Observation of an Extremely High Dioxin Level in a Human Serum Sample from Ukraine by DR CALUX® which was Confirmed to be 2,3,7,8-Tetrachlorodibenzo-p-dioxin by GC-HRMS.

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Introduction

In the fall of 2004 a Ukrainian politician was hospitalized in the Rudolfinerhaus of Vienna General Hospital, suffering from severe abdominal and back pains, with an unknown etiology. In November of 2004 facial images of the Ukrainian politician were circulating on the internet, which clearly showed acnegenic disfiguration of the face. The facial disfiguration resembled a syndrome called "chloracne", which might have been caused by acnegenic chemicals, such as dioxin. In late November, BioDetection Systems (BDS) contacted the Viennese doctors who were in charge of treating the Ukrainian patient and offered to analyze a blood sample from the patient for the presence of dioxins, or dioxin-like chemicals. A serum sample of patient VY was analysed for dioxin, or dioxin-like activity by BDS' DR CALUX® bioassay and confirmatory analysis was performed in two laboratories by GC-HRMS analysis. Here we report on the results obtained on the serum sample of the patient VY.

Materials and Methods

On Tuesday December 6th of 2004 a DHL package arrived at BDS in Amsterdam, containing two tubes, one filled with about 8 ml of serum and one filled with about 8 ml of blood from the Ukrainian patient, VY. A CD-ROM containing information on the facial and chest disfiguration of the skin was also included. An aliquot of 2 ml was taken from the serum containing tube for solvent extraction and silica-sulphuric-acid clean-up according to BDS' low volume serum protocol[1]. The final residue was taken up in 8 μl of DMSO.

BDS' DR CALUX® assay:

Final serum residue was assessed using the DR CALUX® bioassay, consisting of a rat hepatoma H4IIE cell line stably transfected with an AhR-regulated luciferase gene construct. Conditions for cell culture and a procedure for the DR CALUX® bioassay has been described in detail elsewhere[1]. Data are expressed as pg DR CALUX®-TEQ/g fat, using a serum fat percentage of 0.78% determined by BDS. To evaluate the stability of the DR CALUX®-TEQ activity, measurements were performed at 24 and 48 h of exposure of the cells to the serum residue. Repetition of the extraction, clean-up and analysis of the serum sample was performed, due to the fact that initial results obtained were too high to allow adequate quantification.

GC-HRMS confirmatory analysis:

One ml aliquots were taken from the serum containing tube of patient VY and sent to two GC-HRMS laboratories for confirmation analysis. These laboratories were the RIKILT Laboratory for Food Safety, Wageningen, The Netherlands and the Eurofins/GfA laboratory for dioxin analysis in Münster, Germany.

At RIKILT a mixture of $^{13}C_{12}$-labelled dioxins and dioxin-like PCB standards were added to the one ml aliquot of serum. After incubation, 2 ml of isopropanol was added, followed by two times extraction with 4 ml hexane/diethyl ether (97:3 v/v). The hexane fraction was cleaned up on an alumina column and fractionated on a PGC carbon column. The final residue was concentrated to 10 μl and analysed by GC-HRMS with a mass resolution of 10 000.