# EXPRESSION OF CYP1A1 AND 1B1 mRNA IN BLOOD LYMPHOCYTES FROM TWO DISTRICT POPULATIONS IN SLOVAKIA COMPARED TO TOTAL TEQS IN BLOOD AS MEASURED BY THE DRE-CALUX® ASSAY.

Rocio F.Canton[1], Harry T. Besselink[2],J. Thomas Sanderson[1], Sara Botschuyver[2], Bram Brouwer[3], Martin van den Berg[1]

[1]Institute for Risk Assessment Sciences, IRASUtrecht University, Utrecht, The Netherlands.
[2]Biodetection Systems B.V. (BDS) Badhuisweg 3, 1031 CM Amsterdam, The Netherlands.
[3]Institute for Environmental Studies, Vrije Universiteit, De Boelelaan 1115, 1081 HV Amsterdam, The Netherlands.

## Introduction

Cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) are members of the P450 1 family and are involved in the bioactivation of a broad range of xenobiotics and endogenous compounds such as estrogens. Induction of these enzymes has been associated with a various biological and toxicological responses, including carcinogenesis.
Both genes are dioxin-inducible genes and are regulated by the aryl hydrocarbon receptor (AhR) which shows a high binding affinity for certain chlorinated dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). The induction of these two enzymes is one of the more sensitive biological effects of dioxin-like compounds. Thus, the measurement of CYP1A1 and CYP1B1 in humans and wildlife is considered to be useful in establishing relationships between dioxin exposure and possible low-level adverse health effects.
In vitro studies have demonstrated a dose-dependent induction of CYP1A1 and CYP1B1 (mRNA??) in human peripheral lymphocytes by TCDD (Spencer et al., 1999). This suggests the possibility of using CYP1A1 or CYP1B1 mRNA levels in blood cells as a biological marker of exposure of AhR ligands such as dioxins, PCBs and polycyclic aromatic hydrocarbons (PAHs). A recent study of Chinese coke oven workers indeed associated these CYP1A1 and 1B1 mRNA levels with elevated exposure to PAHs (Hanaoka et al., 2002)
The objective of our present study was to quantify CYP1A1 and CYP1B1 mRNA levels in human lymphocytes from two different human populations in Slovakia. The blood samples were collected from an area that was highly PCB-polluted and one intended to represent background exposure in the rest of the country.
We are presently evaluating the CYP1A1 and 1B1 mRNA levels in blood lymphocytes from these two populations as possible biomarkers of human exposure to dioxin and related compounds. We determined the mRNA levels of CYP1A1 and CYP1B1 by a quantitative reverse transcription-PCR method in these lymphocytes. In addition, we examined if there was an association between the individual level of CYP1A1 and 1B1 expression, which could be expected based on the similar mechanism of induction mediated via the AhR.
We also examined a possible relationship between CYP1A1 and 1B1 mRNA levels and TEQs in blood plasma as determined using an Ah receptor-dependent reporter (DRE-CALUX) system.

## Methods and Materials

Study population and blood sampling:
This study included 300 individuals from whom blood samples were taken in duplicate. The people were selected in equal numbers from two differents areas in Slovakia, namely "*Michalovce District*, a polluted area, and "*Stropkov District*" a less polluted reference area.
The blood samples were collected in an EDTA-2Na tube, and fractionated into a buffy coat, plasma and red blood cells by centrifugation. White blood cells were collected from the buffy coat by osmotic hemolysis. The samples were stored at –70 ºC until further treatment.
RNA extraction:
The total RNA was isolated with chloroform and isopropanol using a procedure as described by "Eurogentec" (RNA Instapure, Eurogentec, Maastricht, The Netherlands). Isolated RNA was resuspended in 30µl. of sterile water, quantified by measuring absorbance at 260 nm with a UV-visible recording spectrophotometer (Shimadzu corporation, Kyoto, Japan) and stored at –70ºC prior to analysis. Sufficient

total RNA was obtained from these lymphocytes for simultaneous quantification of CYP1A1 and CYP1B1 genes with QRT-PCR.

TaqMan technology (QRT-PCR):
CYP1A1 and 1B1 mRNA levels were determined by TaqMan technology (QRT-PCR) using an ABI Prism 7000 Sequence Deterction System (PE biosystems). The principle of this technique was described elsewhere (Gibson and Heid,1996). Shortly, this technology uses a fluorogenic probe to generate a sequence-specific fluorescent signal during the PCR. When the cycle-dependent increase of measured signal is statistically significant, the cycle number at which this occurs is defined as the Ct value; the Ct value assigned to a particular sample measurement thus reflects the point during the reaction at which a sufficient number of amplicons have accumulated. Hence, the Ct value is inversely related to the starting number of copies of the target sequence. High number of starting copies of the gene gives low Ct values and vice versa. Primers and internal probes for amplification of CYP1A1 and CYP1B1, as well as for the endogenous gene β-actin, were designed with Primer Express software (PE, Applied Biosystem, Nieuwekerk a/d IJssel, The Netherlands), which selected the theoretically optimized sequences for this system.
Statistical analysis:
The expression of the CYP1A1 and 1B1 genes from these 300 individual blood samples was calculated by using a relative quantification method, as described elsewhere (Comparative Ct method, User Bulletin ·2, PE Biosystems). Briefly, in this method the amount of copies (target) is normalized to a constant amount of copies from an endogenous reference (β-actin) and relative to a standard. The Ct values for the endogenous gene expression levels (β-actin) were subtracted from the Ct values determined for CYP1A1 and 1B1 (ΔCt) and then compared with the standard value (ΔΔCt).
Standard data were obtained by using a serial dilution of mRNA from TCDD-induced human cells (MCF-7 cells) due to the fact that there was no information available about the actual amounts of CYP1A1 and 1B1 mRNA in the blood samples.
Ah receptor-dependent reporter assay:
This assay which determines the amount of dioxin or dioxin-like compounds in a given matrix is rapid, straight forward and is described elsewhere (Murk et al., 1996). After sample collection, a simple extraction method is used to extract the dioxins and other dioxin-like compounds. The extract is cleaned-up and fractionated if necessary, after which the extract is dissolved in DMSO. BDS DR-CALUX® cells are cultured and finally grown in 96-well plates under standardised conditions. Once a confluent monolayer is obtained, the cells are exposed to the diluted cleaned extracts for 6-48 hrs. After lysis and addition of luciferin, the luciferase activity is quantified using a luminometer. The detected luminescence from the analysed samples is compared to that from a TCDD standard curve.to determine the amount of TCDD TEQs in the sample.

## Results and Discussion

In the present study, CYP1A1 and 1B1 mRNA was constitutively detected in human lymphocytes from both populations using Taqman-PCR technology.
The degree of expression in human blood of CYP1A1 and 1B1 mRNA varied up to 10-fold among individuals, regardless of their origin. The variation in mRNA levels could not be explained by geographical origin as we did not observe significantly increased expression of CYP1A1 or 1B1 in the region with high PCB pollution (CYP1A1 $R^2$=0.02, $p<0.0002$; CYP1B1 $R^2$=0.002, $p<0.001$).
On the other hand, when we compared the intraindividual expression of both genes a distinct relationship was observed between the two genes, reflecting the similar pathway of gene regulation as was expected based on the mechanism of action of the AhR (Figure 1).
It was shown (Safe, 1994) that most of toxic actions caused by `dioxin-like' compounds (such as PCDDs, PCDFs and PCBs) are mediated via the AhR signal transduction pathway. In order to obtain information on the individual exposure of the studied populations, dioxin-like toxic equivalents (TEQs) were determined) using a genetically modified mammalian cell line (DRE-Calux), (**BioDetection Systems b.v.** Amsterdam, The Netherlands). As with CYP1A1 and 1B1 levels total TEQs in plasma could vary up to 10-fold. However, we did not find statistically significant increases in TEQs in plasma samples from the region with high PCB pollution ($R^2$= 0.014, $p<0.002$).

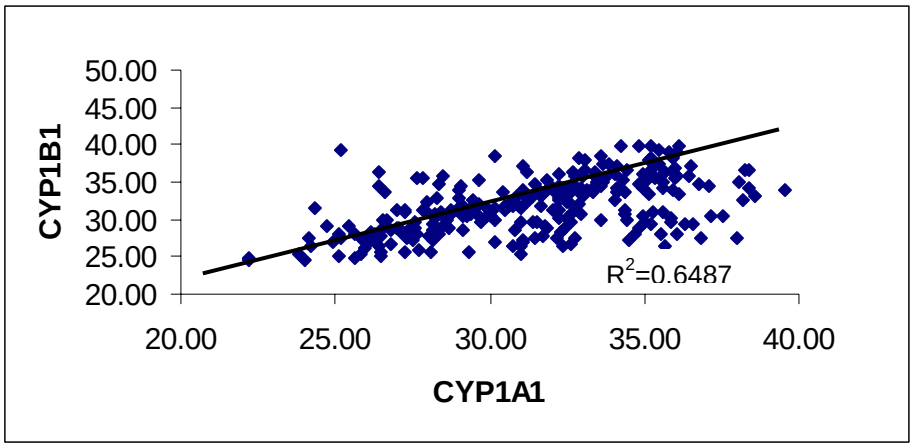


**Figure 1**. Representative Ct values for CYP1A1 and CYP1B1.

We also did not find a relationship between CYP1A1 and/or CYP1B1 expression levels and total TEQs when comparing all individuals from the two areas (CYP1A1, $R^2$=0.003, p<0.001; CYP1B1 $R^2$= 0.01, p<0.01).

In conclusion, the TaqManRT-PCR method is useful to determine changes in very low levels of messenger RNAs, such as those of the human genes of CYP1A1 and 1B1 in human blood. The interindividual levels of CYP1A1 and 1B1 mRNA were highly variable, which may be due to a number of factors such as interindividual genetic differences in response to exposure, but also factors such as smoking and diet. One major individual factor could by the polymorphisms in CYP1A1 and 1B1 genes that have recently been identified, although until now it is not known if there is a relationship between these polymorphisms and CYP1A1 and 1B1 expressions (K.Inoue et al., 2000).

Further studies are needed to identify the possible role of CYP1A1 and 1B1 expressions in blood as biomarkers for exposure to dioxin like compounds in relation to polymorphism.

**Acknowledgements**:

We wish to thank Tomas Trnovec and Jan Petrick who supllied the blood samples. This work was supported financially by European Union project (PCBRISK) number QLK4-CT-2000-00488.

**References**:

1. DianeL.Spencer, Scott A., Masten, Karh M.,Lanier, Xinping Yang, Jean A.Grassman, Chris R.Miller, Thomas R.Sutter, George W.Lucier, and Niger J. Walker (1999) "Quantitattive analysis of constituteve and 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1B1 expression in humn lymphocytes" *Cancer Epidemiology, Biomarkers and Prevent.* vol. 8, pp.139-146
2. Gibson and Heid. (1996) U.E.Gibson, C.A.Heid and P.M.Williams, A novel method for real time quantitative RT-PCR. Genome Res 6 (1996), pp. 995-1001
3. Hanaoka et a., (2002) "Cytochrome P450 1B1 mRNA levels in peripheral blood cells and exposure to polycyclic aromatic hydrocarbons in Chinese coke oven workers". *The Science of the total Environment*. vol.296, issues 1-3, pp.27-33
4. Inoue K., T. Asao and T. Shimada (2000) "Ethnic-related differences in the frequency distribution of genetic polymorphisms in the CYP1A1 and CYP1B1 genes in Japanese and Caucasian populations" *Xenobiotica*, vol.30, no.3, pp. 285-295
5. Kenneth J.Livak and Thomas D, Schmittgen (2001)"Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method" Methods 25,pp.402-408.
6. Masahi Iwanari, Miki Nakajima, Ryoichi Kizu, Kazuichi Hayakawa and Tsuyochi Yokoi (2002) "Induction of CYP1A1, CYP1A2 and CYP1B1 mRNAs by nitropolycyclic aromatic hydrocarbons in various human tissue-derived cells: chemical-cytochrome P450 isoform-, and cell-specific differences" *Toxicokin. and Metab*. Archives of toxicology
7. Murk,A.J.,Legler,J., Denison,M.S., Giesy,J.P., vanden Guchte,C., and Brouwer,A. (1996) Chemical-Activated Luciferase Gene Expression (CALUX): "A novel in vitro bioassay for Ah receptor active compounds in sediments and pore water". *Fundam.Appl.Toxicol*.33,149
8. Nigel J. Walker (2001) "Real-Time and Quantitative PCR: Applications to mechanism-based toxicology" *J.Biochem.Molecular Toxicology*. vol.15, number 3, 2001

9. Safe et al., (1994) "Nonsteroidal estrogens antagonists: mechanism of action and health implications" *J. Natl. Cancer Inst*. 86(23), pp. 1741-2
10. Spencer et al., (1990) D.L. Sutter, Y.M. Tang, C.L. Hayes, Y.Y. Wo, E.W. Jabs, X.Li, H.Yin, C.W.Cody and W.F. Greenlee, "Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2". *J.Biol.Chem* 262 (1994), pp.13092-13099