

## ARE ELEVATED DIOXIN LEVELS IN MILK OF WOMEN FROM IVORY COAST AND CONGO DUE TO THE USE OF CLAY DURING PREGNANCY?

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### Introduction

At DIOXIN 2010 we reported on the intentional consumption of soil and clay materials by humans, a practice called geophagy. This practice has already been described by Aristotle (1), Dioscorides and Avicenna in 40 BC and 1000 AD respectively (2) and subsequently by anthropologists, colonial physicians and explorers like von Humboldt and Livingstone in South America and Africa respectively (3). The practice has apparently been abandoned in Western societies but it is still very common in Africa. Pregnant women e.g. consume clay as a cure against morning sickness but possibly also as a source of minerals, like iron. Various clay products can be purchased in shops for this reason. Also in the Netherlands and the UK women from ethnic minorities still consume these clay materials. Amounts as high as 300 gram per day were described as being consumed, but average daily intakes seem to be more around 30-80 gram. Whether this practice has actual beneficial effects has never been described.

In 2002 the FSA reported that various clay products in the UK contained elevated concentrations of heavy metals like lead. In the Netherlands the VWA started an investigation and obtained similar results. In addition certain clay products have caused problems in the food chain due to high levels of dioxins. The use of kaolinic clay was quite popular as an aid for mixing vitamins and minerals in animal feed but was terminated after detection of high dioxin levels by Jobst et al. (4). A similar practice occurred in the US where ball clay was used for this purpose. In 2004 the use of kaolinic clay for sorting potatoes, caused a small incident of increased dioxin levels in milk in the Netherlands due to the feeding of dioxin contaminated potato peels to dairy cows (5). Knowing the problems, the VWA also investigated some samples of so-called 'pregnancy clays' for the presence of dioxins. Some of the samples were highly contaminated. Since in Africa this practice is much more common, the question arose whether the clays used in Africa might also be contaminated, implying a potential risk for the unborn child. Therefore, samples were also collected in Africa and examined for heavy metals and dioxins. Some of these samples were shown to contain high levels of dioxins. At DIOXIN 2010 also data were presented of the WHO/UNEP study on human milk samples from different countries, including a number of African countries (6). It was therefore of interest to compare these data in order to evaluate whether there were any indications that the use of contaminated clay may add to the dioxin exposure of children.

### Materials and methods

#### *Materials*

Clay products were obtained at local (ethnic) shops in the Netherlands and various countries in Africa.

#### *GC/HRMS*

GC/HRMS on clay samples was performed by RIKILT as previously described. In brief, samples were spiked with <sup>13</sup>C labeled standards and extracted under elevated temperature and pressure using the ASE (Dionex). This was followed by clean up on the automated PowerPrep™ system (FMS) which was composed of disposable pre-

packed columns (Jumbo silica, mixed bed silica, alumina and carbon) to aid in the isolation of the different fractions. The clean up resulted in the collection of two purified fractions, one with mono-ortho PCBs and indicator PCBs and the other with dioxins, furans and non-ortho PCBs. The fractions were concentrated using the Turbo-Vap (Buchi, Germany) and analyzed by GC-HRMS using an Agilent (Wilmington, USA) gas chromatograph 6890N (GC column DB5 MS 60m, 0.25mm i.d., 0.25 $\mu$ m; J&W, Folson, USA) and an AutoSpec Ultima high resolution mass spectrometer (Waters, Milford, USA) operated in electron impact ionization mode using selected-ion monitoring and controlled by Masslynx data system. HRGC-HRMS data was processed using DIOXNOP software to determine the concentrations and subsequently the TEQ levels were calculated based on WHO TEFs 1998.

GC/MS on human milk samples was performed by CVUA Freiburg as described previously (6).

## Results and discussion:

### *Dioxin levels in clay samples*

Samples of clay were first screened using the DR CALUX bioassay, eight from the Netherlands and 20 from African countries. This revealed a number of suspected samples both from the Dutch market and those collected in different African countries. The suspected samples were further investigated using GC/HRMS. Four of these samples showed relatively high levels of 66 (Mabele Cameroon, sampled in the Netherlands), 66 (Mabele, Democratic Republic of Congo (DR Congo), sampled in the Netherlands), 75 (Kaolin, Côte d'Ivoire) and 103 ng TEQ/kg product (labeled as Mabele sale, sampled in the Netherlands, country of origin unknown). Only PCDDs contributed to the levels but patterns were not the same for all clay samples. Figure 1 shows the pattern for the PCDD/Fs in these samples. In addition there was a sample from Nigeria (Nzu) with a level of 24 ng TEQ/kg product and another sample from Cameroon (Mabele) with a level of 4.5 ng TEQ/kg product. Levels in other samples were much lower, showing that not all clays contain high levels of dioxins.

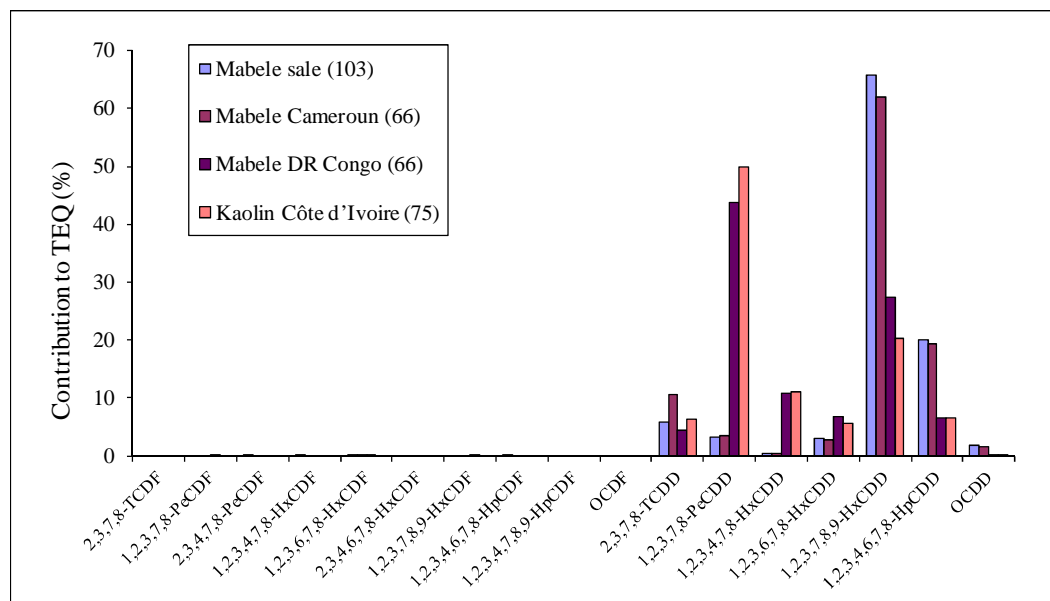


Figure 1. PCDD/F patterns in a number of clay samples collected in The Netherlands (Mabele clays) and Côte d'Ivoire. Patterns are presented in terms of contribution to the dioxin TEQ level (total TEQ-level in parenthesis in the legend in ng TEQ/kg product).

### Dioxin levels in human milk samples from Africa

Pooled human milk samples from different African countries were analyzed for PCDD/Fs. Highest levels were observed in samples from Côte d'Ivoire and the Democratic Republic of Congo, being respectively 11.1 and 12.5 pg TEQ/g fat. The milk sample from Senegal showed a level of 7.2 pg TEQ/g fat but that from other countries were much lower, being in the range of 1.5 to 3.9 pg TEQ/g fat. Figure 2 shows the patterns observed in the human milk, expressed as the relative contribution of different PCDD/Fs to the TEQ-level. Although in most samples also 2,3,4,7,8-PeCDF contributes significantly, it is clear that the PCDDs contribute most to the TEQ, varying from 57% for Nigeria to 85 and 90% for Côte d'Ivoire and DR Congo. Another interesting observation is the high contribution of 1,2,3,7,8,9-HxCDD in the sample from DR Congo. The two highest human milk samples were therefore examined in more detail and compared to the congener patterns observed in the different clays.

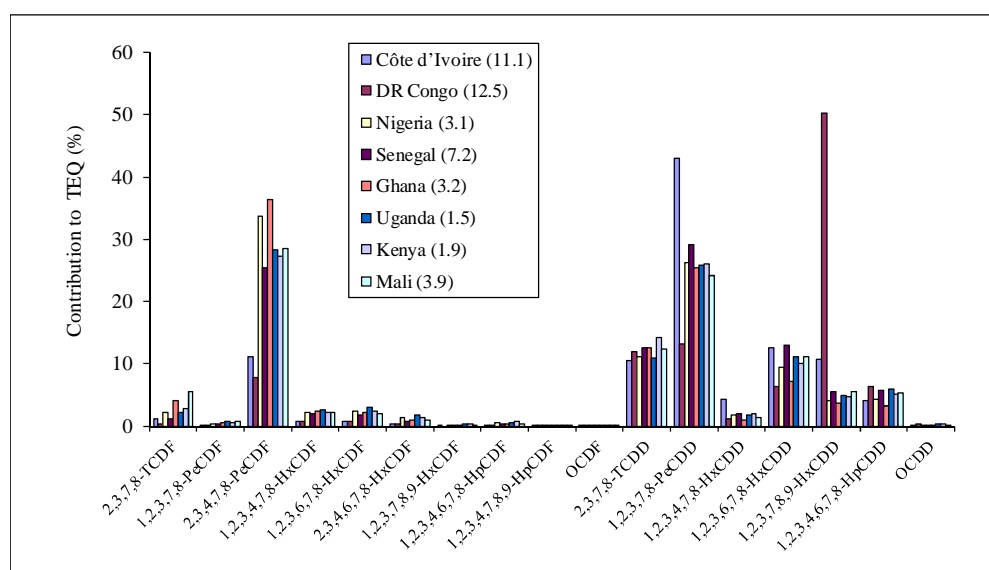


Figure 2. Congener patterns observed in different African human milk samples, expressed as contribution to the PCDD/Fs TEQ level (dioxin TEQ levels in parenthesis in the legend in pg TEQs/g fat).

Figure 3 shows the congener pattern of human milk from DR Congo compared with three different clays that were characterized by a very specific high contribution of 1,2,3,7,8,9-HxCDD. The patterns are remarkably similar and suggest a contribution of the intake of contaminated clay to the relatively high dioxin levels in human milk.

A similar comparison was made for the milk sample from Côte d'Ivoire (Figure 4). The pattern was highly comparable with the kaolinic clay used in the 2004 contamination incident in the Netherlands and the clay collected from Côte d'Ivoire and labeled kaolinite. Also the Mabele clay from DR Congo showed a close resemblance. It should however be pointed out that this congener pattern seems a bit less specific than the one observed in the milk from the DR Congo (see Figure 2).

### Conclusion

Both the relatively high levels of PCDDs in human milk from Congo and Ivory Coast and the similarity of the congener patterns with those from the clays strongly suggest that the use of clays during pregnancy contributes to these high levels in the milk. Regarding the susceptibility of the developing fetus and young child to dioxins the use of contaminated clays should be prevented.

### Acknowledgements:

We gratefully acknowledge the support of the National Coordinators for collection of the human milk samples and funding from the Secretariat of the Stockholm Convention on Persistent Organic Pollutants..

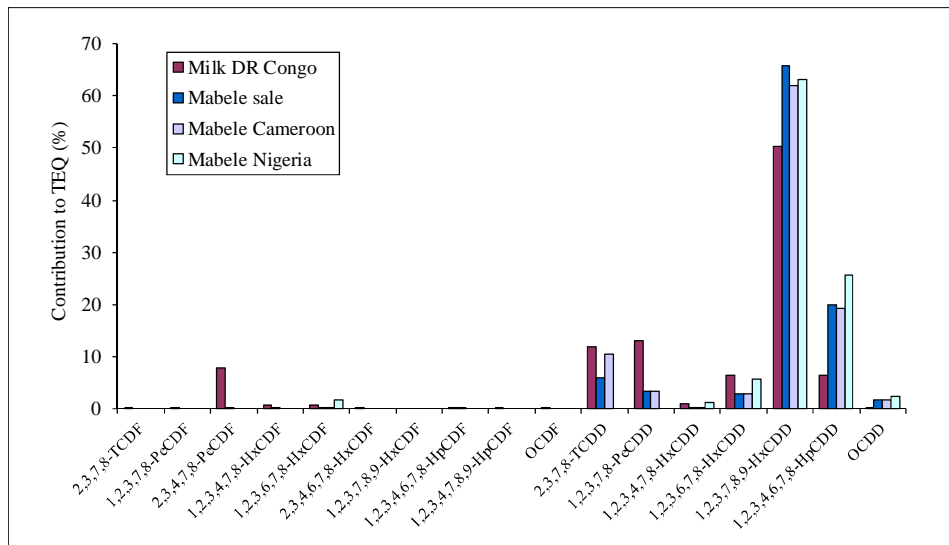


Figure 3. Comparison of the congener pattern in human milk from the Democratic Republic of Congo with the patterns observed in three different clay samples.

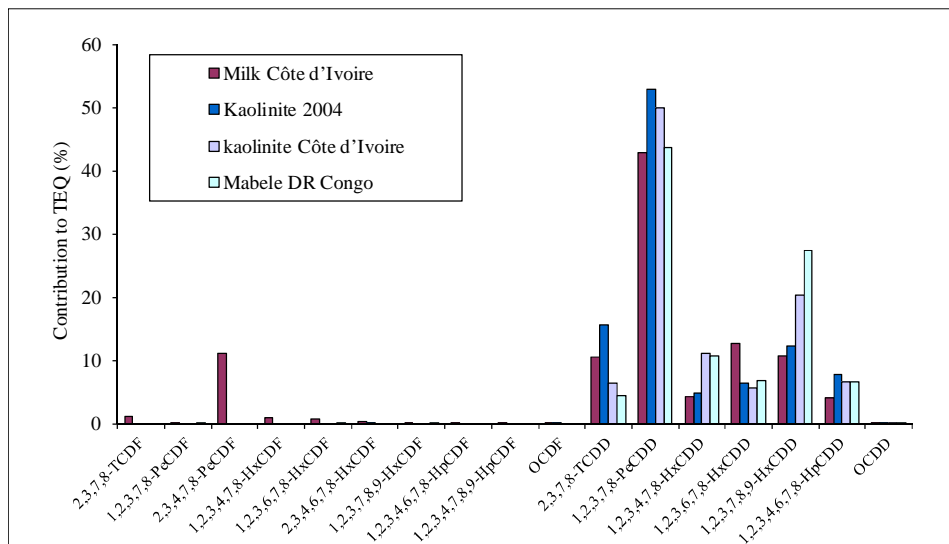


Figure 4. Comparison of the congener pattern in human milk from Côte d'Ivoire with the patterns observed in three different clay samples.

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