Paternal carcinogen exposures and genetic risk in their offspring

Proposal for the CEFIC-LRI Innovative Science Award

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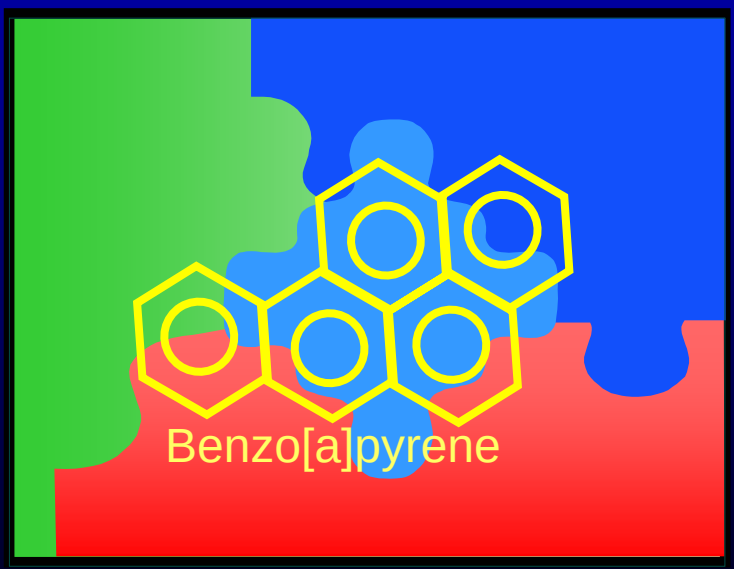
Benzo[a]pyrene

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

Ubiquitous environmental & occupational pollutant

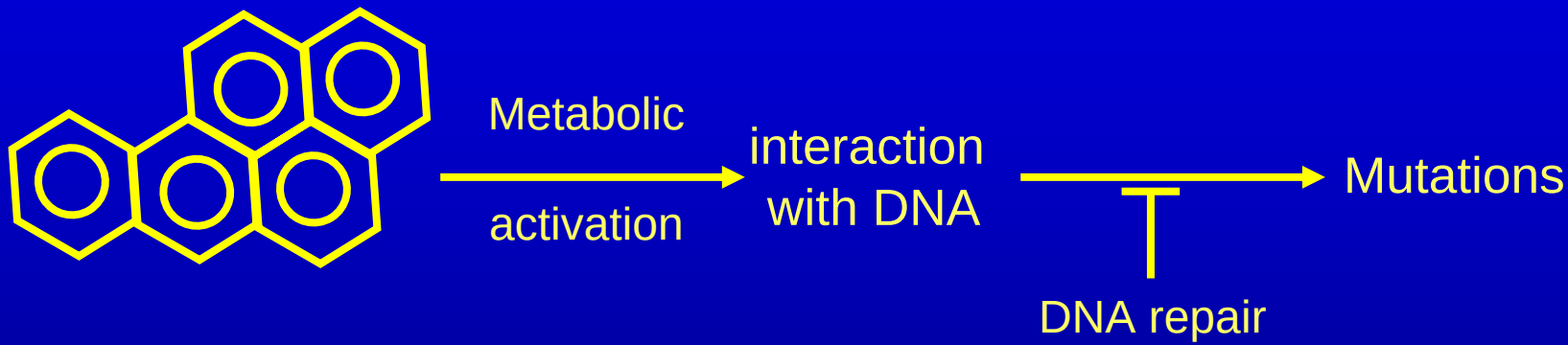


Over 70 years of research on Benzo[a]pyrene

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:

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

Crossing at several time points after exposure:

Stemcells ⟶ Mature sperm

Methods

Assessment of following parameters:

DNA damage in germ cells of male mice

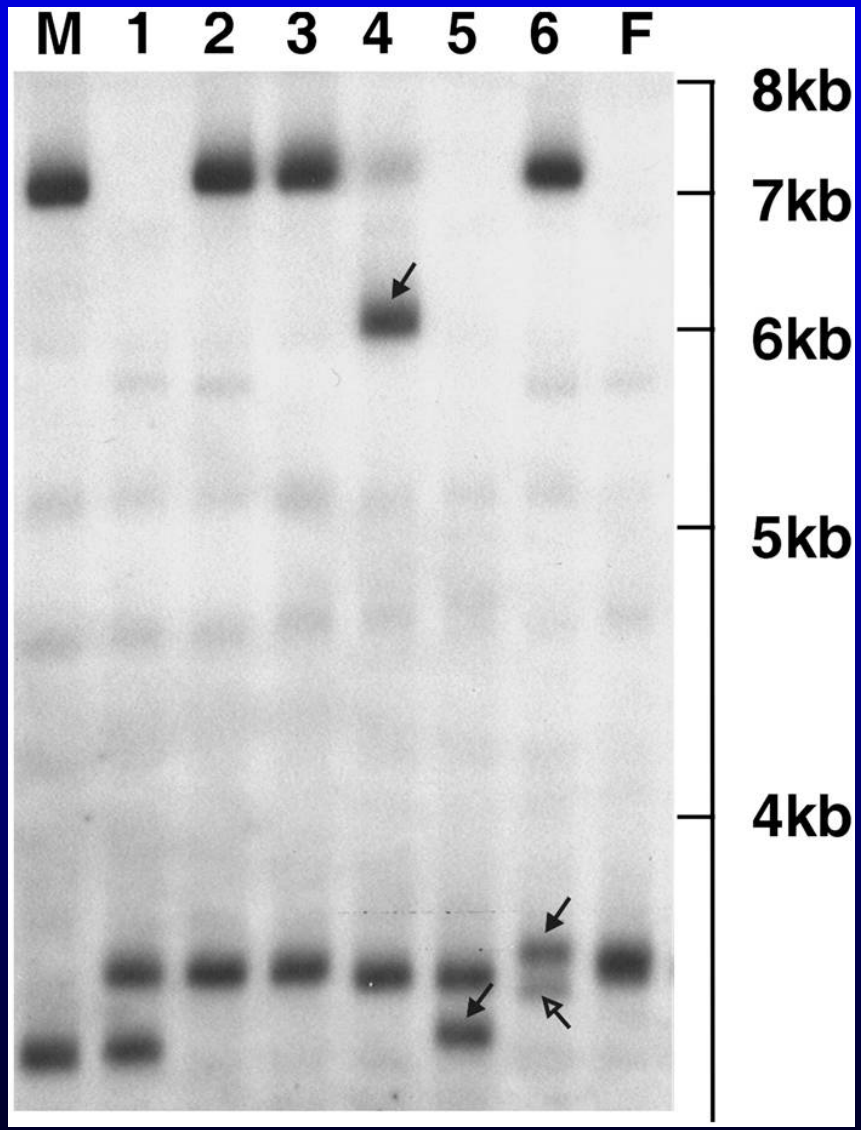
minisatellite mutations in offspring

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

Transcriptomics

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

Variable Number Tandem Repeats



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

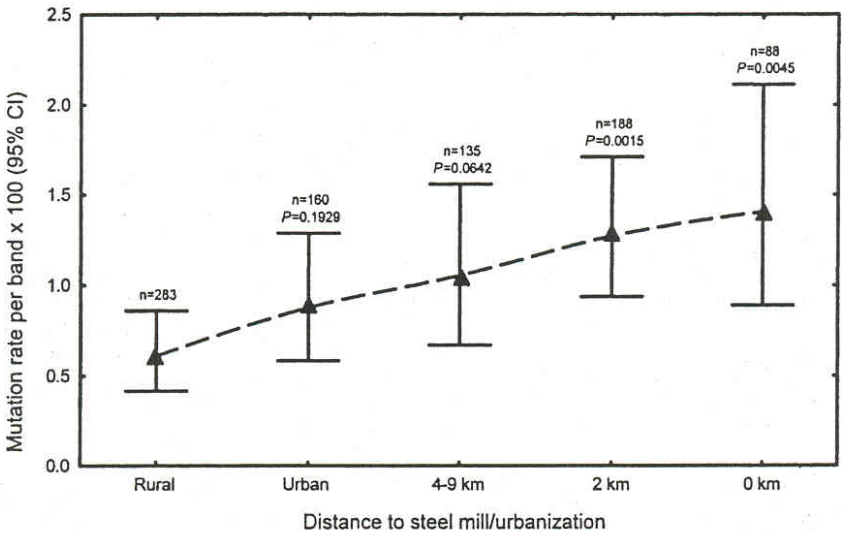


Fig. 2. Minisatellite mutation rates in herring gulls as a function of distance to industrial coking ovens and urbanization. The *P*-values given over each group refer to the Fisher' Exact test (two-tailed) difference between that group and the rural sites. The '*n*' indicates the number of nestlings sampled within each group.

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

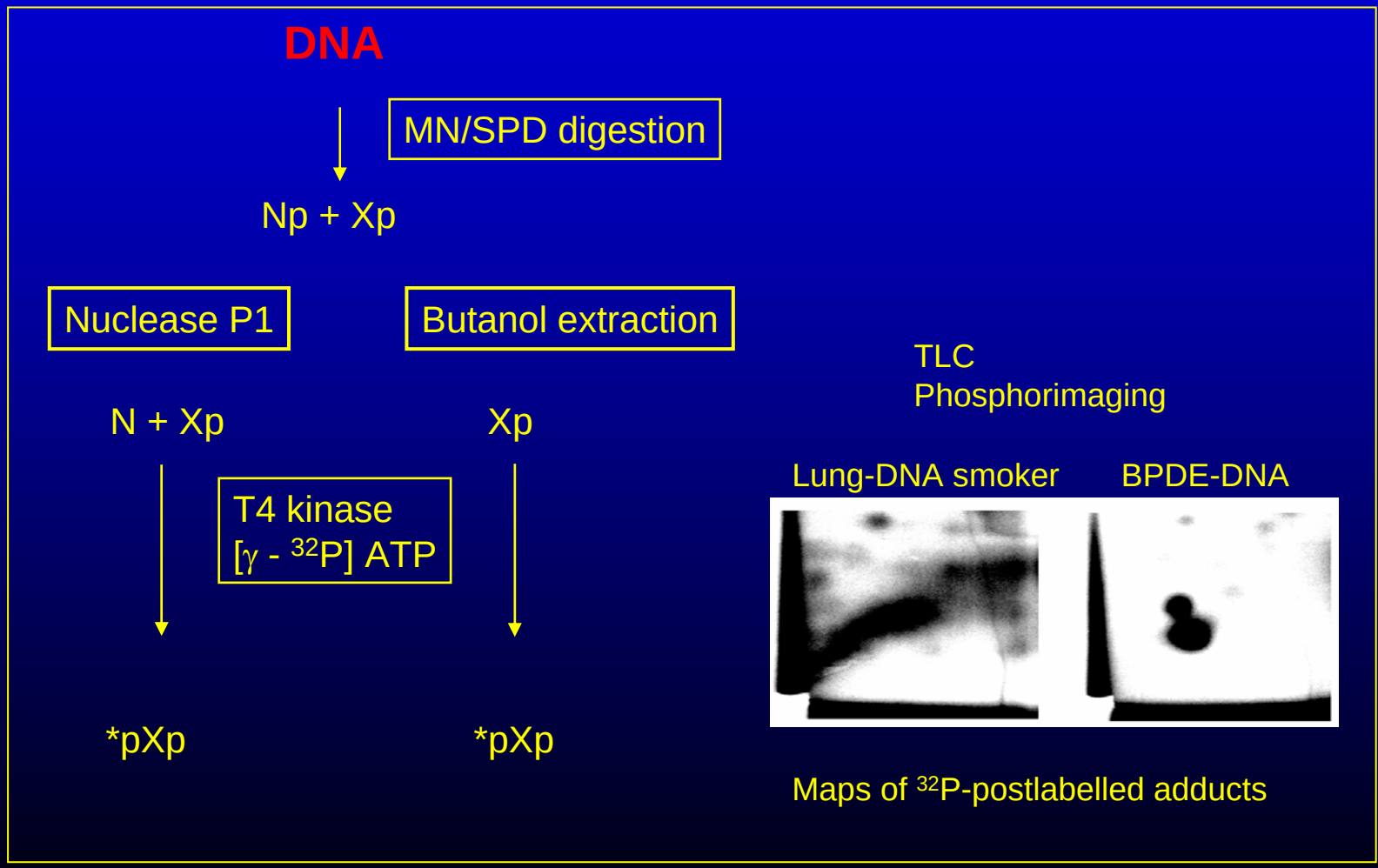
- Mice

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

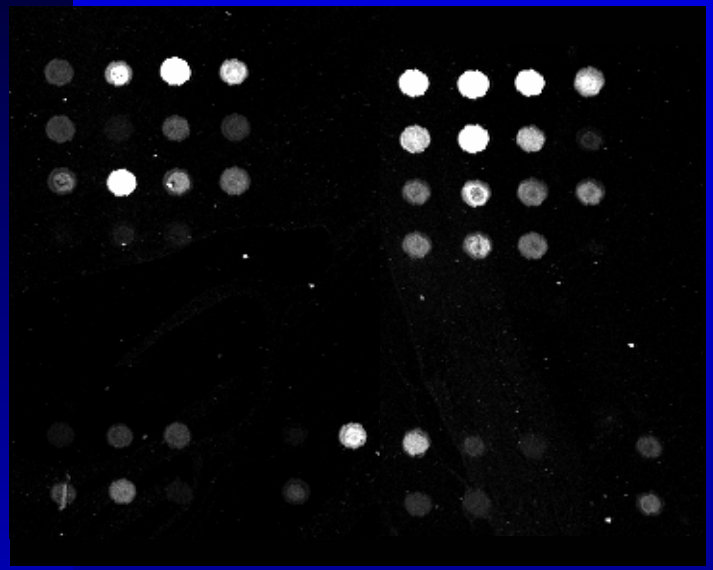
Somers, C.M., McCarry, B.E., Malek, F. and Quinn,J.S. (2004) Science **304**: 1008-1010.

^{32}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-/- males with offspring of exposed wildtype controls.
- DNA adduct levels in testis

Objective 3 *Protective mechanisms*

- Comparing gene-expression in offspring of exposed XPA-/- 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

Dr. Roger Godschalk	University of Maastricht
Prof. Dr. Frederik-Jan van Schooten	University of Maastricht
Dr. Joost van Delft	University of Maastricht
Dr. Harry van Steeg	RIVM

Collaborations

Netherlands Toxicogenomics Center	Prof. Dr. Jos Kleinjans
Genome Center Maastricht	Prof. Dr. Bob Vlietink