Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

# Toxicity profiles of heterocyclic aromatic amines

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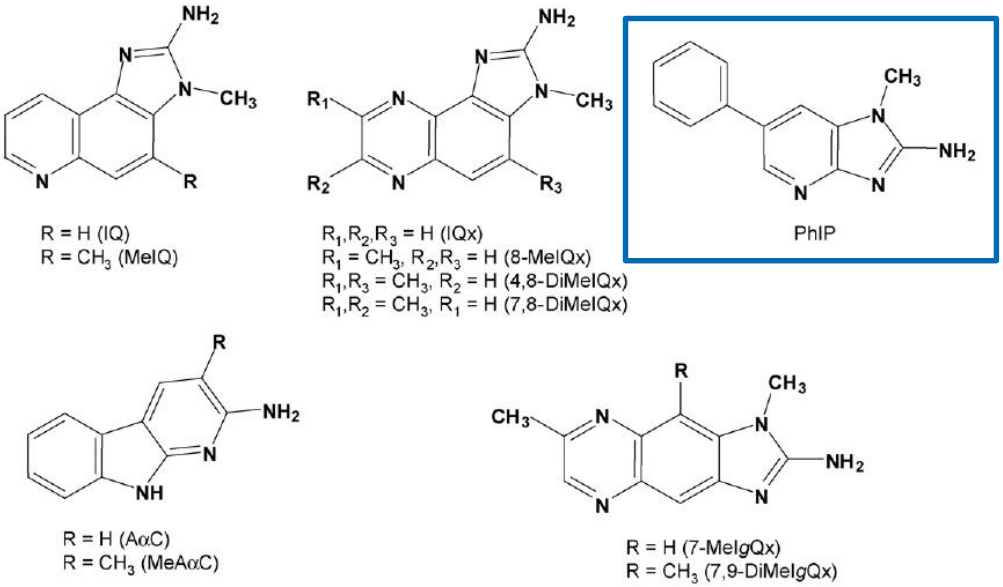
# Mutagenic compounds present in strongly heated meat

- **polycyclic aromatic hydrocarbons**
- **heterocyclic aromatic amines**
- **exogenously formed *N*-nitroso compounds**
- **endogenously formed *N*-nitroso compounds**

HCAs are formed during the heating of meat, fish and poultry, by condensation of creatinine with amino acids.

# Heterocyclic aromatic amines: Introduction




The consumption of **HCAs** increases the risk to develop colon, prostate and breast cancer.

## Target organs of carcinogenic heterocyclic aromatic amines in the rat

| HCA | Target organs/tissues |
|---|---|
| **Trp-P-1** | liver |
| **Trp-P-2** | liver, urinary bladder |
| **Glu-P-1** | liver, small and large intestine, Zymbal gland, clitoris |
| **Glu-P-2** | liver, small and large intestine, Zymbal gland, clitoris |
| **AαC** | liver, blood vessels |
| **MeAαC** | liver |
| **IQ** | liver, small and large intestine, Zymbal gland, clitoris, skin |
| **MeIQ** | large intestine, Zymbal gland, skin, oral cavity, mammary gland |
| **MeIQx** | liver, Zymbal gland, clitoris, skin |
| **PhIP** | large intestine, mammary gland, prostate |

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| **Trp-P-1** | **liver** |
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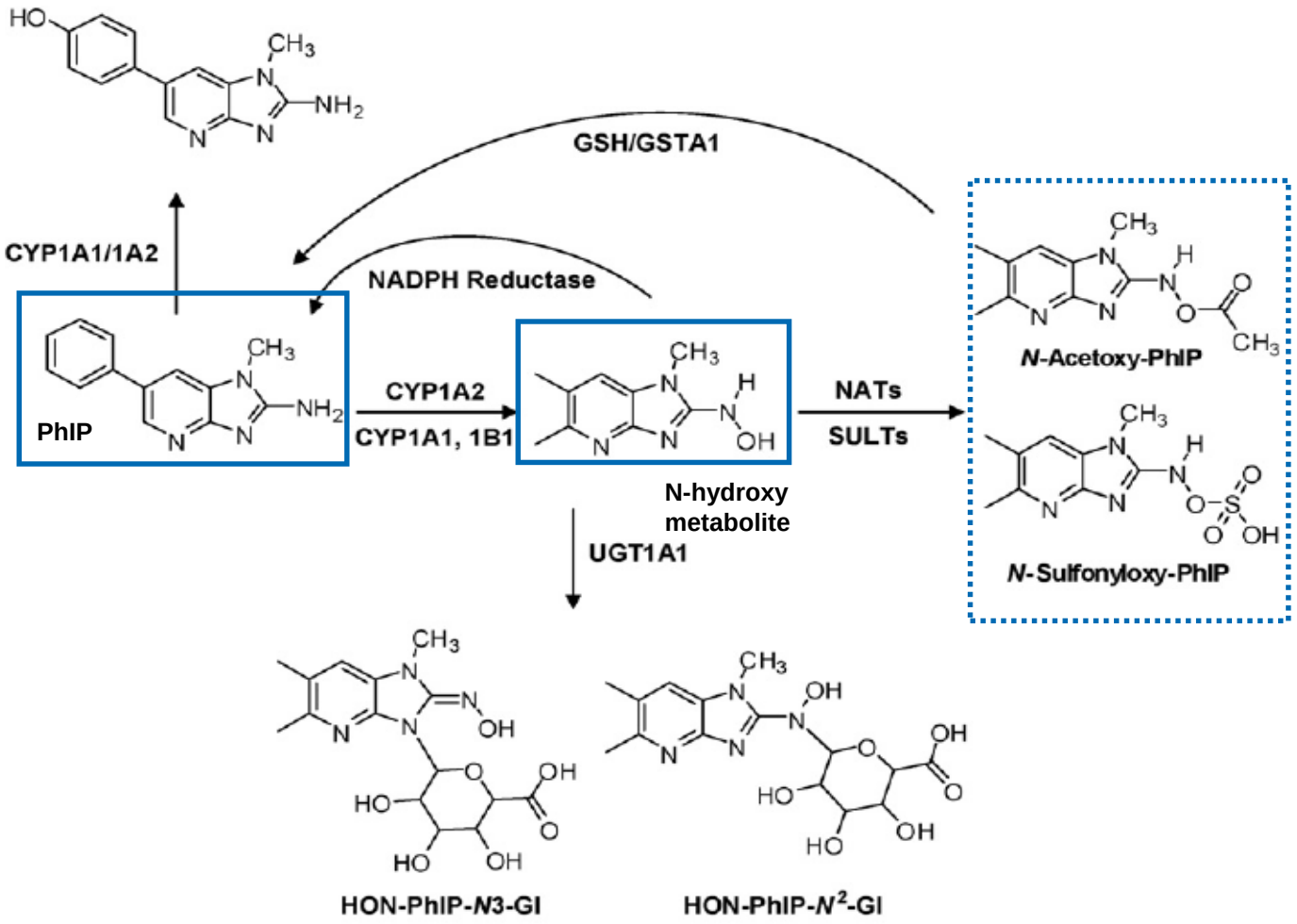
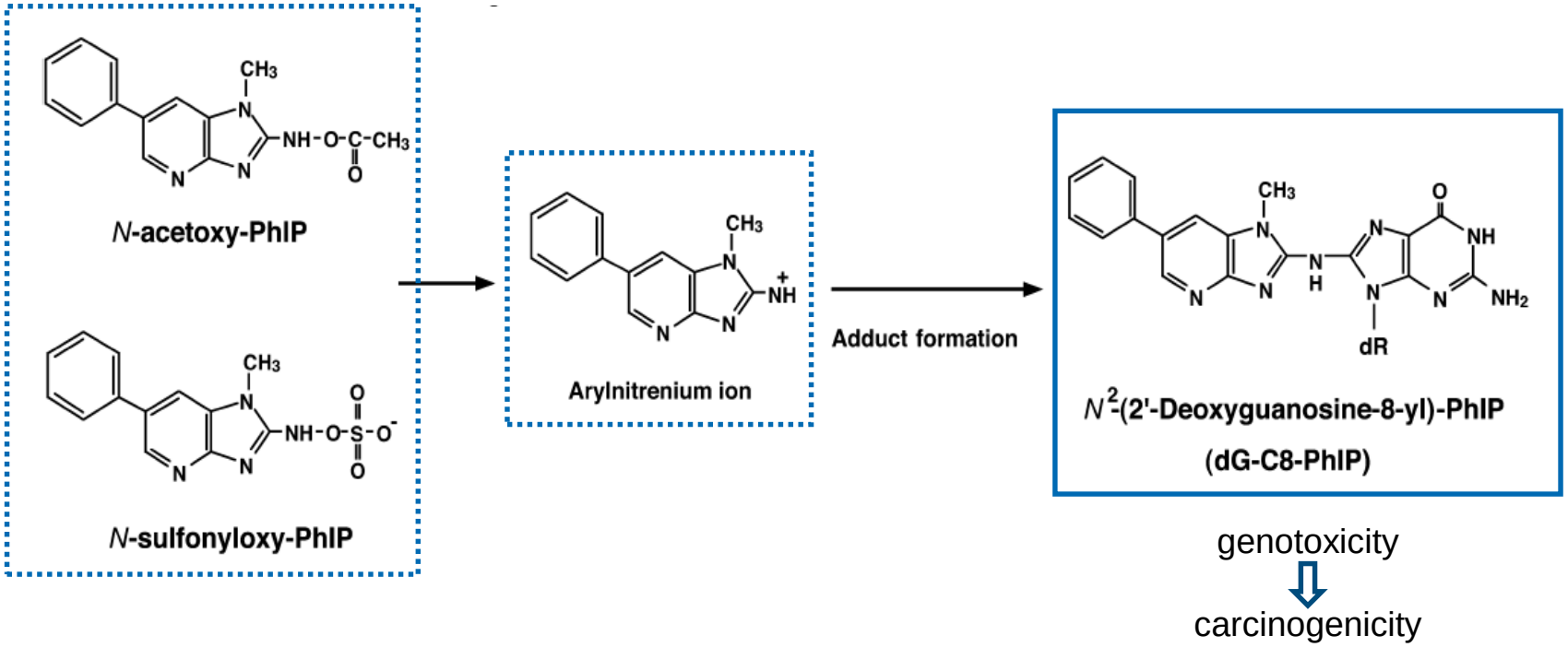
| **Glu-P-1** | **liver**, small and **large intestine**, Zymbal gland, clitoris |
| **Glu-P-2** | **liver**, small and **large intestine**, Zymbal gland, clitoris |
| **AαC** | **liver**, blood vessels |
| **MeAαC** | **liver** |
| **IQ** | **liver**, small and **large intestine**, Zymbal gland, clitoris, skin |
| **MeIQ** | **large intestine**, Zymbal gland, skin, oral cavity, **mammary gland** |
| **MeIQx** | **liver**, Zymbal gland, clitoris, skin |
| **PhIP** | **large intestine**, **mammary gland,** prostate |

# PhIP metabolism




**PhiP** is activated by the CYP1 family and further esterified by NATs and SULTs into unstable products.

# Formation of the dG-C8-PhIP adduct



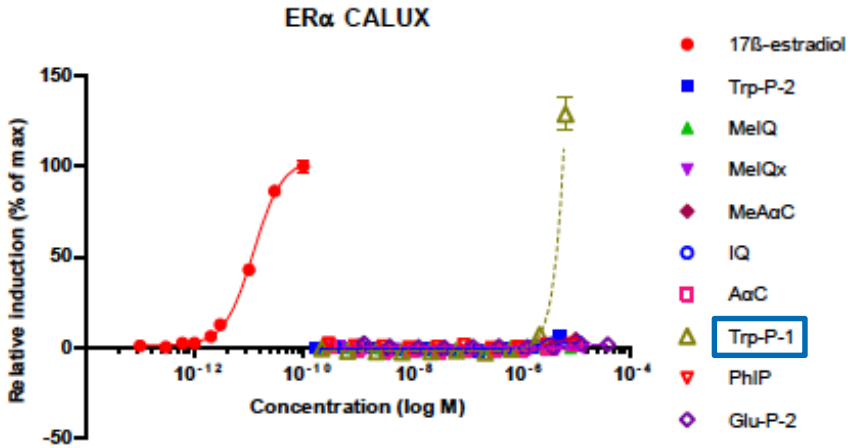

genotoxicity

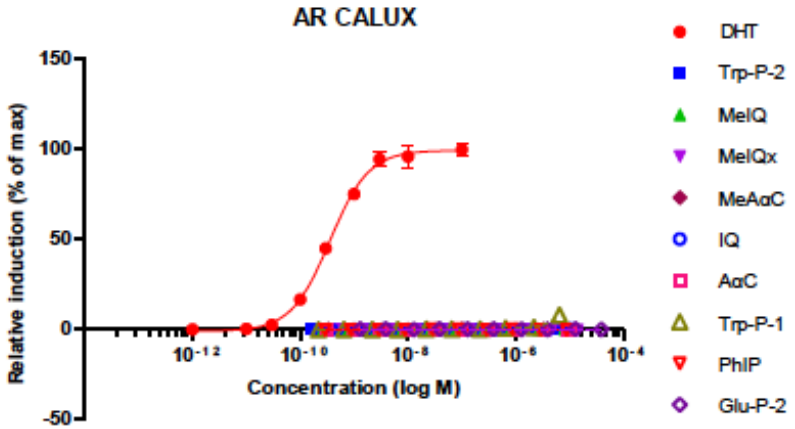
⇩

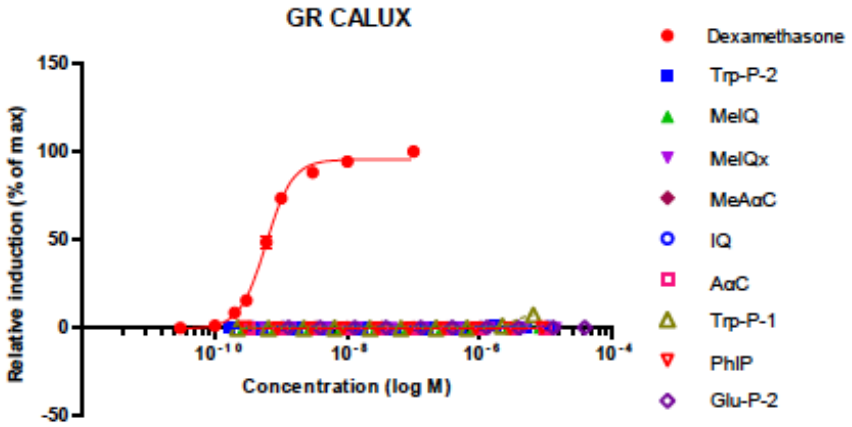
carcinogenicity

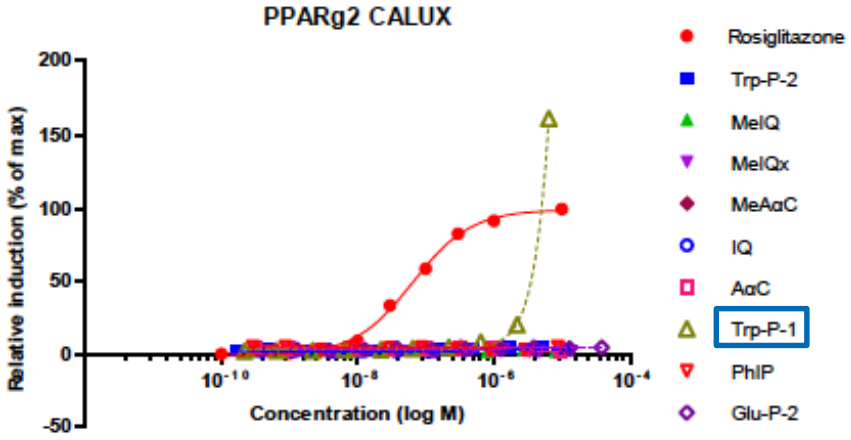
unstable DNA-reactive products of **PhiP** can lead to mutations by adduct formation.

# Heterocyclic aromatic amines: Results (I)

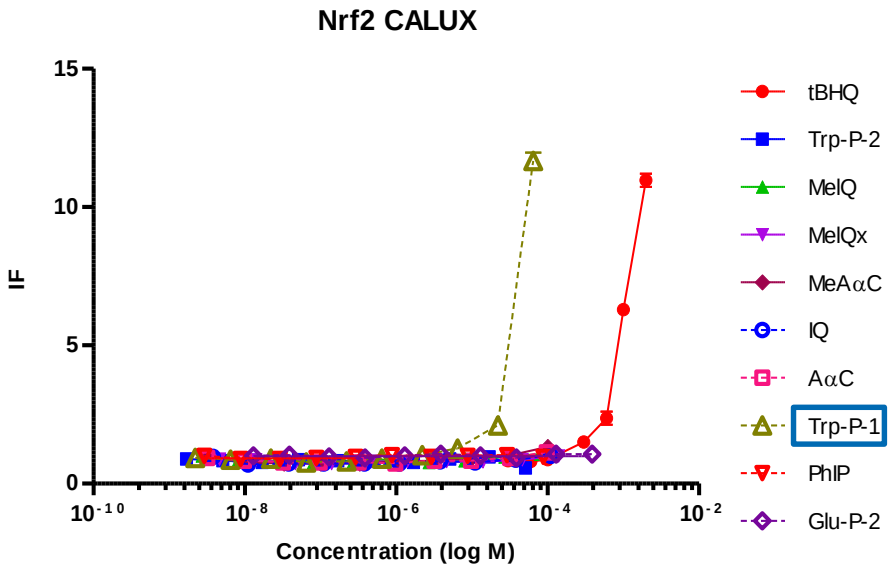


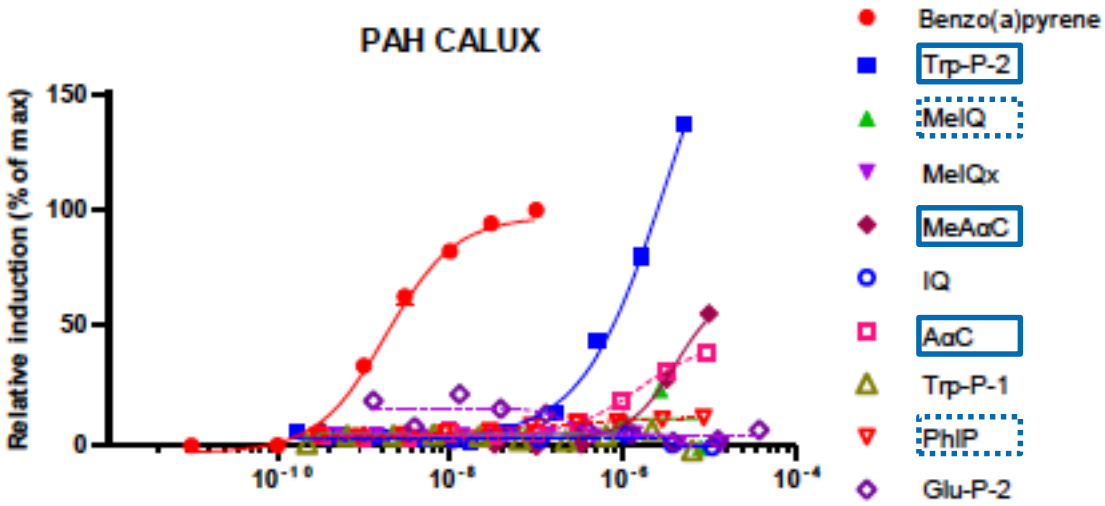



# Heterocyclic aromatic amines: Results (II)



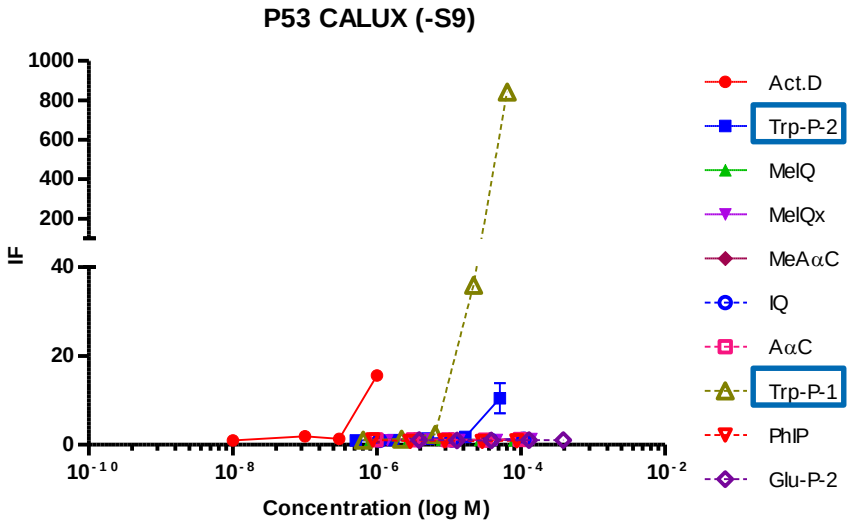

**Trp-P-1** was the only tested HCA that led to a positive response in the ERα, PPARγ2 and Nrf2 CALUX® assays.

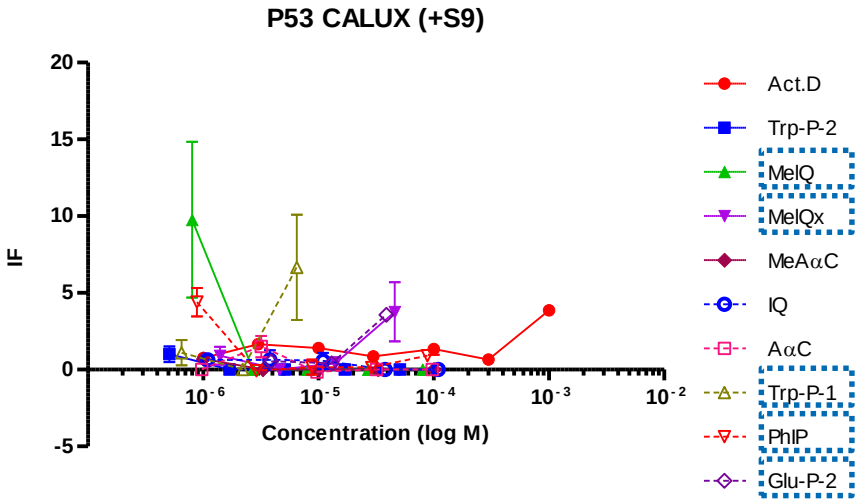
# Heterocyclic aromatic amines: Results (III)




**Trp-P-2, MeAαC** and **AαC** induced a clear positive effect in the PAH CALUX® assay.
**MeIQ** and **PhIP** induced luciferase activity to a limited extent and in a concentration-independent way.

# Heterocyclic aromatic amines: Results (IV)




Only **Trp-P-1 and Trp-P-2** enhanced luciferase expression in the p53 CALUX® assay.
When a **metabolic activation step** was coupled, **Trp-P-1, Glu-P-2, MeIQ, MeIQx** and **PhIP** induced a positive response.

## Heterocyclic aromatic amines: Summary of the results obtained

| | ERα | AR | GR | PPARγ2 | PAH | Nrf2 | p53 (-S9) | p53 (+S9) |
|---|---|---|---|---|---|---|---|---|
| **IF** | | | | | | 11 | 16 | 4 |
| **Trp-P-1** | + (130) | - | - | + (160) | - | + (12) | + (840) | + (10) |
| **Trp-P-2** | - | - | - | - | + (140) | - | + (10) | - |
| **Glu-P-2** | - | - | - | - | - | - | - | + (3.6) |
| **AαC** | - | - | - | - | + (40) | - | - | - |
| **MeAαC** | - | - | - | - | + (55) | - | - | - |
| **IQ** | - | - | - | - | - | - | - | - |
| **MeIQ** | - | - | - | - | + (24) | - | - | + (15) |
| **MeIQx** | - | - | - | - | - | - | - | + (5.6) |
| **PhIP** | - | - | - | - | + (12) | - | - | + (4.4) |

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| **Glu-P-2** | - | - | - | - | - | - | - | + (3.6) |
| **AαC** | - | - | - | - | + (40) | - | - | - |
| **MeAαC** | - | - | - | - | + (55) | - | - | - |
| **IQ** | - | - | - | - | - | - | - | - |
| **MeIQ** | - | - | - | - | + (24) | - | - | + (15) |
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**Trp-P-1** was the only tested HCA that led to a positive response in the ERα, PPARγ2 and Nrf2 CALUX® assays.

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| **Glu-P-2** | - | - | - | - | - | - | - | + (3.6) |
| **AαC** | - | - | - | - | + (40) | - | - | - |
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| **IQ** | - | - | - | - | - | - | - | - |
| **MeIQ** | - | - | - | - | + (24) | - | - | + (15) |
| **MeIQx** | - | MeIQx elicited **no** ERα-related activity<br>Lauber et al. (2004) | | | | | - | + (5.6) |
| **PhIP** | - | PhiP elicited ERα-related activity<br>Gooderham et al. (2002) and Lauber et al. (2004) | | | | | - | + (4.4) |

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| **IQ** | - | - | - | - | - | - | - | - |
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| **MeIQx** | - | - | - | - | - | | | |
| **PhIP** | - | - | - | - | + (12) | | | |

By means of the **PAH** CALUX® assay activation of the **AhR** is quantified.

Activation of the AhR and interaction with xenobiotic response elements could lead to an **induction of CYP enzymes**.

Trp-P-2 and AαC did not lead to an activation of the AhR to a DNA binding form in a gel retardation assay
Kleman et al. (1992)

Gene expression profiles of PhiP and MeIQx in HepaRG™ cells showed responses of downstream targets of the AhR: CYP 1A1, 1A2, 1B1 and dehydrogenase 3A1
Dumont et al. (2010)

# Heterocyclic aromatic amines: Summary of the results obtained

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| **IQ** | - | - | - | - | - | - | - | - |
| **MeIQ** | - | - | - | - | + (24) | - | - | + (15) |
| **MeIQx** | - | - | - | - | - | - | - | + (5.6) |
| **PhIP** | - | - | - | - | + (12) | - | - | + (4.4) |

The **p53** CALUX® assay is able to detect **genotoxic** compounds.

Most **HCAs** require metabolic activation to form DNA adducts and acting mutagenically in bacterial as well as mammalian cell-based genotoxicity assays.

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| **MeAαC** | - | - | - | - | + (55) | - | - | - |
| **IQ** | - | - | - | - | - | - | - | - |
| **MeIQ** | - | - | - | - | + (24) | - | - | + (15) |
| **MeIQx** | - | - | - | - | - | - | - | + (5.6) |
| **PhIP** | - | - | - | - | + (12) | - | - | + (4.4) |

The **Nrf2** CALUX® assay provides information on the **mechanism** underlying the genotoxic effect.

Activity in the **p53** and **Nrf2** CALUX® assays shows that the compound acts via a toxicity pathway involving **oxidative stress**.

## Conclusions

- The results obtained show that the battery of CALUX® assays performed in the present study can successfully be used to **screen for molecular cell targets of carcinogenic compounds** such as HCAs.
- Due to the **particular responsive elements** present in the different promoters, the cell lines specifically respond to the pathway of interest, and the interpretation of the results is much easier than when utilizing complex promoters.

## Acknowledgements

**Thank you for your attention!**

Thanks to

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BDS BioDetection Systems

# References


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Lauber, S.N., Ali, S., Gooderham, N.J., 2004. The cooked food derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine is a potent oestrogen: a mechanistic basis for its tissue-specific carcinogenicity. Carcinogenesis 25, 2509-2517.