



## Reply to letter to the Editor

**Reply to Koppe and Ten Tusscher's letter to the Editor concerning the use of human peripheral blood lymphocytes to determine relative effect potencies for dioxin like compounds**


Dear Editor,

Koppe and Ten Tusscher express their concern with our recent paper about differential relative effect potencies of some dioxin-like compounds in human peripheral blood lymphocytes and murine splenic cells (van Ede et al., 2014). Their main concern appears to be related to the use of human peripheral blood lymphocytes (PBLs) as an experimental model to determine relative effect potencies (REPs) for dioxin-like compounds (DLCs). According to Koppe and Ten Tusscher, only a small subset of helper Th17 cells within the PBLs express the aryl hydrocarbon receptor (AHR), which weakens this model for testing effects of dioxin-like compounds in humans and challenges the results and conclusion of our paper. They suggest the use of polymorphonuclear leukocytes (PMNs).

The authors agree with Koppe and Ten Tusscher that within the population of peripheral blood lymphocytes, expression of AHR is primarily restricted to the subpopulation of CD4<sup>+</sup> Th17 cells (Veldhoen et al., 2008). Nonetheless, it must not be ignored that upon isolation, other cell types are also present in the upper layer of the Ficoll, like regulatory T cells, Th subset cells (depending on individual background), B cells as well as monocytes and dendritic cells that also express the AHR (Lawrence and Kerkvliet, 2007; Quintana et al., 2008; Sherr and Monti, 2013). However, the latter two cell types do not belong to the lymphoid branch and in this perspective, we realize that the name PBLs might have been confusing. In retrospect, the name peripheral blood mononuclear cells (PBMCs) would have been more appropriate in our paper.

Having said this, we like to emphasize that our aim was to determine relative effect potencies of DLCs and compare these between two species, namely human and mouse. Our aim was never to identify AHR functionality in different subpopulations of the immune system nor to predict human *in vivo* immune responses. Therefore, we disagree with Koppe and Ten Tusscher that PMNs should have been used as an *in vitro* model to determine REPs. Nonetheless, Koppe and Ten Tusscher raise a relevant question: do the REPs determined in our PBMC model, provide an adequate prediction of REPs in other (immune) cells of the human body? Without a doubt, tissue-specific differences due to variations in regulation of cofactors (e.g. ARNT), chaperone proteins or other intrinsic AHR factors may modulate AHR functionality. However,

such differences would not only affect AHR activation by DLCs, but also activation by the reference compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In this respect, the arguments of Koppe and Ten Tusscher fall short in relation to our aim of the study to determine relative effect potencies of DLCs in two species. The fact that only a subpopulation of the PBMCs may have a functional AHR response could have influenced the limit of detection of our experiments, but not our measured REPs for DLCs. Apparently, Koppe and Ten Tusscher fail to recognize that TCDD was always used simultaneously as a reference to determine REPs for other DLCs. Therefore, we still have confidence in our conclusions that the REPs observed for the DLCs tested are a realistic representation for human and rodent REPs.

We would like to add, that this conclusion is strengthened by the fact that for example a lower human REP for PCB 126 is not confined to our experimental model with PBMCs, but has also been found in other studies using human primary hepatocytes, keratinocytes and hepatoblastoma cells (Silkworth et al., 2005; Sutter et al., 2010; Zeiger et al., 2001). Therefore, our stance remains unchanged that human cells respond differently to some DLCs than rodent cells and this should be included in future risk assessments of DLCs.

### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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