Polycyclic Aromatic Hydrocarbons (homocyclic PAHs) are known ubiquitous environmental contaminants. Humans are exposed from various sources including food. Because of their carcinogenic properties, PAHs have been widely studied and are currently strictly regulated. Recently, PAH-nitro derivatives, also called PANHs or Azaarenes, have been reported to occur at low levels in food materials. These molecules are structurally very similar to PAHs (carbon substituted by a nitrogen) (Figure 1) and have been anticipated to exhibit similar or higher toxic potential. However, very little information is available on their toxic properties as compared to PAHs. It is currently very difficult to establish the level of safety concern associated with the presence of PANHs in food. In this context, the main objective of the present work was to compare the in vitro toxicity properties of PANHs with their respective PAH structural analogues.

**METHODODOLOGY**

Several PANHs and PAHs structural counterparts were selected (Table 1). They were studied using a battery of in vitro toxicity bioassays (Table 2 & 3). Benzo(a)pyrene (B(a)P) was used as positive/prototypic control. Based on the literature, the most relevant in vitro bioassays endpoints were the following: (1) nuclear receptors activation/inhibition (Estrogen receptor α (ERα), Estrogen receptor β (ERβ), Androgen receptor (AR), Aryl Hydrocarbon Receptor (AhR)) using CALUX assays, (2) genotoxicity potential in absence and presence of metabolic activation (S9) (Gadd45α using the Bluescreen (Gentronix), p53 induction using the CALUX assay and Histone phosphorylation (H2AX) (ToxInsight-ThermoFisher)), and (3) cell viability in absence and presence of S9 (mitochondrial, lysosomal activity and protein synthesis effect) from Xenometrix.

**RESULTS**

Dose-response curves for each molecule were obtained in each bioassays. In house and CRO’s data were concordant. The results obtained for the reference compound B(a)P were in agreement with reported data from the literature confirming the suitability of the battery of tests.

**Cytotoxicity:** there was no specific tendency in cytotoxicity potency with respect to the ring number or PANHs vs PAHs (Table 2).

**Genotoxicity:** there was no simple trends in genotoxicity with respect to the ring number (although the most potent were amongst the highest number of ring) or PANHs vs PAHs. Metabolic activation was a pre-requisite step (Figure 1 and Table 2).

**Nuclear receptor-mediated effects:**

- AhR was the most sensitive parameter with a direct correlation between AhR activation potency and the ring number (Fig. 2a).
- Inverse correlation between anti-AR activity and ring number (Fig 2b).
- Only one compound with anti-ERα activity (Fig 2c).
- Inverse correlation between ERα activation potency and the ring number (Fig 2d).

**CONCLUSION**

The data did not identify any correlation between cytotoxicity, genotoxicity and receptor-mediated effects. PAHs and their derivatives seem to act mainly as ligands for the AhR receptor. Data allowed concluding that compared to PAH analogues, the tested PANHs exhibit similar toxicological profiles and are likely to raise similar toxicological concern. However, PANHs may not bring significant additional risk burden since exposure seems much lower than for PAHs. This would need further analytical confirmation and proper exposure assessment to better estimate the level of safety concern. Further work should involve the evaluation of additional PANHs and PAHs, in isolation and in mixtures, as well as the investigation of the role of metabolism on the effects on nuclear receptors.

Finally, the current data show the applicability of an in vitro battery to compare structurally similar chemicals and to set priorities for further work. Such an approach is also aligned to the 3Rs initiative for reduction of animal testing.