

# Paternal carcinogen exposures and genetic risk in their offspring

Proposal for the CEFIC-LRI Innovative Science Award

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# Benzo[a]pyrene

- 1930 Kennaway & Cook  
benzo[a]pyrene isolated from coal tar
- 1933 Yamagiwa & Ichikawa  
benzo[a]pyrene is carcinogenic in rabbits

Ubiquitous environmental & occupational pollutant

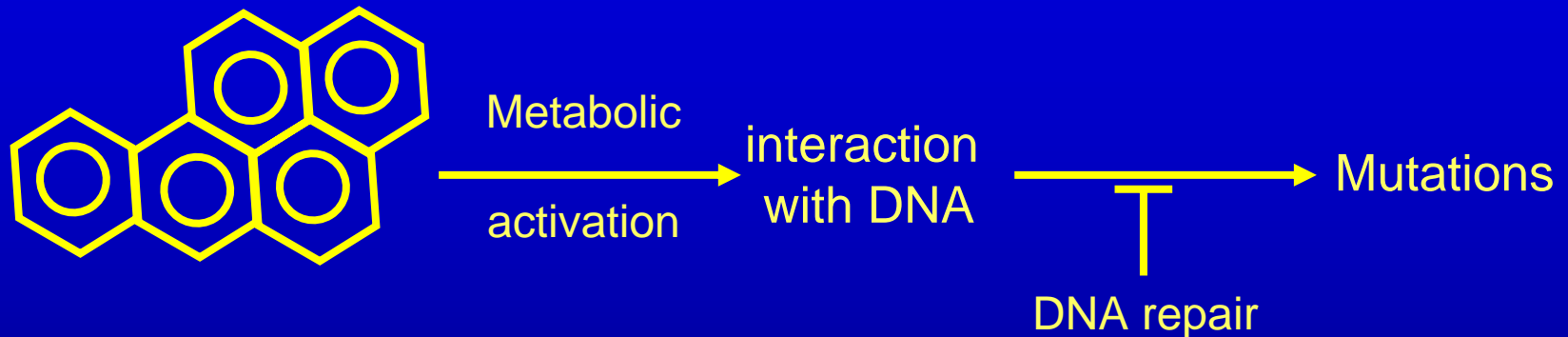


Over 70 years of research on  
Benzo[a]pyrene

What is missing?

# Germ-line mutations

Role of B[a]P-DNA adducts in inducing mutations in somatic cells is undisputed:



.....but their role in inducing germ-line mutations is not thoroughly investigated:

- No human studies
- Limited research in experimental animals

## OBJECTIVE 1

1) Impact of DNA damage in gametes induced by paternal low dose exposure to benzo(a)pyrene on the formation of germ line mutations

# Protective mechanisms

Germ cells can be protected against parental exposures to carcinogens, via a complex network of molecular mechanisms:

- DNA repair                      Removal of damage
- P53                                Provide time for repair/ apoptosis
- Heat Shock Proteins        Essential for gametogenesis

To study the role of protective mechanisms; Knock-out DNA-repair

## OBJECTIVES 2 & 3

- 2) Does modulation of DNA repair affect germ line mutagenesis?
- 3) To investigate potential protective mechanisms of sperm against exposures to benzo(a)pyrene



# Overall study design

Paternal exposure to B[a]P (and unexposed controls) is followed by crossing these animals as follows:

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<i>+B[a]P males:</i>	Wt	XPA(-/-)	XPA(-/-)
	x	x	x
<i>-B[a]P females:</i>	Wt	Wt	XPA(-/-)

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Crossing at several time points after exposure:

Stemcells → Mature sperm

# Methods

Assessment of following parameters:

## *DNA damage in germ cells of male mice*

minisatellite mutations in offspring

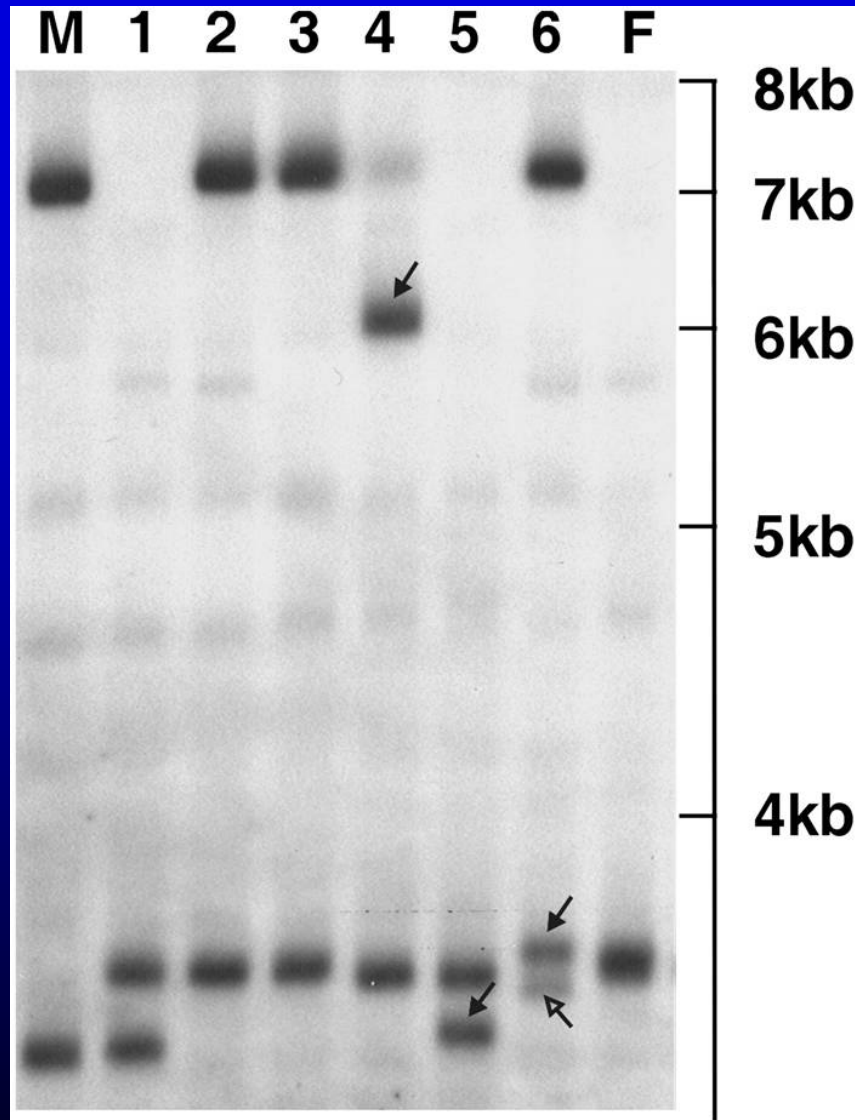
B[a]P-DNA adducts by  $^{32}\text{P}$ -postlabeling

## *Transcriptomics*

changes in gene-expression profiles to elucidate potential protective mechanisms in testis



# V<sub>ariable</sub> N<sub>umber</sub> T<sub>andem</sub> R<sub>epeats</sub>



0.1-20 kb long, 6 repeat units

\* in nuclear genome highly repeated DNA sequences; tandem repeats

\* mostly transcriptionally inactive

\* non-coding DNA

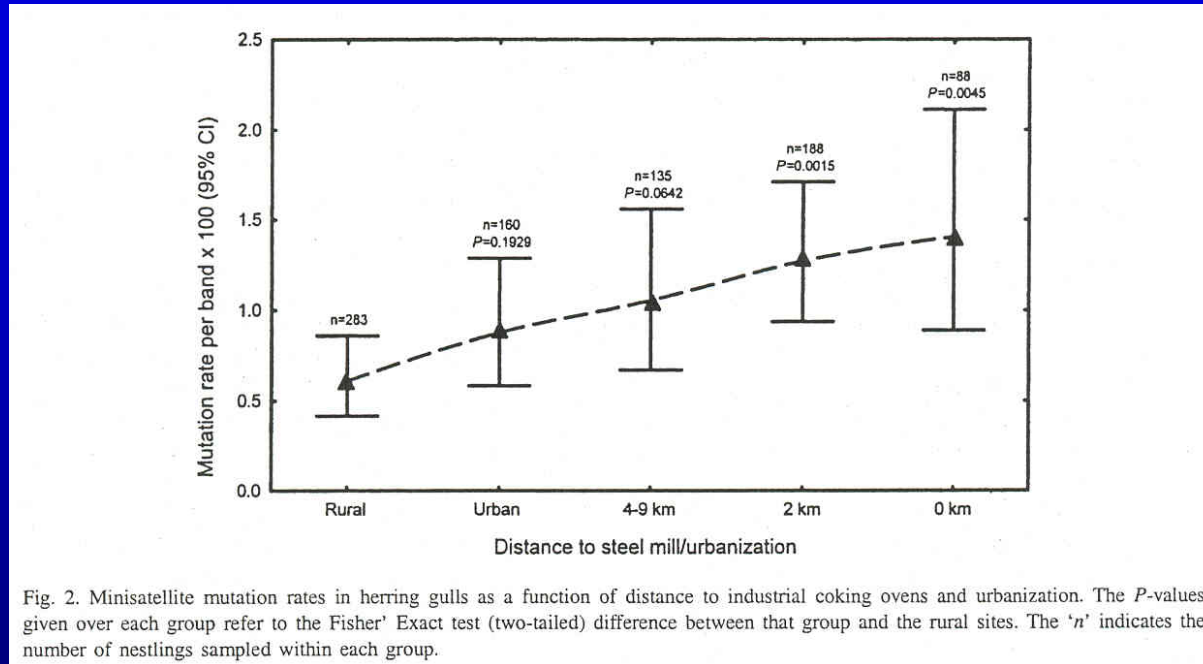
\* high rate of mutation

\* mutation rate correlates with rate at coding loci



# Proof of concept.....

## - Herring gulls



Yauk, C.L., Fox, G.A., McCarry, B.E., and Quinn, J.S. (2000) *Mutation Research* **452**: 211-218.

## - Mice

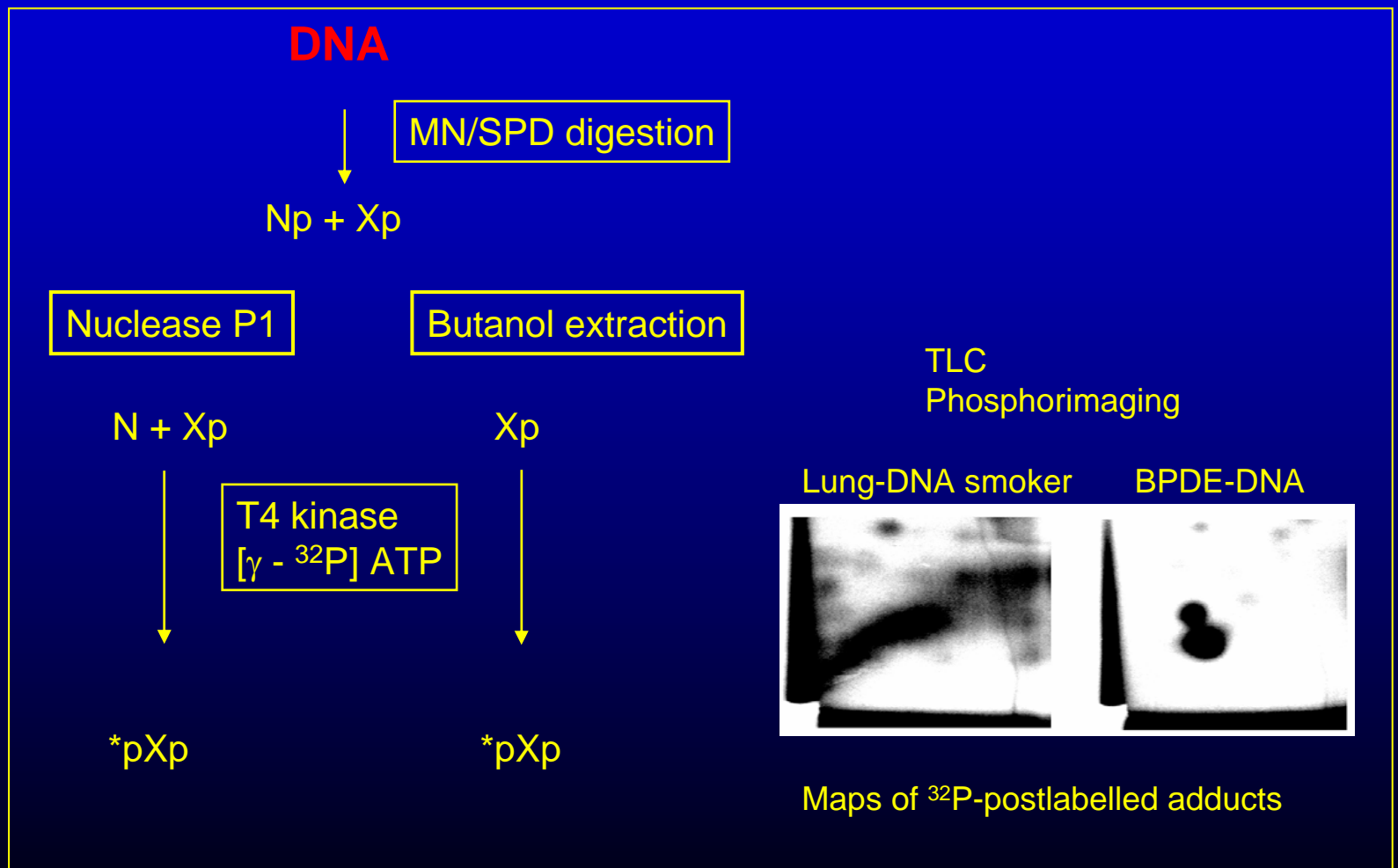
Somers C.M., Yauk, C.L., White, P.A., Parfett, C.L., Quinn JS (2002) *PNAS* **99**: 15904-7

Somers, C.M., McCarry, B.E., Malek, F. and Quinn, J.S. (2004) *Science* **304**: 1008-1010.

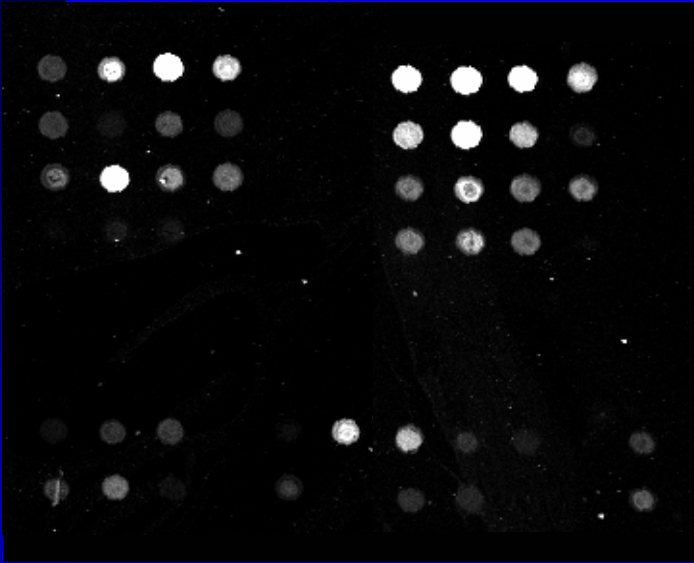


# $^{32}\text{P}$ -postlabeling

- Currently most sensitive assay for the detection of B[a]P - DNA adducts
- Routinely applied in our laboratory



# Transcriptomics



Example of micro-array

1. Selfmade mouse microarray  
Based on the PHASE I  
human microarray, containing  
>600 genes

2. Toxicologically relevant,  
Several pathways, including:

- Inflammation
- DNA damage & repair
- Oxidative stress
- cell-proliferation / apoptosis

3. Validation by RT-PCR

Identification of new protective pathways?

# Reaching the objectives

## Objective 1 *Role of B[a]P in germ line mutagenesis*

- Comparing mutation frequencies in offspring of exposed males with offspring of unexposed controls.
- DNA adduct levels in testis (Biologically Effective Dose)

## Objective 2 *Role of DNA repair in germ line mutagenesis*

- Comparing mutation frequencies in offspring of exposed XPA<sup>-/-</sup> males with offspring of exposed wildtype controls.
- DNA adduct levels in testis

## Objective 3 *Protective mechanisms*

- Comparing gene-expression in offspring of exposed XPA<sup>-/-</sup> males with offspring of exposed wildtype controls.
- DNA adduct levels in testis



# Timeframe

## **0 - 4 months**

Experimental conditions will be optimised before the actual breeding experiment starts

## **4 - 14 months**

Main breeding experiment and collection of tissues

## **9 – 20 months**

Analysis of samples

## **20-24 months**

Data collection and statistical analysis  
Writing of report & scientific publications



# Dissemination

- Publications in scientific journals
- Presentations at scientific meetings
- Special website created for this proposal
- Communication to CEFIC



# Benefit for CEFIC, Science & Society

- Further completion of the B[a]P-puzzle
- Towards a future with reduced animal usage for toxicity testing?
- Improved knowledge → Better protection of workers

## Project Research Team

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RIVM

## Collaborations

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Prof. Dr. Jos Kleinjans

*Genome Center Maastricht*

Prof. Dr. Bob Vlietink

