevarer blev det på baggrund af ældre data anslået, at den gennemsnitlige eksponering af bly fra kaffe svarede til 40 % af blyindtaget fra drikkevarer og dermed næsten 20% af det totale indtag af bly fra drikke- og fødevarer. Da hovedparten af det totale indtag af bly stammer fra kosten, udgør det estimerede bidrag fra kaffe således et betragteligt bidrag til danskernes samlede indtag af bly. En nylig tysk undersøgelse gennemført af BfR viste et højt indhold af bly i kaffe brygget på espresso- og espresso maskiner af den type, der anvendes på cafeer. Nærværende projekt har derfor til formål at estimere indtaget af bly fra kaffe i den danske befolkning, samt at vurdere, hvor stor en del, der kommer fra henholdsvis kaffe, vand og kaffebryggere. Dette indtag er blevet sammenlignet med det samlede indtag af bly fra drikkevarer/fødevarer. Det er desuden blevet undersøgt, om der er en systematisk geografisk variation af blyindholdet i kaffe.

For at sikre at de valgte kaffe typer, der blev udtaget, var representative for det danske marked, blev der gennemført et udtræk fra Dansk Husstandspanel (GfK), der bygger på en meget detaljeret registrering af en række husstandes indkøb af de 566 forskellige varenumre for kaffe, der findes på det danske marked. Det blev desuden sikret, at de udtagne prøver repræsenterede kaffe fra forskellige dele af verden, således at en eventuel geografisk forskel i blyindholdet kunne fastslås. I alt blev der analyseret 44 kaffebønne produkter, heraf 9 af de 10 mest solgte varenumre, og 7 instant kaffe produkter.

For at fastslå, om forskellige kaffebrygningsmetoder har indflydelse på indholdet af bly i drikkeklar kaffe, blev der analyseret på kaffe brygget efter følgende seks metoder: Filttkaffe brygget på kaffemaskine, stempelkande kaffe, kogekaffe, kaffe brygget på mokkakande, hjemmebrygget espresso og espresso fra cafeer. Der blev desuden udført analyser af det vand, der blev anvendt til brygningen.

Til beregning af den samlede eksponering for bly via kaffe blev de fundne blykonzentrationer sammenholdt med konsumdata for kaffe, der blev udtrukket fra den danske kost database af afdeling for risikovurdering og ernæring ved DTU Fødevareinstituttet.

Det er almindelig kendt, at analyser af bly er vanskelige og let fører til overestimering af indholdet på grund af en række diffuse kilder. Derfor blev der i dette projekt gjort et omfattende arbejde for at kvalitetssikre data.

Analyserne viste et indhold af bly i de 44 prøver af kaffebønner på 4,5 – 65,3 ng/g med et gennemsnit på 15,9 ng/g. Dette er noget lavere end det, der er blevet fundet i de fleste andre studier, der er gennemført verden over. I disse undersøgelser er bly blot en enkelt af mange sporelementer der er analyseret, og kvalitetssikringen er ikke beskrevet i detaljer. Denne forskel i resultater kompromitterer derfor ikke validiteten af det aktuelle studie. I instant kaffe blev der i de 7 prøver fundet i gennemsnit 17,9 ng/g. Med undtagelse af kogekaffen blev det fundet, at uanset hvilken brygometode, der blev anvendt, blev alt den bly der var tilstede i kaffebønnerne ekstraheret ud i den drikkeklare kaffe. For kogekaffen ser det umiddelbart ud til at kun ca. halvdelen ekstraheres,
men på grund af de relativt høje analyseusikkerheder kan det ikke udelukkes, at alt bly også ekstraheres ved denne bryggeteknik.

Der blev ikke observeret afsmitning fra bryggeudstyret i de kaffer, der blev brygget hjemme. På de målinger, der er udført på café kaffe, er der kun analyseret på den drikkeklare kaffe.

Indtaget af kaffe blev for danske mænd bestemt til 706 ml/dag i gennemsnit med en 95 percentil på 1657 ml/dag. For danske kvinder var de tilsvarende tal 550 ml/dag og 1371 ml/dag. På baggrund af dette blev det beregnet, at mænd i gennemsnit indtager 0,74 µg bly/person/dag og at 95 percentilen indtager 1,73 µg/person/dag. Kvinder indtager i gennemsnit 0,58 µg bly/person/dag med en 95 percentil på 1,35 µg bly/person/dag. Det gennemsnitlige indtag svarer til 9,1 % af det samlede blyindtag fra drikkevarer for mænd og 7,1 % for kvinder. Dette er væsentligt lavere end det tidligere estimat på 40 %. Årsagen til dette er, at der i denne undersøgelse er fundet lavere indhold af bly i kaffe end i analyser af kaffe gennemført i de tidligere undersøgelser.

I de 8 prøver af drikkeklar kaffe, der blev taget på cafeer, blev der generelt fundet et blyindhold på niveau med det der blev fundet i hjemmebrygget kaffe. Der var dog en enkelt prøve, hvor indholdet var 67 ng/g. Der blev senere taget en ny prøve fra samme cafe, brygget på samme maskine og den viste et væsentligt lavere indhold. Dette indikerer, at det høje indhold kan skyldes en relativ nylig afkalkning da brugen af syre til afkalkning kan frigøre bly fra lødninger i kaffemaskinen, hvis det er tilstede. Fra en sundhedsmæssig betragtning er indholdet dog ikke så højt, at det vil være sundhedsmæssigt problematiskt at indtage en kop kaffe dagligt med dette indhold.

Indtaget af bly fra kaffe svarer samlet set til hhv. 4,2% og 3,3% af det totale blyindtag med kosten for danske mænd og kvinder. Generelt kan det konkluderes, at indtaget af bly fra kaffe er lavt i forhold til indtaget fra andre fødevarer, og at det ikke udgør en væsentlig del af det samlede indtag af bly med kosten.
3. Summary and conclusion

In the latest monitoring report on chemical contaminants in food from DTU FOOD it was estimated that the average exposure of lead from coffee corresponded to 40% of the lead intake from beverages and thus to almost 20% of the total dietary intake of lead. This estimate was based on old data. A recent German study conducted by BfR showed a high content of lead in coffee brewed at professional espresso machines of the types frequently used in cafes. The aim of this project was to estimate the intake of lead from coffee in the Danish population, and to assess the amount contributed from coffee, water and coffee brewers, respectively. This intake has been compared with the total intake of lead from drinks/food. It has also been investigated whether there is a systematic geographic variation of the lead content in coffee.

To ensure that the selected coffee types were representative for the Danish market, data were extracted from the Danish Household Panel (GfK) based on a very detailed record of a number of household purchases of the 566 different code numbers for coffee found on the Danish market. It was also ensured that the coffee selected represented beans from different parts of the world so that any geographical difference in lead content could be determined. In total 44 coffee bean products (among them 9 of the 10 most sold coffees in Denmark) and 7 instant coffee products were analysed.

To determine whether different coffee brewing methods influence the content of lead in ready to drink coffee, coffee samples based on each of the following six brewing methods were analysed: Filter coffee brewed coffee maker, French press coffee, boiling coffee, coffee brewed in mocha pot, home-brewed espresso and espresso from cafes. The water used for the coffee brewing was also analysed.

To calculate the total exposure to lead from coffee, the measured lead concentrations was compared to consumption data for coffee in Denmark extracted from the Danish Food Database by the Division of Risk Assessment and Nutrition at DTU FOOD.

It is generally known that analysis of lead is difficult and easily leads to overestimation of the contents due to a number of diffuse sources. Therefore extensive efforts were made in this project to ensure quality control of the data.

The analysis indicated that the content of lead in the 44 samples of coffee beans was ranging from 4.5 to 65.3 ng/g with an average of 15.9 ng/g. This is somewhat lower than what has been found in most other studies conducted worldwide. In these studies lead was just one of many trace elements analyzed and quality assurance is not described in detail. These differences in the results reported do thus not compromise the validity of the current study. In the instant coffee an average of 17.9 ng/g was found in the 7 samples. With the exception of the coffee boiling it was found that regardless of the brewing method used, all the lead present in the coffee beans was extracted to brewed coffee. For boiling coffee, it initially appeared that only about half of the lead was extracted, but because of the relatively high analytical uncertainties in this analysis it cannot be excluded that all lead present is extracted by this brewing method as well.

There was no indication that lead was extracted from home brewing equipment. In those measurements conducted on café brewed espresso only ready to drink coffee was analysed.
The intake of coffee in Danish men is on average 706 ml/day on average, with a 95 percentile of 1,657 ml/day. For Danish women, the corresponding figures are 550 ml/day and 1,371 ml/day respectively. Based on this, it was calculated that on average, men have an intake of from coffee of 0.74 µg of lead/person/day with a 95 percentile of 1.73 µg/person/day. Women have an average intake of lead of 0.58 µg/person/day with a 95 percentile of 1.35 µg/person/day. The average intake is equal to 9.1% of the total lead intake from beverages for men and 7.1% for women. This is substantially lower than the previous estimate of 40%. The reason for this is, that in the current study the concentration of lead found in coffee was lower compared to previous studies.

In the 8 samples of coffee from cafes the lead concentrations was generally at the same level as it was in home-brewed coffee. A single sample had a concentration 67 ng/ml. In a new sample from the same cafe, brewed on the same machine at a later time the measured content of lead was significantly lower. This indicates that the high content may be due to a relatively recent descaling of the machine as the use of acid for de-calcification is assumed to release lead from weldings in the machine, if it is present. From a health perspective, the content is not so high that it would be problematic from a health point of view to drink a cup of coffee every day even if the concentration of lead remains at 67 ng/ml.

The intake of lead from coffee corresponds to 4.2% and 3.3% of the total dietary intake for Danish men and women, respectively. In general, it can be concluded that the intake of lead from coffee is low compared to the intake from other dietary sources, and that it does not constitute a major part of the total dietary intake lead.
4. Introduction

Lead (Pb) is an element which is found in the environment from both natural and industrial sources. Especially the previous use of tetraethyl lead as an additive in gasoline has been a considerable source of lead in the environment. Lead is absorbed in crops from the soil and is thus directly transmitted to humans by consumption of these crops or indirectly through consumption of animals given feed with a high lead content. Drinking water is another potential source of lead in humans. Lead in drinking water partly arises from the ground water and partly from migration from pipes, fixtures and appliances which contain lead either in pure form or as part of alloys used for weldings. Other sources of lead are work place air, migration from consumer products such as toys, clothes, electronics and food contact materials and from environmental pollution in general. In Denmark and EU lead is, however, heavily regulated in e.g. consumer products, building materials, drinking water and industrial emissions etc. It is assessed by the National Food Institute in Denmark (DTU FOOD) that food and beverages is the major contributor to the intake of lead in Denmark (A.Petersen et al., 2013).

In the Danish population the intake of lead has been declining since lead was phased out as an additive in almost all gasoline in the late 1970'ies. The latest assessment by DTU FOOD from 2013 shows an average intake of lead of 0.25 µg/kg body weight/day with a 95 percentile of 0.46 g/kg body weight (b.w.)/day (see Table 1) (A.Petersen et al., 2013). Beverages represent about half of the total intake of lead (see figure 1). Based on rather old analytical data DTU FOOD has estimated that approximately 40% of the lead exposure from beverages is from coffee. Based on these data the intake of lead from coffee thus corresponds to approximately 20% of the total dietary intake of lead. Lead in coffee may originate from the brewing water, extraction from the coffee bean itself or from lead present in metal alloys in the brewing equipment (e.g. in weldings). A recent, but small study from Germany suggests that lead migration from coffee brewing equipment is usually low, but that there may be a significant migration of lead from certain types of new professional espresso brewers or newly decalcified brewers (Bundesinstitut für Risikobewertung, 2014). The german study includes only eight differences coffee machines and it is thus not possible to draw general conclusions based on these results.
TABLE 1. INTAKE OF LEAD IN THE DANISH POPULATION BASED ON THE DANISH MONITORING PROGRAMME (A.Petersen et al., 2013).

<table>
<thead>
<tr>
<th>µg/kg bw/day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.25</td>
</tr>
<tr>
<td>sd</td>
<td>0.11</td>
</tr>
<tr>
<td>N</td>
<td>0.069</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentile</th>
<th>µg/kg bw/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1974</td>
</tr>
<tr>
<td>5</td>
<td>0.102</td>
</tr>
<tr>
<td>25</td>
<td>0.17</td>
</tr>
<tr>
<td>50</td>
<td>0.23</td>
</tr>
<tr>
<td>75</td>
<td>0.30</td>
</tr>
<tr>
<td>95</td>
<td>0.46</td>
</tr>
<tr>
<td>99</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Minimum 0.03, Maximum 1.05

The most important contributors to dietary exposure to lead in the Danish population are the food groups: beverages (47 %), fruit and fruit products (17.4 %), vegetable and vegetable products (9.1 %), and cereals and cereal products (8.4 %) (see figure 1) (A.Petersen et al., 2013).

FIGURE 1. SOURCES OF LEAD IN PERCENT OF THE TOTAL INTAKE FROM FOOD IN DENMARK (A.Petersen et al., 2013)

Toxicity of lead
The gastro intestinal absorption of lead is estimated to be 15 – 20 % in adults and a little higher in children. There are large intra individual differences in the absorption mainly due to differences in nutritional status. Deficiency in elements like iron or calcium may increase the absorption of lead. After absorption lead is transported through the blood stream by metal transporters and accumulates in the bones and to a lesser degree in the liver and the kidney (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010).
In children effects on the developing central nervous system are considered the critical effect of lead. EFSA has calculated that an intake of 0.50 μg/kg b.w./day may decrease the IQ at an age of 7 by 1 %. EFSA concluded that it was not possible to define a lower level of exposure without any adverse effect, and a sufficient margin of exposure (MOE) could thus not be established (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010). The MOE is by definition the Benchmark dose (BMDL)/actual exposure. The BMDL is the dose which is calculated to induce a given magnitude of an effect. In this case the BMDL01 is the dose which may decrease the IQ in children by 1 %. If the MOE is 1 the actual exposure may decrease IQ of 1 % in children. If the MOE is 10 the actual exposure is 10 times below the dose which is expected to decrease the IQ by 1 %. There is no common agreement on what MOE should be considered acceptable. If a BMDL01 is based on human data, a MOE of 10 will usually be sufficient. If the BMDL value is based on animal experiments a MOE of 100 will usually be sufficient. However, the chosen response (in this case 1 %), and the nature of the toxicological endpoint has large influence on which MOE should be considered sufficient.

For adults EFSA consider effects on systolic blood pressure and kidney effects as the critical effects of lead. Based on benchmark dose modelling EFSA calculated that dietary lead intake in adults of 1.50 μg/kg b.w./day may affect the cardiovascular system by increasing the systolic blood pressure by 1 %. EFSA concluded that a margin of exposure of 10 or greater would be sufficient to ensure that there is no appreciable risk of a clinically significant effect on systolic blood pressure. Indeed, even at MOEs greater than 1.0 the risk would be very low (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010).

Based on animal studies EFSA calculated a BMDL10 for chronic kidney disease of 0.63 μg/kg b.w./day. EFSA concluded that a margin of exposure of 10 or greater would be sufficient to ensure that there is no appreciable risk of a clinically significant change in the prevalence of chronic kidney disease. Indeed, overall, the risk at MOEs of greater than 1.0 would be very low (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010).

With the use of European data for concentrations of lead in food and European consumption data EFSA has calculated the MOE for the 3 critical effects in different part of the population. For non-breast feed children the MOEs are below for 1 even for average consumers. For adults the MOEs for nephrotoxicity are around 1 for the average consumer and below 1 for what EFSA consider high consumers (95 percentile) (see table 2). These data indicate an urgent need to decrease the exposure to lead from food in the whole population. If coffee is a significant contributor to the total intake of lead consumer guidance could be a valuable tool in decreasing the exposure.

The significance of in utero exposure is not yet been elucidated.
TABLE 2. ESTIMATED MARGINS OF EXPOSURE (MOES) FOR DIFFERENT ENDPOINTS BY TYPE OF POPULATION. THE MOE RANGE FOR IN UTERO EXPOSURE WAS DERIVED FROM THE 20 TO 40 YEAR OLD FEMALE CONSUMER DATA (FROM EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010)

<table>
<thead>
<tr>
<th>Population/diet</th>
<th>Endpoint</th>
<th>Average consumer</th>
<th>High consumer (95 percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Cardiovascular effects</td>
<td>1.2 - 4.2</td>
<td>0.62 - 2.1</td>
</tr>
<tr>
<td></td>
<td>Nephrotoxicity</td>
<td>0.51 - 1.8</td>
<td>0.26 - 0.86</td>
</tr>
<tr>
<td>Vegetarians</td>
<td>Cardiovascular effects</td>
<td>1.2 - 3.3</td>
<td>0.67 - 1.9</td>
</tr>
<tr>
<td></td>
<td>Nephrotoxicity</td>
<td>0.50 - 1.4</td>
<td>0.28 - 0.79</td>
</tr>
<tr>
<td>Specific diet (Game meat)</td>
<td>Cardiovascular effects</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nephrotoxicity</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>infants 3 months breast milk</td>
<td>Developmental neurotoxicity</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>infants 3 months infant formulae</td>
<td>Developmental neurotoxicity</td>
<td>0.79 - 1.9</td>
<td>0.53 - 1.3</td>
</tr>
<tr>
<td>children 1-3 years</td>
<td>Developmental neurotoxicity</td>
<td>0.16 - 0.45</td>
<td>0.09 - 0.29</td>
</tr>
<tr>
<td>children 4-7 years</td>
<td>Developmental neurotoxicity</td>
<td>0.19 - 0.63</td>
<td>0.10 - 0.38</td>
</tr>
<tr>
<td>In utero exposure (a)</td>
<td>Developmental neurotoxicity</td>
<td>0.39 - 1.3</td>
<td>0.19 - 0.74</td>
</tr>
</tbody>
</table>

MOE: margin of exposure. (a) For cardiovascular effects the MOE was calculated by dividing the BMDL01 intake value of 1.50 μg/kg b.w. /day by the respective dietary exposure estimates taken from Table 29; (b) For nephrotoxicity the MOE was calculated by dividing the BMDL10 intake value of 0.63 μg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29; (c) For neurodevelopmental toxicity, the MOE was calculated by dividing the BMDL01 intake value of 0.50 μg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29. The MOE range for in utero exposure was derived from the 20 to 40 year old female consumer data;

**Literature studies**
Beside the BfR study already mentioned, only 6 relevant studies published within the last 25 years with analytical data on the concentration of lead on coffee beans or coffee infusion have been identified (see table 3).

- In a Brazilian study 30 samples of coffee beans were measured using ICP-EAS. Concentrations of several hundred ng lead/g were found (C.A.P.Schmidt et al., 2009).
- Concentrations of metals, including lead were determined in 11 coffee infusions analysed by ICP-MS made from coffees purchased in Bosnia and Herzegovina, Brazil, Lebanon and Poland (Nedzarek et al., 2013).
- In a study assessing the daily intake of trace elements from foodstuffs in Rio de Janeiro, 3 samples of coffee were analysed for lead using ICP-MS (Santos et al., 2004).
- Lead could not be detected (method AAS: detection limit 100 ng/g) in a study of ground coffee, decaffeinated coffee, flavoured coffee, pure Arabica coffee or instant coffee (Grembecka et al., 2007).
- In a study on differentiate geographic growing origins the concentration of lead in coffee was below the detection limit (500 ng/g) (Anderson and Smith, 2002).
- A study on nutritional and non-nutritional elements in Brazilian coffee did not detect lead above the detection level of 450 ng/g using ICP-AES (Santos and Oleiveira, 2001).
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of samples</th>
<th>Method</th>
<th>Lead concentration ng/g</th>
<th>Comment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee beans</td>
<td>15</td>
<td>ICP-EAS</td>
<td>470 ± 850</td>
<td>Coffee beans grown in basalt containing soil</td>
<td>Smidt et. al, 2009</td>
</tr>
<tr>
<td>Coffee beans</td>
<td>15</td>
<td>ICP-EAS</td>
<td>350 ± 540</td>
<td>Coffee beans grown in sandstone containing soil</td>
<td>Smidt et. al, 2009</td>
</tr>
<tr>
<td>Infusion 1 g coffee in 27 ml water</td>
<td>11</td>
<td>ICP-MS</td>
<td>858 ± 193</td>
<td>Coffee purchased in different countries</td>
<td>Nedzarek et al, 2013</td>
</tr>
<tr>
<td>Infusion 183 g coffee in 6 L water</td>
<td>3</td>
<td>ICP-MS</td>
<td>230 - 308</td>
<td>Assessment of trace element intake in Rio de Janeiro</td>
<td>Santos et al, 2004</td>
</tr>
<tr>
<td>Coffee beans and Infusion</td>
<td>F-AAS</td>
<td>ND</td>
<td>Differentiation of marked coffee</td>
<td>Grembecka et al, 2007</td>
<td></td>
</tr>
<tr>
<td>Coffee beans</td>
<td>ICP-AES</td>
<td>ND</td>
<td>Differentiation of geographic growing origins of coffee</td>
<td>Anderson, 2002</td>
<td></td>
</tr>
<tr>
<td>Coffee beans</td>
<td>ICP-AES</td>
<td>ND</td>
<td>Determination of nutritional and non-nutritional elements in Brazilian coffee</td>
<td>Santos and Oliveira, 2001</td>
<td></td>
</tr>
</tbody>
</table>
5. Study design and methods

Introduction
In this chapter the study design with a description of the sampling, brewing and analytical methods as well as the quality assurance applied in the project will be presented.

Collected coffee samples
Coffee beans: In total 51 samples of coffee beans were purchased in 2014 on the Danish retail market and given internal sample codes (no. 1-51). These 51 samples included 29 ground coffee beans, 15 whole beans and 7 instant coffees. Of the top 10 most used coffee beans in Danish households 9 of these beans were represented among the 51 samples. Ten of the samples were organic produced coffees, whereas rest was conventionally grown. Beans from areas with different geoactivity were targeted, including e.g parts of South America and Southeast Asia where relative high lead content in the soils was expected and parts of Brazil and Africa where lower lead levels were expected. See Table 3A in the appendix for specific information on all the coffee beans included in this study.

Coffee drinks: Six different home brewing methods (M1-M6) were tested for three coffee bean brands (sample no. 17, 37 and 49, which represented normal (average) lead levels in the range 11-20 ng/g). One was within the top 10 most used coffee beans (nr. 49) and duplicate brewing were performed for quality control (nr. 37). Different brewing principles were covered, including filtration, stepping, boiling and pressure brewing. For the brewing procedures (M1-M5) 65 g ground beans per litre tap water were used. Furthermore ready-to-drink espresso coffee from 8 cafes and gas stations were purchased in the Copenhagen area. See TABLE 4 for more details on the coffee drinks included in this study.

Brew water: Tap water (Søborg, Denmark) was poured into in a 5 L glass flask to ensure uniform brewing water for the brewing methods (M1-M5). The tap water had a mean lead (Pb) content of 0.62 ng/ml (n=2, 7% relative standard deviation, RSD).

### TABLE 4. COFFEE DRINKS: DESCRIPTION OF BREWING METHODS, WHICH WERE TESTED FOR 3 SELECTED COFFEE BRANDS (AND SAMPLES FROM CAFES FROM WHICH READY-TO-DRINK ESPRESSOS WERE PURCHASED)

<table>
<thead>
<tr>
<th>Method</th>
<th>No.</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtration</td>
<td>M1</td>
<td>Automatic drip – new</td>
<td>13 g beans pr 200 ml tap water, plastic filter</td>
</tr>
<tr>
<td>Filtration</td>
<td>M2</td>
<td>Automatic drip – pre used</td>
<td>13 g beans pr 200 ml tap water, paper filter</td>
</tr>
<tr>
<td>Stepping</td>
<td>M3</td>
<td>French press</td>
<td>200 ml boiling tap water was left in the pot without heating for 30 sec. and was then poured over 13 g beans. The stepper was pressed after 3.5 min brewing.</td>
</tr>
<tr>
<td>Boiling</td>
<td>M4</td>
<td>Norwegian</td>
<td>13 g beans and 200 ml tap water were boiled 5 min in a pot.</td>
</tr>
<tr>
<td>Pressure</td>
<td>M5</td>
<td>Mocha pot</td>
<td>6 g beans and 100 ml tap water heated in aluminium mocha pot on a hotplate.</td>
</tr>
<tr>
<td>Pressure</td>
<td>M6</td>
<td>Espresso, homemade</td>
<td>-</td>
</tr>
</tbody>
</table>
Determinaton of lead
The method described below is accredited according to ISO17025 by the Danish accreditation body, DANAK.

Reagents: Water was ultra-purified using a Millipore system (Molsheim, France) and all reagents used were of per analysis quality or better. Nitric acid (HNO₃) (PlasmaPure, SCP Science, France), hydrochloric acid (HCl) (PlasmaCal, SCP Science) and methanol (MeOH) (Rathburn, UK) were used for sample preparation and analysis. Standard stock solutions of lead (Pb), tin (Sn) and bismuth (Bi) were obtained from SCP Science (Courtaboeuf, France). Sodium hydroxide (NaOH) from (Merck, Darmstadt, Germany), acetic acid (CH₃COOH) from (Merck) and HCl were used for pH buffering of water for blind brewing.

Coffee beans: Subsamples (homogenized beans 0.4-0.6 g) were digested in high-pressure quartz vessels using microwaves (Multiwave 3000, Anton Paar, Austria) with 2 ml water and 4 ml concentrated nitric acid. The digests were diluted to a volume of 20 ml with water. Prior to analysis sample aliquots were further diluted 5 times with a 2% HNO₃, 1% HCl aqueous solution.

Certified reference materials: The following certified reference materials (CRMs) were used in the study to evaluate the analytical quality. BCR281 Rye grass and BCR62 Olive leaves (Institute of Reference Materials and Measurements, Geel, Belgium) and NBS1572 Citrus leaves (National Institute of Standards and Technology, USA). Subsamples of the CRM were digested following the same procedure for the coffee.

Coffee drinks: The coffee solutions were filtrated with single use hydrophilic syringe filters (0.45 µm, Minisart, Sartorius, Göttingen, Germany) and prior to analysis aliquots (1 ml) were further diluted to a volume of 6 ml with a 2% MeOH aqueous solution.

ICPMS determination: The lead, tin and bismuth content was determined by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 8800 ICP-MS instrument (Agilent Technologies, Waldbronn, Germany) run in no gas mode (m/z Pb208, Sn118 and Bi209 with 0.2 sec. integration time/mass). The ICP-MS was equipped with a Micro mist glass concentric nebuliser (Agilent), and a Scott-type double-pass water-cooled spray chamber (Agilent). Typical plasma conditions were 1,500 W RF power, 15 L min⁻¹ plasma gas, 0.88 L min⁻¹ carrier gas and 0.32 L min⁻¹ makeup gas. Acceptable daily performance of the instrument was ensured by autotuning using a solution of 1 µg/L each of Li, Mg, Y, Ce, Ti and Co in aqueous 2% HNO₃ (Agilent). Calibration standards were prepared in the same acid concentration as the sample test solutions and were in the range 1-40 µg L⁻¹ for Sn and 0.1-20 µg L⁻¹ for Pb. Bismuth was used as an internal standard and was added on-line (5 µg L⁻¹) via a t-piece using the peristaltic pump. Quantification was done by external calibration using linear calibration curves of the relative responses to bismuth. Certified reference material and blank samples were analysed together with the samples. No corrections were made for the reported results.
Quality assurance parameters
Detailed information on the quality assurance data can be found in the appendix (Tables 2 and 3).

Coffee beans: The certified reference materials (CRMs) were analysed in two dilutions in concentrations within the calibration curve (0.25 and 2.5 ng/ml). The obtained results agreed well with certified target values (94-114% mean recoveries) and good precision (<5% RSD) were obtained for CRMs and standards (2 ng/ml and 1 ng/ml) analysed throughout the sequences. The calibration curves were prepared in measurement solution (2% HNO₃, 1% HCl) and showed linearity with good linear correlation (R=0.9998) over the full calibration range (0.1 – 20 ng/ml). The conversion factor between the measurement solution (ng/ml) and the original coffee bean sample (ng/g) was ~ 186 ml/g. Hence the lowest calibration standard (0.1 ng/ml) corresponds to ~19 ng/g lead in a coffee bean sample. However, due to the obtained accuracy of low calibration points and the satisfying precision obtained for duplicate analysis of the real samples, levels as low as 4.5 ng/g lead in coffee bean (0.02 ng/ml in solution) are reported in the current report. Since lead is present in our environment and consequently in dust particles in the laboratory a risk of false positive samples exist. Therefore re-dilution of coffee bean extracts or a new digestion of a new subsample followed by a re-analysis was performed in cases of bean sample contents > 21 ng/g. The mean values were reported if the RSD was not unacceptable high (>35%), otherwise the results with high RSD values (>35%) were discarded as false positives. The obtained RSD values for the double determinations (n=25) were between 3-43%, which is pooled to an average relative standard deviation of 23% (RSDpooled).

Coffee drinks: Possible bias caused by matrix effects was evaluated by spike experiments. Coffee drinks from homebrew methods M1-M5 (n=1) using coffee bean no 49 were spiked with 1 ng/ml lead. Mean spike recovery of 94% and 6% RSD (n=5) were obtained and hence no bias was identified despite the high carbon level introduced into the ICP-MS (due to sample preparation by dilution instead of the traditional wet digestion). The BCR281 CRM was analysed in two dilutions in concentrations within the MeOH calibration curve (0.25 and 2.5 ng/ml). Good accuracy (104% mean recovery) and good precision (13% RSD) were obtained also for the acid digested BCR281. The limit of quantification (LOQ) was estimated to approximately 1.9 ng/ml coffee drink for which a satisfying RSD (<25%) could be obtained, at lower levels (0.8 ng/ml) the RSD was higher (47%). The RSD for the duplicate brewings (n=5) for brand no. 37 were between 9-47% and was pooled to an average relative standard deviation of 25% (RSDpooled).

1 186 ml/g = 20 g x 5 dilution / (0.5 g * 1.07 g/ml) = g ekstrakt x diluted / (g sample * density of solution)
6. Results and discussion

Coffee beans:
Figur 2 shows the results from the analysis of lead in coffee beans. The lead contents were in the range 4.5-65.3 ng/g. The overall mean of ground coffees and whole beans was 15.9 ng/g, the mean of beans within 9 of the top 10 brands used in 15.7 ng/g and the mean of instant coffee was 17.9 µg/L. The analytical uncertainty associated with the data was estimated as 23% (RSD pooled), which is an acceptable precision because the levels are near the limit of quantification (LOQ).

The hypothesis was that beans from soils with high geological activity (expected to have high lead content) would be reflected in lead levels detected in the coffee beans. However, with the present dataset it was not possible to link geographical origin with lead levels in the coffee beans. From Ethiopia, which is an area with volcanic activity (NASA, 2015 and Tom, 2015) the three brands (no. 22, 35 and 13) contained between 4.5-21.3 ng Pb/g bean. The brand with the highest lead content was from the Chinese province Yunnan and grown in 700-1100 meters above sea level. Both Ethiopia and Yunnan cover very large geographical areas with only some sites near volcanic activity and since anthropogenic activities also can cause elevated soil lead levels (Markus and McBratney 20012) much more sample details are needed to identify a trend.

FIGURE 2. LEAD (MEAN +/- STANDARD DEVIATION POOLED) IN COFFEE PRODUCTS PURCHASED IN 2014 ON THE DANISH RETAIL MARKET. IN TOTAL 51 BRANDS INCLUDING 9 OF THE 10 MOST USED BRANDS IN THE DANISH HOUSEHOLDS (HIGHLIGHTED WITH BRIGHT COLOR).
Coffee drinks

Homebrewing: Figure 3 shows the results from the analysis of lead in homebrewed coffee drinks. The mean lead contents were in the range <LOQ – 4.2 ng/ml. The overall mean was 2.2 ng/ml for homebrewed coffee drinks. The analytical uncertainty associated with the numbers is 25% RSD pooled but this is expected because the levels are near the coffee drink LOQ (1.9 ng/ml).

**FIGURE 3.** LEAD (MEAN +/- STANDARD DEVIATION POOLED) IN COFFEE BREWED ON THREE SELECTED BRANDS OF BEANS USING SIX DIFFERENT HOME BREWING METHODS. METHODS: FILTER MACHINE NEW (M1), FILTER MACHINE PRE-USED (M2), FRENCH PRESS (M3), POT BOILING (M4), MOCHA POT (M5) AND ESPRESSO, HOMEMADE (M6). THE REPPLICATE BREWINGS FOR NO. 37 WERE USED TO DETERMINE THE OVERALL RELATIVE STANDARD DEVIATION (RSDPOOLED).

**Estimation of carry-over from bean to coffee drinks.** From the coffee beans and coffee drinks concentrations, the carry-over from bean to drink was determined. First the lead from the tap water (0.62 ng/ml) was subtracted and then the coffee drink concentrations (ng/ml) were converted into bean concentrations (ng/g) using the fact that 0.065 g bean/ml coffee drink was used for brewing methods M1-M5. The mean mass balance for the different methods indicates a carry-over of lead from beans to the coffee drinks for automatic drip filter machines (M1, M2), pot boiling (M4) and mocha pot (M5). The French press (M3) stepping procedure gave less than 100% carry over from bean to drink. However, since the detected levels in these coffee drinks were below the LOQ and hence there is a very high uncertainty in this dataset and the exaction factor could consequently not be exactly estimated. Likewise, the carry-over factors for several experiments were above 100% due to the high uncertainty in these numbers. See Figure 4 for the comparison of results from the beans and brewing data for carry-over estimation of lead in beans into coffee drinks.
FIGURE 4. COMPARISON OF BEAN AND BREWING RESULTS IN THE UNIT ng/g FOR CARRY-OVER ESTIMATION OF DIFFERENT BRANDS AND BREW METHODS. METHODS: FILTER MACHINE NEW (M1), FILTER MACHINE PRE-USED (M2), FRENCH PRESS (M3), POT BOILING (M4) AND MOCHA POT (M5).

Café espresso

Figure 5 shows the results from the analysis of lead in café espressos. The lead contents varied from <LOQ (1.9 ng/ml) up to 67 ng/ml in one sample of double shot espresso from café 5. Two café 5 espressos were sampled on two different occasions and these samples had significant different lead content (67 and 11 ng/ml, respectively). The espresso from café 5 with the high lead content had also a significantly higher tin (Sn) level than the rest of the espressos. This suggests that lead is leached along with tin most probably from lead-contaminated tin-weldings in the coffee machine. Previously elevated lead levels in espresso brewed on professional expresso machines have been reported in a German study. The high lead levels were associated with the cleaning procedure where use of acids for de-calcification was suspected to release lead from tin-weldings used in the coffee machines (Bundesinstitut für Risikobewertung, 2014).
FIGURE 5. LEAD IN CAFÉ ESPRESSO WAS DETERMINED IN ONE REPLICATE EXCEPT FOR CAFE 5 WHICH WAS SAMPL ED ON TWO OCCASIONS (DOTTED OUTLINE = 2ND TIME). THE TIN (Sn) CONTENT IS PLOTTED FOR THE FIRST SAMPLING ROUND RELATIVE TO CAFÉ 5 (=100%).

Exposure assessment
As the amount of coffee used to brew 1 L coffee for the different home-brewing methods is identical (except for instant coffee) and as the analysis indicates that the extraction of lead is complete for all the home-brewing methods, the exposure assessment for home-brewed coffee can be performed without taking the brewing method into account.

The lead concentration in instant coffee is very low considering that only about 12 g of instant coffee is used for 1 L water whereas 65 g is used for 1 L coffee using coffee beans. Therefore, and because the consumption of instant coffee is low compared with the consumption of coffee brewed on beans, a specific exposure assessment for instant coffee has not been performed.

Consumption of coffee:
DTU FOOD has extracted the Danish consumption of coffee from the Danish Food Database (see table 5). As expected adults has by far the highest consumption of coffee per person. Children up to the age of 17 in general have a low consumption. Even if only the coffee consumers are considered the consumption in this age group is far below the consumption for adults.
TABLE 5. CONSUMPTION OF COFFEE IN THE DANISH POPULATION DIVIDED INTO MEN, WOMAN AND DIFFERENT AGE GROUPS (NON-PUBLISHED DATA FROM THE NATIONAL FOOD INSTITUTE BASED ON THE DANISH MONITORING PROGRAM).

<table>
<thead>
<tr>
<th>Consumer group</th>
<th>Age group</th>
<th>4 - 9 years</th>
<th>10 - 17 years</th>
<th>18 - 75 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN ml/day</td>
<td>P95 ml/day</td>
<td>MEAN ml/day</td>
<td>P95 ml/day</td>
<td>MEAN ml/day</td>
</tr>
<tr>
<td>Men, all</td>
<td>1</td>
<td>-</td>
<td>18</td>
<td>71</td>
<td>706</td>
</tr>
<tr>
<td>Women all</td>
<td>1</td>
<td>-</td>
<td>21</td>
<td>171</td>
<td>550</td>
</tr>
<tr>
<td>Men, consumers only</td>
<td>57</td>
<td>86</td>
<td>217</td>
<td>943</td>
<td>824</td>
</tr>
<tr>
<td>Women, consumers only</td>
<td>26</td>
<td>51</td>
<td>170</td>
<td>429</td>
<td>686</td>
</tr>
</tbody>
</table>

A realistic exposure assessment was performed using the mean concentration of lead in coffee. As a worst case scenario based on a high brand loyalty toward a coffee with a high lead concentration was assumed. That is probably a very conservative assumption as it is likely that coffee wholesalers choose to buy coffee from different producers depending on the price. If that is the case, the mean lead concentration within a single brand will over time move toward the mean concentration of all brands. The highest concentration was 65.3 ng/g. However, as the consumption of this coffee is extremely low (not even quantified) the coffee with a concentration of 23.2 ng/g (sample no. 48) was used in the assessment.

The mean intake of lead from all sources in Denmark is 0.25 µg/g b.w./day which is equivalent to 17.5 µg/person using a standard weight of 70 kg. It has previously been estimated by DTU FOOD that about 40 % of the lead exposure from beverage is from coffee. As 46.6 % of the total intake of lead is from beverages, the intake of lead from coffee should thus correspond to an average intake of 3.3 µg lead/person (or about 20% of the total dietary intake of lead).

Based on the current study the mean intake of lead from brewed coffee is 0.74 µg lead/day (9.1 % of total contribution from beverages) for men 0.58 µg lead/day (7.1 % of total contribution from beverages) for women (see table 6).

TABLE 6. CALCULATED INTAKE OF LEAD IN µG/PERSO N FROM COFFEE, INCLUDING BREWING WATER BASED ON THE AVERAGE MEASURED CONTENT OF LEAD (15.9 NG/G). THE COFFEE IS BREWED USING 65 G COFFEE PER L AND IT IS ASSUMED THAT ALL LEAD (100%) IS EXTRACTED FROM THE COFFEE DURING BREWING.

<table>
<thead>
<tr>
<th>Consumer group</th>
<th>Age group</th>
<th>4 - 9 years</th>
<th>10 - 17 years</th>
<th>18 - 75 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ml/day</td>
<td>P95 ml/day</td>
<td>Mean ml/day</td>
<td>P95 ml/day</td>
<td>Mean ml/day</td>
</tr>
<tr>
<td>Men, all</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.07</td>
<td>0.74</td>
</tr>
<tr>
<td>Women all</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.18</td>
<td>0.58</td>
</tr>
<tr>
<td>Men, consumers only</td>
<td>0.06</td>
<td>0.09</td>
<td>0.23</td>
<td>0.99</td>
<td>0.86</td>
</tr>
<tr>
<td>Women, consumers only</td>
<td>0.03</td>
<td>0.05</td>
<td>0.18</td>
<td>0.45</td>
<td>0.72</td>
</tr>
</tbody>
</table>

For a person with a brand loyalty towards the coffee with the high lead concentration the lead intake from coffee was calculated to be 1.06 µg lead/day (13% of total contribution from beverages) and 0.83 µg lead/day (10% of total contribution from beverages) for men and women respectively (see table 7).
TABLE 7. CALCULATED INTAKE OF LEAD IN µG/PERSON FROM COFFEE, INCLUDING BREWING WATER BASED ON THE HIGHEST MEASURED CONTENT OF LEAD FOUND IN A COFFEE WITH A SIGNIFICANT CONSUMPTION (23.2 NG/G). THE COFFEE IS BREWED USING 65 G COFFEE PER L AND IT IS ASSUMED THAT ALL LEAD (100%) IS EXTRACTED FROM THE COFFEE DURING BREWING.

<table>
<thead>
<tr>
<th>Age group</th>
<th>4 - 9 years</th>
<th>10 - 17 years</th>
<th>18 - 75 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ml/day</td>
<td>P95 ml/day</td>
<td>Mean ml/day</td>
<td>P95 ml/day</td>
</tr>
<tr>
<td>Men. all</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>Women all</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Men. consumers only</td>
<td>0.09</td>
<td>0.13</td>
<td>0.33</td>
<td>1.42</td>
</tr>
<tr>
<td>Women. consumers only</td>
<td>0.04</td>
<td>0.08</td>
<td>0.26</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Not even the 95 percentile of the consumers who always drink coffee with the highest lead concentration will reach 40 % of the lead intake from beverages from coffee. It could be hypothesised that the difference is caused by differences in the lead concentration in the brewing water, but the concentration in water measured in this study of 0.62 ng/ml is similar to the average value for lead in tap water of 0.87 ng/ml as reported in the Danish monitoring study (A.Petersen et al., 2013). The reason for the difference seems to be that the figure used for lead content in ready-to-drink coffee was 6.0 ng/ml. In this study the mean concentration of lead in coffee products was 15.9 ng/ml, which is equivalent to 1.0 ng/ml ready to drink coffee. It has not been possible to find and look through the validation of the result from the high value used in the previous assessment, but it really emphasises the importance of the work done in this study to avoid false positive results and thereby achieve a more accurate estimate of the lead exposure from coffee drinking.

In this study the mean intake of lead from coffee is 4.2 % of total dietary intake of lead for men and 3.3 % for women. Although the total intake of lead in the Danish population is too high from a health point of view, the contribution from coffee is so insignificant that specific consumer guidance concerning intake of the coffee with the aim of decreasing the lead exposure is not considered necessary.

The concentration in ready to drink espresso from cafes was similar to the homebrewed coffee in all but one sample, which had a concentration of 65 ng/ml. Even if a consumer has a daily cup of coffee from this cafe it will not be of health concern. The correlation between tin and lead described earlier indicate that the source of the high value could be a lead containing welding in the espresso machine. The much lower level in a sample taken from the same café at another occasion indicates that the lead concentration is not constant in coffee brewed from this machine. The high concentration may be caused by a recent decalcification of the espresso brewer.

The concentration of lead in coffee in this study is very low compared to the concentrations found in the studies summarised in the introduction. There is no information on the quality assurance in the literature studies including what was done to avoid false positive results. In this study a lot of effort was made to avoid false positive results. The results in this study are therefore considered very reliable even though they do not confirm findings in other studies.

In conclusion, the lead concentrations in coffee found in this study are low and are not considered to present a health risk to the consumer.
References

Ref Type: Generic


Ref Type: Generic


Appendix 1: Quality assurance

**TABEL 2. COFFEE BEANS QUALITY ASSURANCE PARAMETERS: TARGET VALUES AND RANGE, RECOVERY (REC.), RELATIVE STANDARD DEVIATION (RSD), CONCENTRATION IN MEASUREMENT SOLUTION (RAW CONC.), DILUTION FACTOR FOR CONVERSION OF NG/ML IN SOLUTION TO NG/G IN SAMPLE.**

<table>
<thead>
<tr>
<th>QC type</th>
<th>Dilution</th>
<th>n</th>
<th>Rec.</th>
<th>RSD</th>
<th>Raw conc [ng/ml]</th>
<th>Target value [ng/g]</th>
<th>Target range [+/− ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent blank *</td>
<td>1</td>
<td>20</td>
<td></td>
<td></td>
<td>0.161</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Solvent (2% HNO₃+1% HCl) #*</td>
<td>1</td>
<td>36</td>
<td></td>
<td></td>
<td>0.001</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 ng/ml #</td>
<td>1</td>
<td>13</td>
<td>98%</td>
<td>4%</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1 ng/ml *</td>
<td>1</td>
<td>1</td>
<td>97%</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BCR281# (Rye Grass)</td>
<td>967</td>
<td>7</td>
<td>94%</td>
<td>2%</td>
<td>2.5</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281* (Rye Grass)</td>
<td>972</td>
<td>1</td>
<td>95%</td>
<td></td>
<td>2.5</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281+ (Rye Grass)</td>
<td>963</td>
<td>2</td>
<td>97%</td>
<td>3%</td>
<td>2.5</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281# (Rye Grass)</td>
<td>9,675</td>
<td>6</td>
<td>95%</td>
<td>4%</td>
<td>0.2</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281* (Rye Grass)</td>
<td>9,716</td>
<td>1</td>
<td>114%</td>
<td></td>
<td>0.2</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281+ (Rye Grass)</td>
<td>9,631</td>
<td>2</td>
<td>107%</td>
<td>9%</td>
<td>0.2</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR62# (Olive leaves)</td>
<td>7,802</td>
<td>7</td>
<td>104%</td>
<td>2%</td>
<td>3.2</td>
<td>25,000</td>
<td>1,500</td>
</tr>
<tr>
<td>BCR62# (Olive leaves)</td>
<td>78,018</td>
<td>6</td>
<td>105%</td>
<td>5%</td>
<td>0.3</td>
<td>25,000</td>
<td>1,500</td>
</tr>
<tr>
<td>NBS 1572# (Citrus leaves)</td>
<td>4,893</td>
<td>7</td>
<td>99%</td>
<td>4%</td>
<td>2.7</td>
<td>13,300</td>
<td>2,400</td>
</tr>
<tr>
<td>NBS 1572# (Citrus leaves)</td>
<td>48,933</td>
<td>7</td>
<td>97%</td>
<td>2%</td>
<td>0.3</td>
<td>13,300</td>
<td>2,400</td>
</tr>
</tbody>
</table>

Analysis date: 2014-12-01 (#), 2014-12-12 (*) and 2015-01-30 (+)

**TABEL 3. COFFEE DRINKS QUALITY ASSURANCE PARAMETERS: TARGET VALUES AND RANGE, RECOVERY (REC.), RELATIVE STANDARD DEVIATION (RSD), CONCENTRATION IN MEASUREMENT SOLUTION (RAW CONC.).**

<table>
<thead>
<tr>
<th>QC type</th>
<th>Dilution</th>
<th>n</th>
<th>Rec.</th>
<th>RSD</th>
<th>Raw conc [ng/ml]</th>
<th>Target value [ng/g]</th>
<th>Target range [+/− ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent blank*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Solvent (3% MeOH)</td>
<td>1</td>
<td>12</td>
<td></td>
<td></td>
<td>0.0004</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>1</td>
<td>6</td>
<td>121%</td>
<td>17%</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>BCR281</strong></td>
<td>972</td>
<td>1</td>
<td>97%</td>
<td></td>
<td>2.5</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td><strong>BCR281</strong></td>
<td>9,716</td>
<td>1</td>
<td>96%</td>
<td></td>
<td>0.2</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td><strong>BCR281+</strong></td>
<td>963</td>
<td>1</td>
<td>97%</td>
<td></td>
<td>2.5</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td><strong>BCR281+</strong></td>
<td>9,631</td>
<td>1</td>
<td>124%</td>
<td></td>
<td>0.2</td>
<td>2,380</td>
<td>110</td>
</tr>
<tr>
<td>BCR281 mean</td>
<td>4</td>
<td>5</td>
<td>104%</td>
<td>13%</td>
<td>2,380</td>
<td>4</td>
<td>1.2-1.6</td>
</tr>
</tbody>
</table>

* Reagent blank (n=5) for brew method M1, M2, M3, M4 and M5 (brew water pH buffered, filtrated and diluted) all having high lead level due to contaminated pH buffer. Data from 2014-12-12 except marked those marked with (+) which are from 2015-01-30
** Extracted as coffee beans but quantified by the external 3% methanol (MeOH) standard curve.
*** Brew method M1-M5 (n=1) for coffee bean no 49.
### TABEL 1A. MEAN LEAD (NG/ML) IN COFFEE BREWED ON THREE BRANDS OF BEANS USING SIX DIFFERENT HOME BREWING METHODS. METHODS: FILTER MACHINE NEW (M1), FILTER MACHINE PRE-USED (M2), FRENCH PRESS (M3), POT BOILING (M4), MOCHA POT (M5) AND ESPRESSO, HOMEMADE (M6). FOR REPlicate BREWINGS INDICATED BY (N) THE MEAN AND STANDARD DEVIATION IS REPORTED.

<table>
<thead>
<tr>
<th>Bean no.</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 37 (n=2)</td>
<td>1.9 +/-0.2</td>
<td>2.4 +/-0.3</td>
<td>0.8 +/-0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>nd</td>
</tr>
<tr>
<td>No. 49 (n=1)</td>
<td>1.9</td>
<td>4.4</td>
<td>1.0</td>
<td>3.7</td>
<td>3.5</td>
<td>0.3</td>
</tr>
<tr>
<td>No. 17 (n=1)</td>
<td>2.6</td>
<td>3.0</td>
<td>1.2</td>
<td>4.2</td>
<td>3.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Brew, water, filtrated, diluted (n=3)

nd = no data - only one replicate

### TABEL 1A. CARRY-OVER ESTIMATION OF LEAD IN BEANS TO COFFEE DRINK FOR THREE BRANDS OF BEANS USING FIVE DIFFERENT HOME BREWING METHODS. METHODS: FILTER MACHINE NEW (M1), FILTER MACHINE PRE-USED (M2), FRENCH PRESS (M3), POT BOILING (M4), MOCHA POT (M5) AND ESPRESSO, HOMEMADE (M6). TAP WATER CONTENT WAS SUBTRACTED (0.62 NG/ML) AND THE FACTOR 0.065 G BEAN / ML DRINK WAS APPLIED.

<table>
<thead>
<tr>
<th>Bean no.</th>
<th>Bean no. (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean no. 37</td>
<td>20</td>
</tr>
<tr>
<td>Bean no. 49</td>
<td>11</td>
</tr>
<tr>
<td>Bean no. 17</td>
<td>15</td>
</tr>
</tbody>
</table>

Carry-over* 153% 268% 38% 298% 227%

*Calculated as brew concentration / bean concentration (e.g. mean M1 / mean bean).

### TABEL 2A. MEAN LEAD (PB) AND RELATIVE LEVEL OF TIN (Sn) IN ESPRESSOS PURCHASED AT 8 CAFES IN DENMARK.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pb [ng/ml]</th>
<th>Tin (Sn)</th>
<th>No of shot</th>
<th>Espresso machine brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cafe 1</td>
<td>2</td>
<td>1%</td>
<td>Single</td>
<td>La Cimbali</td>
</tr>
<tr>
<td>Cafe 2</td>
<td>4</td>
<td>6%</td>
<td>Single</td>
<td>La Marzocco</td>
</tr>
<tr>
<td>Cafe 3</td>
<td>13</td>
<td>15%</td>
<td>Double</td>
<td>La Maazocco</td>
</tr>
<tr>
<td>Cafe 4</td>
<td>2</td>
<td>1%</td>
<td>Single</td>
<td>Nespesso</td>
</tr>
<tr>
<td>Cafe 5</td>
<td>67 (A) and 11* (B)</td>
<td>100%</td>
<td>Double</td>
<td>La Cimbali</td>
</tr>
<tr>
<td>Cafe 6</td>
<td>1</td>
<td>2%</td>
<td>Single</td>
<td>Coffee Art Plus - Pelican Rouge</td>
</tr>
<tr>
<td>Cafe 7</td>
<td>1</td>
<td>8%</td>
<td>Single</td>
<td>Bentax</td>
</tr>
<tr>
<td>Cafe 8</td>
<td>1</td>
<td>1%</td>
<td>Single</td>
<td>WMF Bistro</td>
</tr>
</tbody>
</table>

*) This espresso was also purchased from café 5 but on a later occasion.
### TABLE 3A: LEAD (Pb mean [ng/g]) in 51 Coffee Bean Purchased in 2014 on the Danish Retail Market including the 9 out of 10 Most Used Brands in Danish Households. Sample Information (Laboratory Number, Coffee Type and Origin) is Linked to the Use in Denmark via EAN-Codes (Institut for Fødevare- og Ressourceøkonomi, 2013). All Lead Values are Associated with Relative Standard Deviation of 23% (RSDPOOLED).

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Country</th>
<th>Continent</th>
<th>Type</th>
<th>Bean</th>
<th>Growth</th>
<th>Rank no.</th>
<th>Use in DK</th>
<th>Pb, mean [ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Ethiopia</td>
<td>Africa</td>
<td>ground</td>
<td>Arabica</td>
<td>3.4</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Various countries</td>
<td>-</td>
<td>ground</td>
<td>Mix</td>
<td>27.7</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>instant</td>
<td>No data</td>
<td>21.9</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>North America</td>
<td>beans</td>
<td>Arabica</td>
<td>-</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Various countries</td>
<td>-</td>
<td>ground</td>
<td>Arabica</td>
<td>2.5</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>India</td>
<td>Asia</td>
<td>beans</td>
<td>Robusta</td>
<td>-</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Peru</td>
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<td>beans</td>
<td>Arabica</td>
<td>-</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mexico</td>
<td>North America</td>
<td>ground</td>
<td>Arabica</td>
<td>19.8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
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<td>Africa</td>
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<td>Arabica</td>
<td>-</td>
<td>8.1</td>
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<td></td>
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<td>51</td>
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<td>-</td>
<td>instant</td>
<td>Arabica</td>
<td>-</td>
<td>8.1</td>
<td></td>
<td></td>
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<td>-</td>
<td>8.2</td>
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<td>14</td>
<td>-</td>
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<td>ground</td>
<td>Arabica</td>
<td>4</td>
<td>384.8</td>
<td>9.1</td>
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<tr>
<td>28</td>
<td>Indonesia</td>
<td>Asia</td>
<td>beans</td>
<td>Arabica</td>
<td>-</td>
<td>9.6</td>
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</tr>
<tr>
<td>50</td>
<td>-</td>
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<td>Variety 1</td>
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</tr>
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</tr>
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<td>-</td>
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</tr>
</tbody>
</table>

- no data
FIGURE 1A. LEAD (MEAN +/- STANDARD DEVIATION POOLED) IN COFFEE PRODUCTS PURCHASED IN 2014 ON THE DANISH RETAIL MARKET FOR WHICH GEOGRAPHICAL ORIGIN WAS DECLARED: CONTINENT, COUNTRY (COFFEE NUMBER).

<table>
<thead>
<tr>
<th>Continental Region</th>
<th>Country/Region</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
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<td>33</td>
</tr>
<tr>
<td>Africa</td>
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<td>49</td>
</tr>
<tr>
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<td>China</td>
<td>14</td>
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<td>Asia</td>
<td>Indonesia</td>
<td>19</td>
</tr>
<tr>
<td>Asia</td>
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</tr>
<tr>
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<td>Brazil</td>
<td>48</td>
</tr>
<tr>
<td>South America</td>
<td>Colombia</td>
<td>46</td>
</tr>
<tr>
<td>South America</td>
<td>Guatemala</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 5. Top 10 coffee bean brands used in Denmark.

1. Institute for Food and Resource Economics, 2013.
Appendix 2: Comments to the extract from the Danish Household Panel (Dansk husholdningspanel, GfK)

Personal communication with Thomas Eisler, Institute from Food- and ressource economy 16/10-2014

Bemærkninger til GfK udtræk vedrørende kaffe

Udtrækket er præsenteret som de enkelte det gennemsnitlige forbrug pr. husholdning af den pågældende varegruppe pr. år. For at undersøge de beregnede mængders robusthed er de beregnet på to alternative måder:
Gennemsnitligt forbrugte mænede(gr.) pr. husstand i GfK-panelet et givet EAN-nummer (g_ean_mgd)
Vægtet gennemsnit af forskellige husholdningstypers forbrugte mængde af et givet EAN-nummer (agg_ean_mgd)

Ved de sidstnævnte beregning er husholdningerne klassificeret i 7 typer, som matcher husstandstyperne i Danmarks Statistik Forbrugsundersøgelser: Enlige under 60 år uden børn; Enlige på 60 år og derover uden børn; Enlige med børn; 2 voksne uden børn hvor hovedpersonen er under 60 år; 2 voksne uden børn hvor hovedpersonen er over 60 år; 2 voksne med børn; og husstande med 3 eller flere voksne. Disse husholdningstyper er vægtet sammen ved at anvende antallet af husstande indenfor de respektive typer, jf. Forbrugsundersøgelsen fra Danmarks Statistik (2011:2013). Et hurtigt blik på tallene tyder dog på en rimelig god overensstemmelse mellem de beregnede andele fra de to beregningsmetoder.
Exposure to lead from intake of coffee

Lead is toxic to human health and the environment. The Danish Environmental Protection Agency has published a survey of lead and lead compounds in 2014 (Environmental Project no. 1539). This survey reports that the blood lead level in the population of Denmark and EU is considered to be within a level that can cause health effects, especially with children. The blood lead levels have, however, been shown a decreasing trend over the past decades.

Food and beverages is one of the primary sources of intake of and exposure to lead, with beverages accounting for almost 50%. Previous studies from Denmark have estimated that the intake of lead from coffee is very high and may contribute to up to 20% of the total lead intake from food and beverages. This estimate is, however, based on older, non-published data. In the current project extensive chemical analyses of coffee beans, drinking water and ready-to-drink coffee have been performed. The results hereof have been compared to calculations of the total intake of lead from food and beverages.

The results show that the intake of lead from coffee is considerably lower than previously estimated and account for 4.2% and 3.3% of the total lead intake from food and beverages for Danish men and women, respectively. It can generally be concluded that the intake of lead from coffee is low in comparison with other types of food, and that it does not constitute a substantial part of the total intake of lead with food and beverages.