# Skin manifestations in German workers with high occupational PCB exposure

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**Introduction**

Polychlorinated biphenyls (PCB) are notorious environmental pollutants and since 2013 classified as human carcinogen group 1 by the International Agency for Research on Cancer (IARC).

Exposure in the normal population occurs mainly through ingestion (90%), however, other roots of exposure (inhalation and through dermal contact) play an important role during occupational exposure[1]. Skin manifestations are one of the first and most visible symptoms of PCB/dioxin exposure.

Studies on populations from the Yusho (Japan 1968) and Yucheng (Taiwan 1978) incidents showed skin manifestations like chloracne and pigmentation of the skin.[2, 3]

Not much is known about the long-term effects of PCBs exposure on the development of cutaneous malignancies. One study found a strong association between non dioxin-like (NDL)-PCB levels and the risk of developing a cutaneous malignant melanoma.[5]

Cutaneous absorption of PCBs was previously observed in animal studies revealing a dynamic time relapse for PCBs between initial skin contact and skin absorption. Analysis showed that 24 h after skin contact only 25% of the initially applied PCBs could be recovered.[4] Since PCBs are strongly lipophilic, a more lipid containing medium like oils in transformers would most probably result in a higher absorption factor.

In spring 2010 high internal exposures for PCBs were discovered in workers of a transformer recycling company in Germany, where PCB-contaminated material was not handled according to proper occupational hygiene.[6]

After ambient and first human biomonitoring studies a comprehensive follow up monitoring and prospective surveillance program for (former) workers, their family members and relatives and subjects working or living in the surroundings of the company was initiated.[6]

In this study, as part of the surveillance program, our aim is to identify effects of dioxin-like (DL) and NDL-PCBs on the skin as well as to examine the expression of genes in the skin on mRNA level in order to provide more insight in the pathophysiological effects of PCBs on the skin.

**Materials and methods**

As a part of the surveillance program[6] yearly blood samples (between July 2010 and October 2012) were obtained from all participants of this PCB exposed cohort. In order to determine the internal exposure to PCB, the serum samples were analyzed for the 6 indicator PCB (28, 52, 101, 138, 153, 180) as well as for 12 coplanar PCB (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) [7]. The limit of detection (LOD) based on a signal-to-noise ratio of 3 was determined to be 0.01 µg/L serum for all investigated analytes. Samples exceeding the calibration range (up to 3 µg/L plasma) were reanalyzed using a smaller volume of plasma. For quality control purposes, bovine serum was spiked with all analytes at a concentration of 0.4 µg/L and included in every analytical series. [7]

The participants underwent a physical examination by a trained physician and dermatologist with an emphasis on the skin using dermatoscopy. PCB-induced suspect skin lesions of exposed persons were photographed.
36 participants with suspect skin lesions approved for a biopsy after informed consent. After local anesthesia with 1% prilocain 4 mm punch biopsies were taken. Biopsies were cut in half and used for RNA isolation and histological analysis, respectively. For RNA isolation tissue samples stabilized in RNAlater (Qiagen, Hilden, Germany) were mechanically disrupted and homogenized by using a tissue lyzer (Qiagen, Hilden, Germany). Total RNA was extracted with Nucleo Spin RNA II (Macherey-Nagel, Düren, Germany) according to the protocol of the manufacturer. Purified RNA was reverse transcribed and taqman application was performed. TaqMan experiments were carried out on an ABI Prism 7300 sequence detection system (Applied Biosystems, Weiterstadt, Germany) using Assays-on-Demand gene expression products for AhRR (Hs01005075_m1), CYP1A1 (Hs00153120_m1), CYP1B1 (Hs00164383_m1), CCL7 (Hs00171147_m1), CCL20 (Hs01011368_m1), CXCL1 (Hs00236937_m1), CXCL2 (Hs00601975_m1), interleukin (IL)-1β (Hs00174097_m1), IL-6 (Hs00985641_m1), IL-8 (Hs00174103_m1), MMP-9 (Hs00234579_m1). RNA of human adult normal skin tissues (n = 8) served as control RNA.

**Results and discussion**

In our cohort of 304 exposed participants, 1 was diagnosed with chloracne (fig. 1). We observed no involvement of the meibomian glands and there were no cysts observed on the scrotum, as seen in other study. [8]
Hyperpigmentations were seen in 37 participants. 10 participants showed unspecific facial papules and pustules.
PCB serum levels were significant elevated in our mainly DL-PCB exposed and mainly NDL-PCB exposed group as compared to control individuals (fig. 2).
Skin biopsies or excisions of skin lesions were performed in 36 participants. One participant showed a cutaneous lymphoma (mycosis fungoides) and one was diagnosed with basal cell carcinoma.
Preliminary results show a significant relation of all measured PCB congeners and the appearance of hyperpigmentation. A significant relation with the appearance of papules or pustules on the skin was related to PCB 52, 101 and 169.

Exposure to higher levels of PCBs is associated with occurrence of skin lesions [2,3,9], as well as with a higher incidence of malignant melanoma[5]. A recent study showed an upregulation of CYP1A1 in the skin, as well as a 4-fold level of TCDD in the skin compared to serum concentrations in a patient with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) intoxication[10]. In this study however, the mentioned patient was exposed to a (very high) single oral dose of TCDD while our patients had a more chronically, lower dose, exposure to multiple PCB congeners.
Preliminary results of our study showed that DL-PCB exposure was significantly related to an increased expression of CYP1A1 ($p<0.05$) and AhRR ($p<0.001$) (fig. 3), which is in agreement with the abovementioned study of Saurat et al. [10]
Cyp1B1 and IL-1ß were not significantly related to DL-PCB exposure.
It has been hypothesized that skin-expressed CYP1A1 plays a direct role in the metabolism of dioxin, which adds to the xenobiotic metabolism in the liver. [10]
Another important finding of our study is the non-significant upregulation of MMP-9 (fig. 3) in relationship with DL-PCB exposure. Upregulation of MMP-9 might be an important factor in the carcinogenetic properties of PCBs. Other studies showed that upregulation of MMP-9 was associated with a poor outcome in malignancies [11]
For NDL-PCB exposure we could not find any relationship with the mentioned marker genes (CCL7, CCL20, CXCL2, IL-6) in the skin.

In this work, as part of a surveillance program on 304 PCB exposed workers, we identified an upregulation of marker genes in the skin for DL-PCB exposed participants, providing the background for further ongoing work on mechanism of PCB-induced toxicological effects on gene level. Upregulation of MMP-9 might be an important factor in the carcinogenetic properties of PCBs. A long term follow up will be performed in order to examine the long term effects of PCB exposure on the skin especially to detect malignancies like malignant melanoma.

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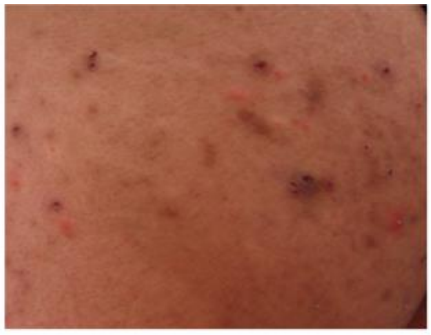

**Figure 1. Chloracne.**
Acne-like eruptions with comedones, pustules and cysts were seen in one of the participants.

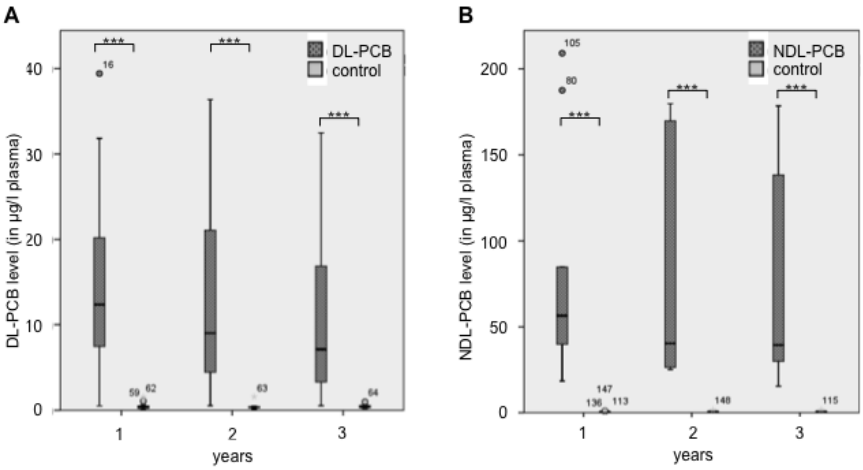


**Figure 2. PCB plasma level of the DL-PCB and NDL-PCB cohort.** (**A**) DL-PCB plasma level (sum of PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) of the predominantly DL-PCB exposed individuals (DL-PCB) compared to the control group (control). (**B**) NDL-PCB plasma level (sum of indicator PCB 28, 52, 101, 138, 153, 180) of the predominantly NDL-PCB exposed individuals (NDL-PCB) compared to the control group (control). Values of $p<0.001$ = *** were considered as significant.

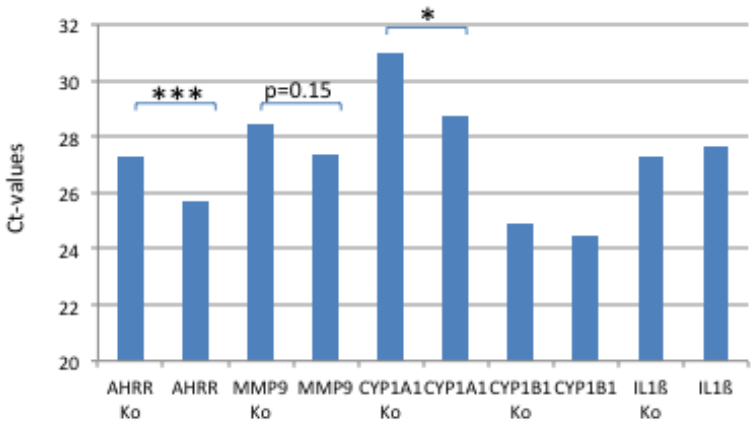


**Figure 3. Gene regulation in PCB exposed human skin.** Gene regulation on mRNA level in skin lesions of DL-PCB exposed cohort (n = 36) versus skin of control individuals (Ko, n = 8). Expression of the indicated genes was analysed with Taqman real-time PCR. Values of $p<0.05$ = * were considered as significant.