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Modern mutagenicity tests, Ames revisited

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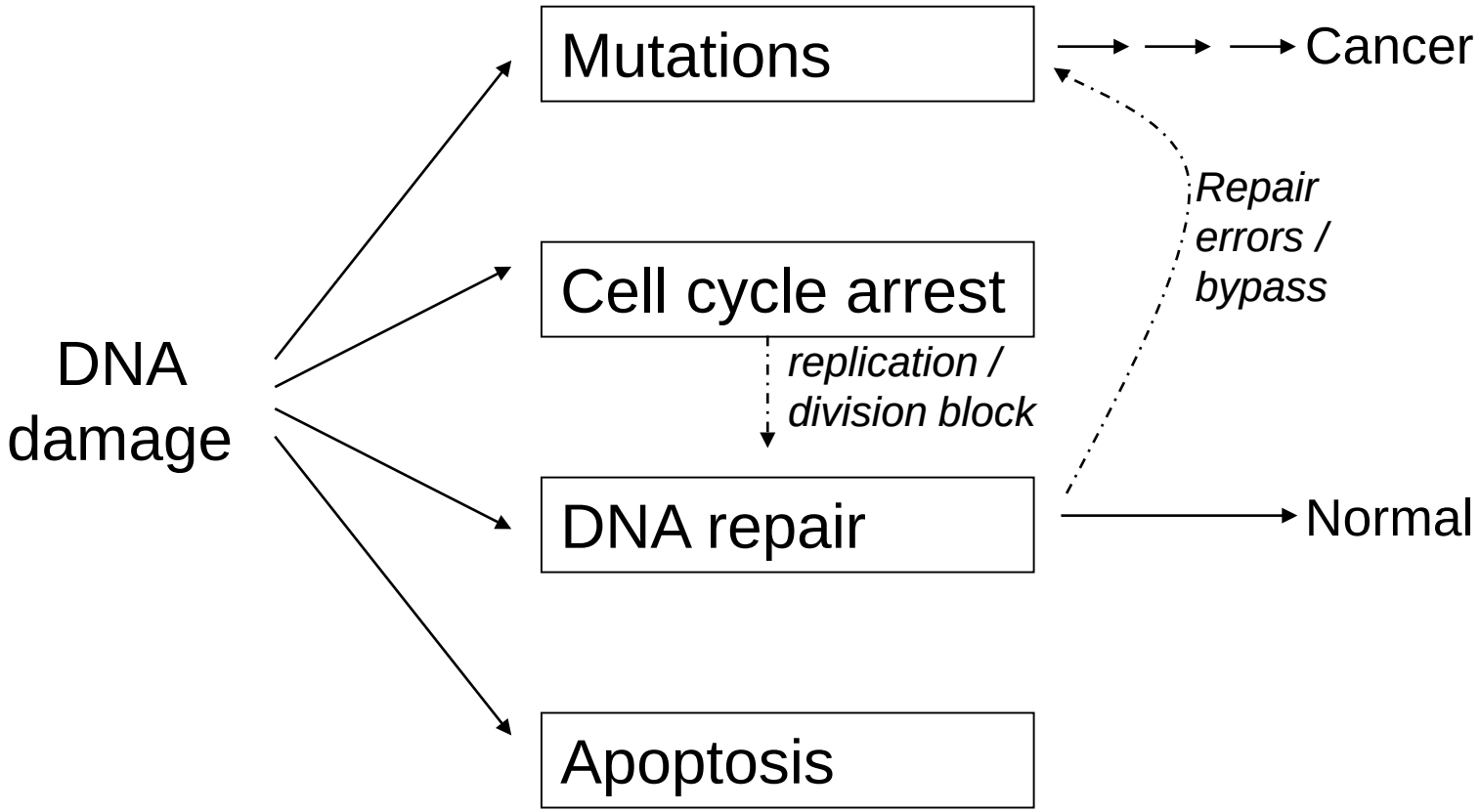
Effect- and toxicity-based assessment of exhausts

March 16, 2018, Empa, Dübendorf, Switzerland

Mitglied der Leibniz-Gemeinschaft



Schins and Hei, 2008

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 3, pp. 782–786, March 1973

An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens

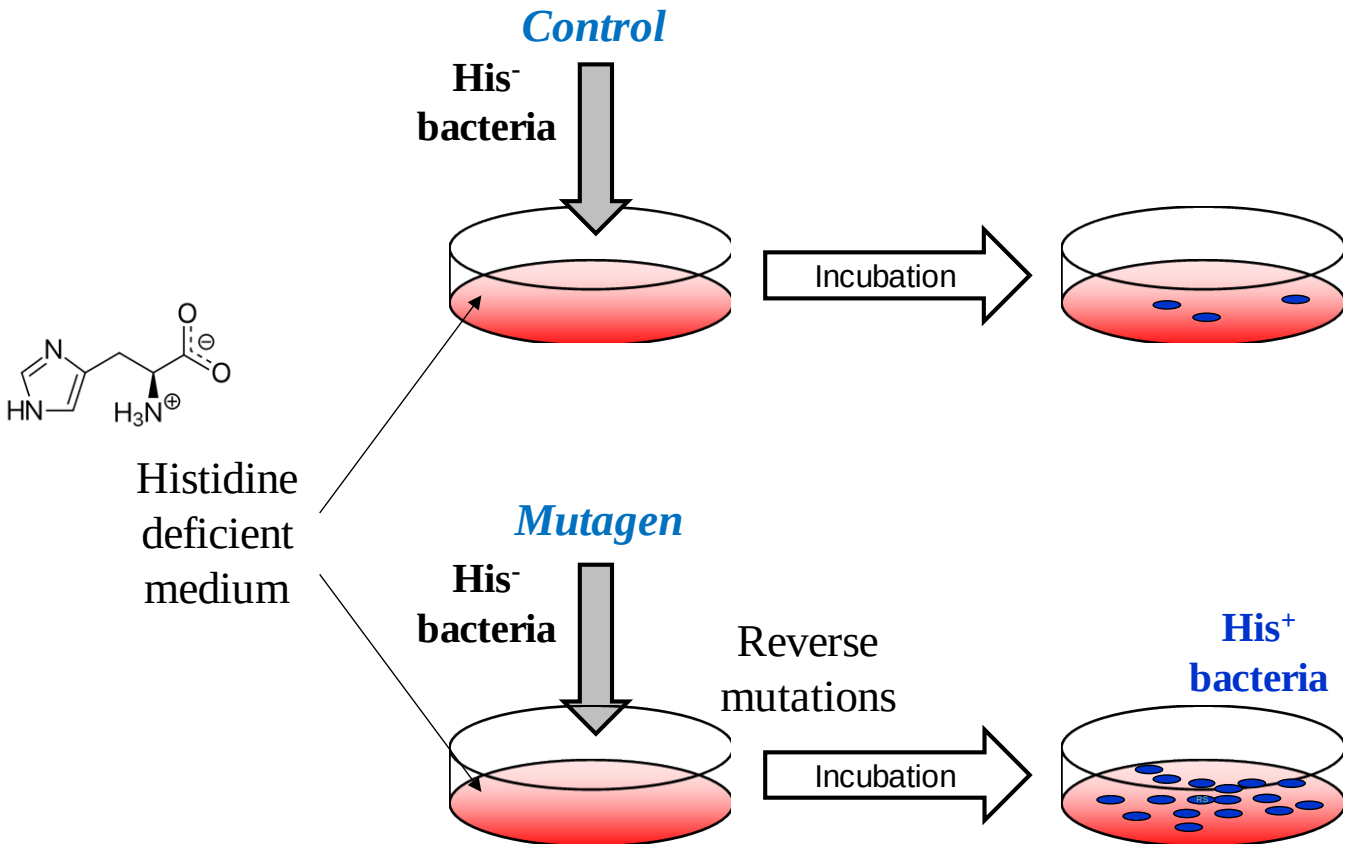
(*Salmonella typhimurium*/lipopolysaccharide/frameshift mutations)

BRUCE N. AMES, FRANK D. LEE, AND WILLIAM E. DURSTON

Biochemistry Department, University of California, Berkeley, Calif. 94720

Contributed by Bruce N. Ames, January 11, 1973

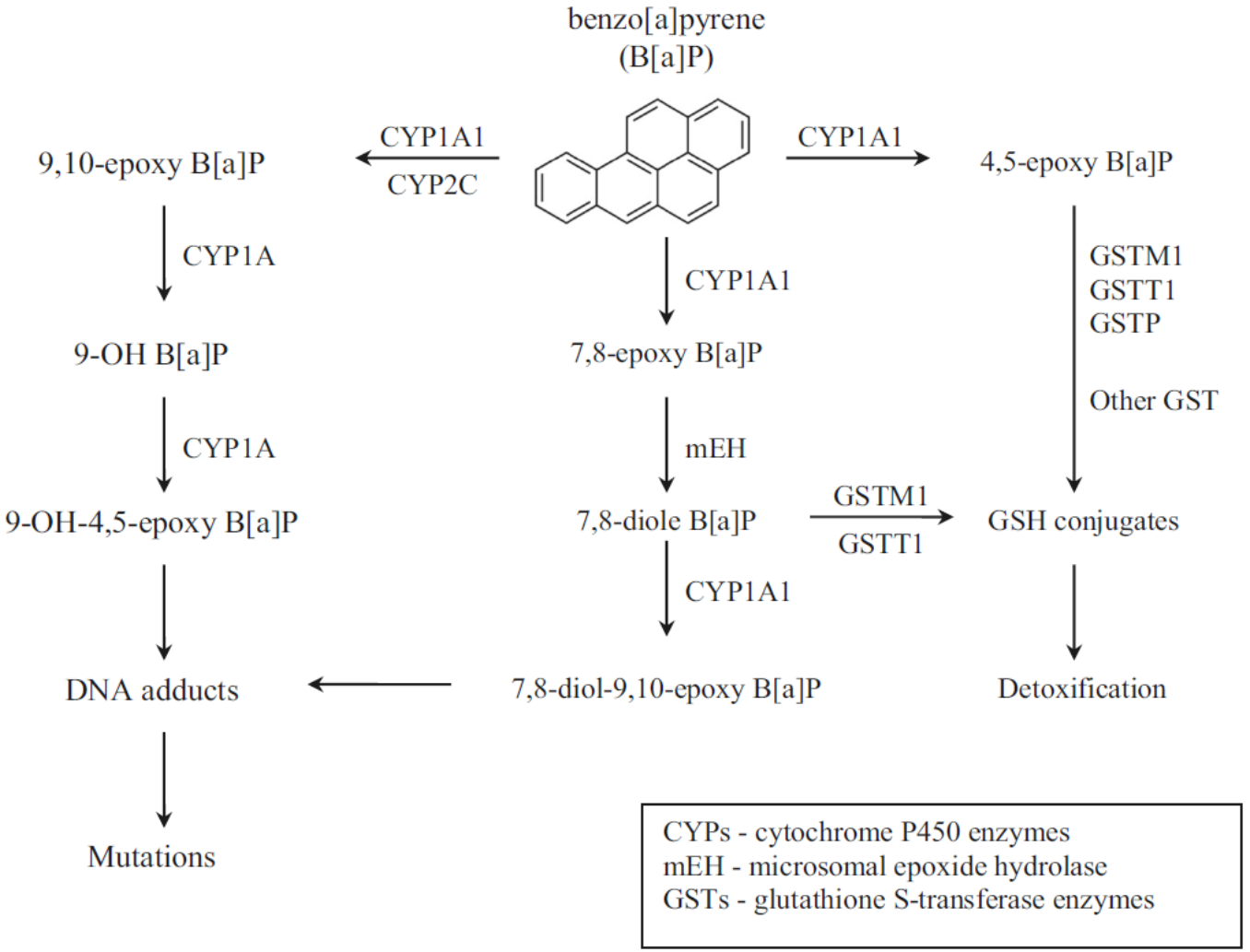
Principle of the Ames Test (Bacterial Reverse Mutation Test)



Bacteria (prokaryotic) versus mammalian cells (eukaryotic)

Metabolism

Polycyclic aromatic hydrocarbon (PAH)-DNA adducts and cancer



Lodovici et al. Cancer Epidemiol Biomark Prev 2004; 13:1342–1348

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2281–2285, August 1973

Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection

(frameshift mutagens/aflatoxin/benzo(a)pyrene/acetylaminofluorene)

BRUCE N. AMES, WILLIAM E. DURSTON, EDITH YAMASAKI, AND FRANK D. LEE

Biochemistry Department, University of California, Berkeley, Calif. 94720

Contributed by Bruce N. Ames, May 14, 1973

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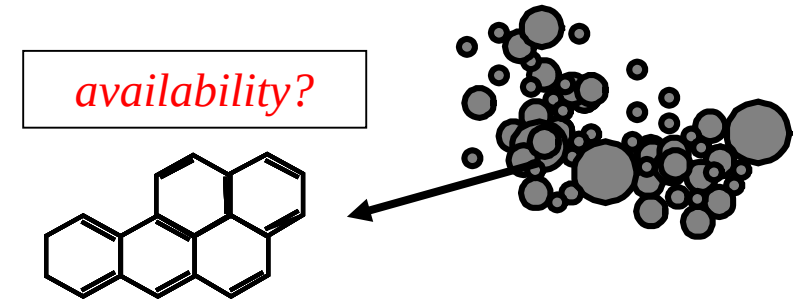
Mutagenesis of inhaled PAH-containing particles



availability?

metabolic activation

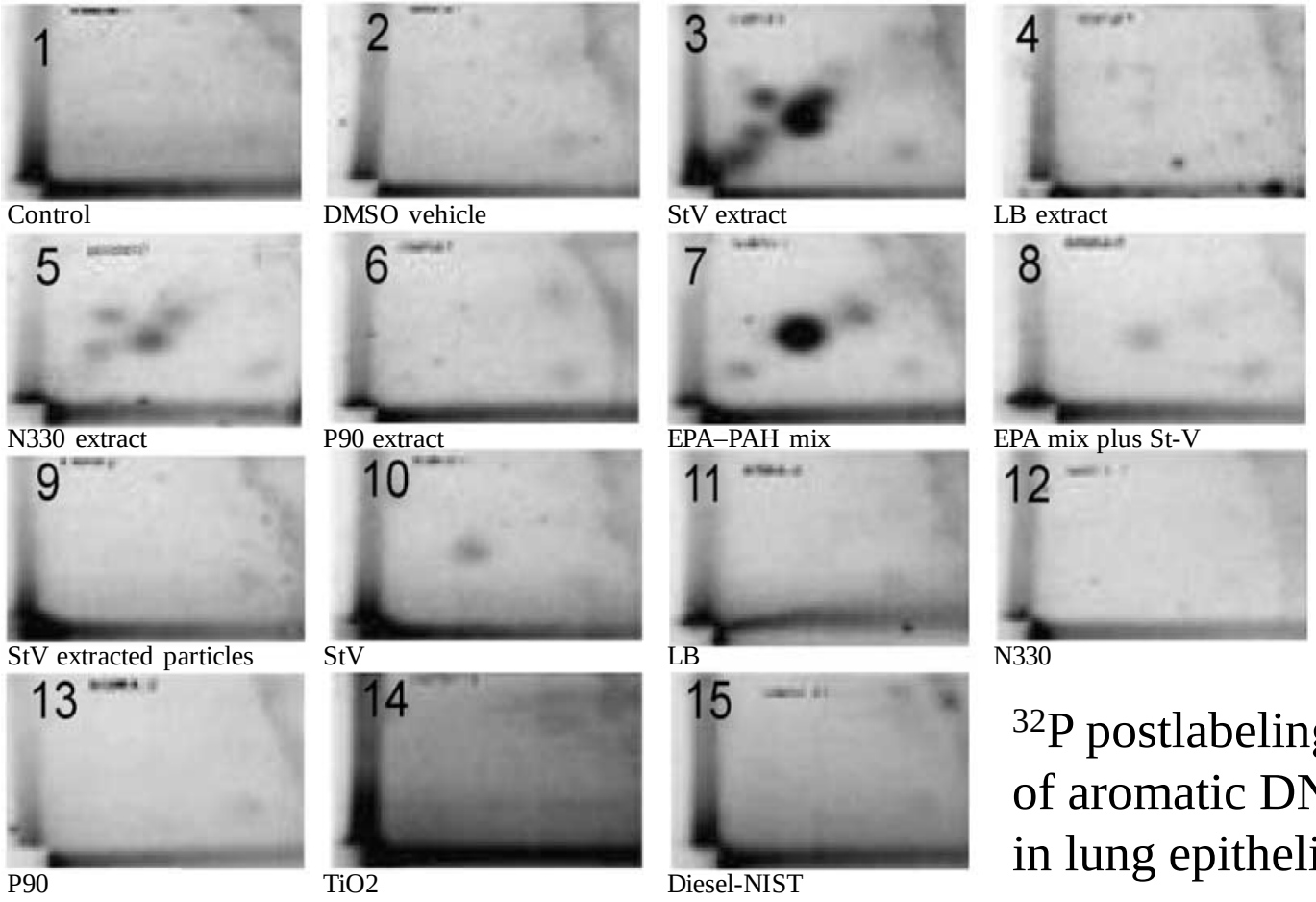
DNA repair, cell cycle arrest apoptosis/necrosis

Bulky DNA-adduct

mutagenesis

Schins and Knaapen (2007) Inhal Toxicol

Bioavailability of polycyclic aromatic hydrocarbons (PAH) from carbon black nanoparticles



^{32}P postlabeling detection of aromatic DNA adducts in lung epithelial cells

Borm et al.(2005) Toxicol Applied Pharmacol

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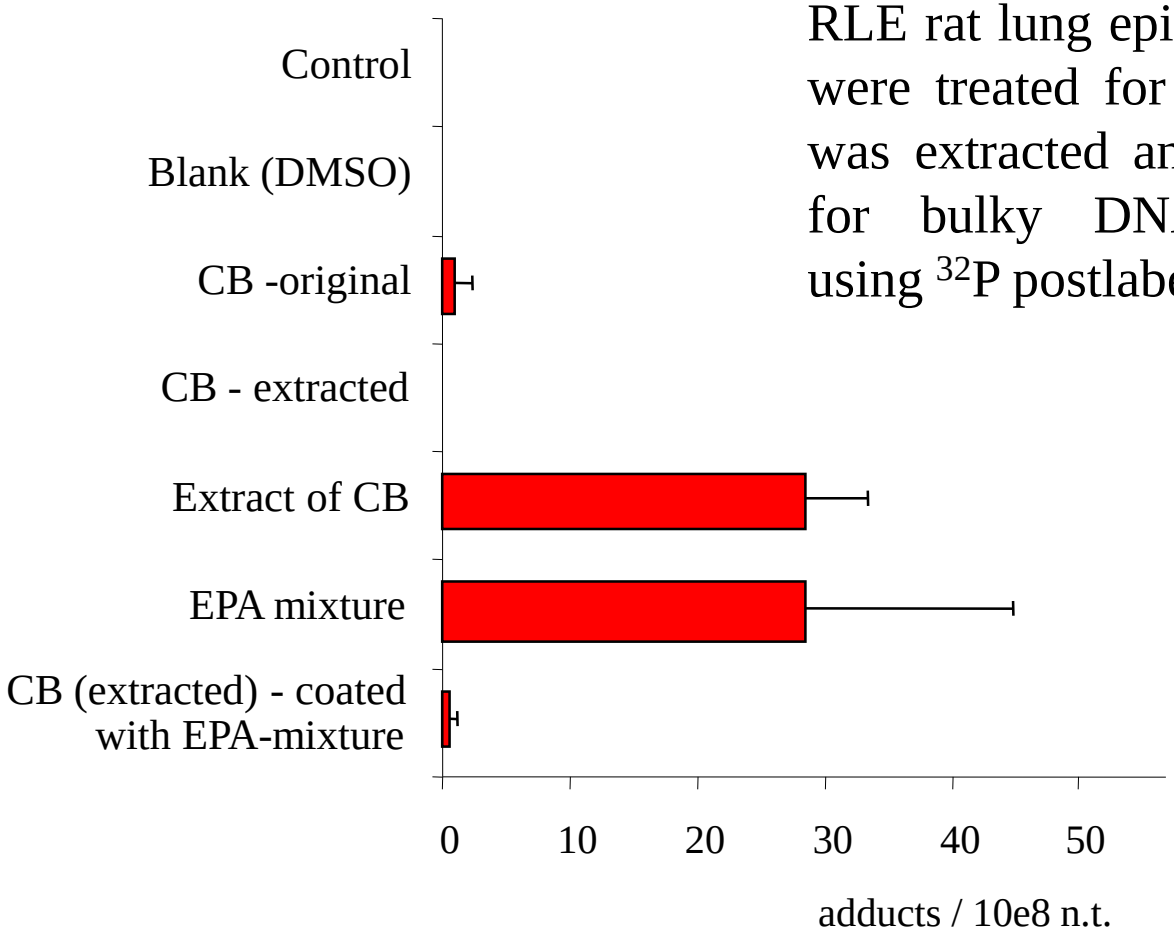
Bioavailability of polycyclic aromatic hydrocarbons (PAH) from carbon black nanoparticles



RLE rat lung epithelial cells were treated for 24h. DNA was extracted and analysed for bulky DNA adducts using ^{32}P postlabelling

Borm et al.(2005) Toxicol Applied Pharmacol

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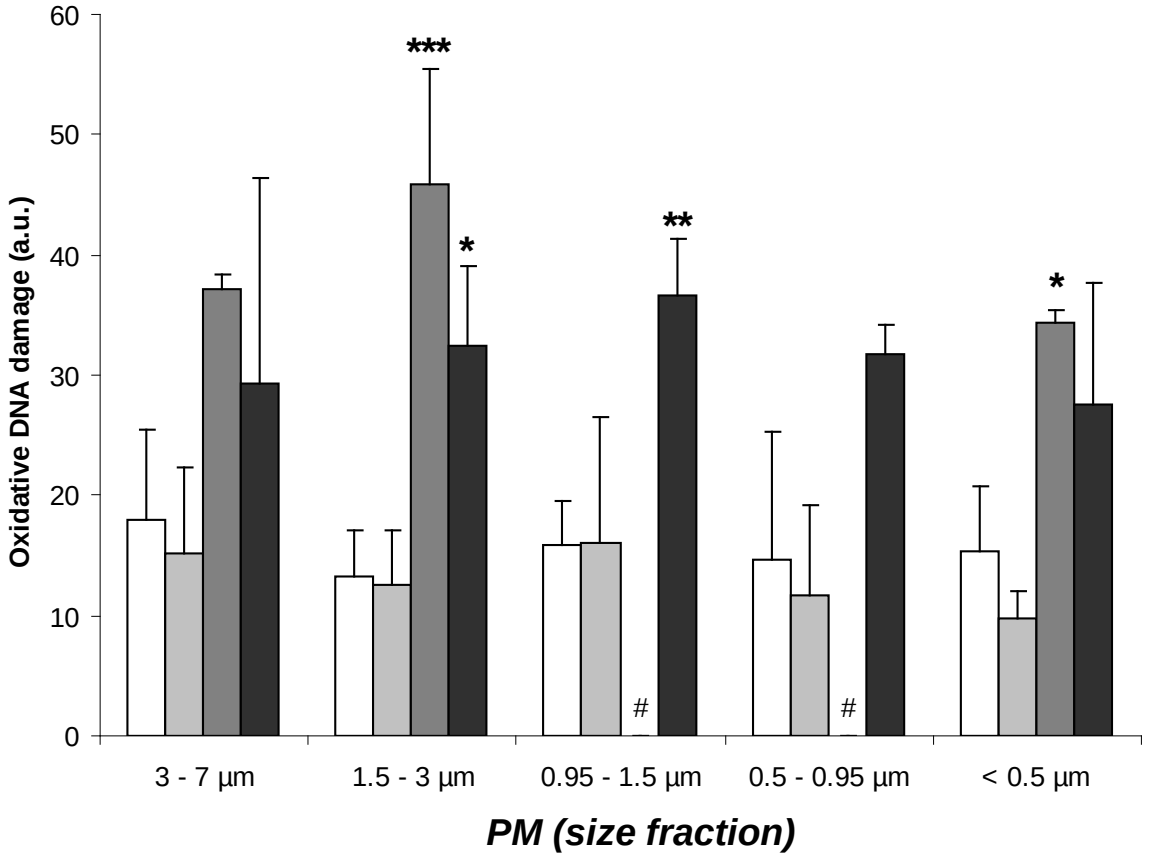
DNA damage by size-fractionated ambient PM in A549 human lung epithelial cells

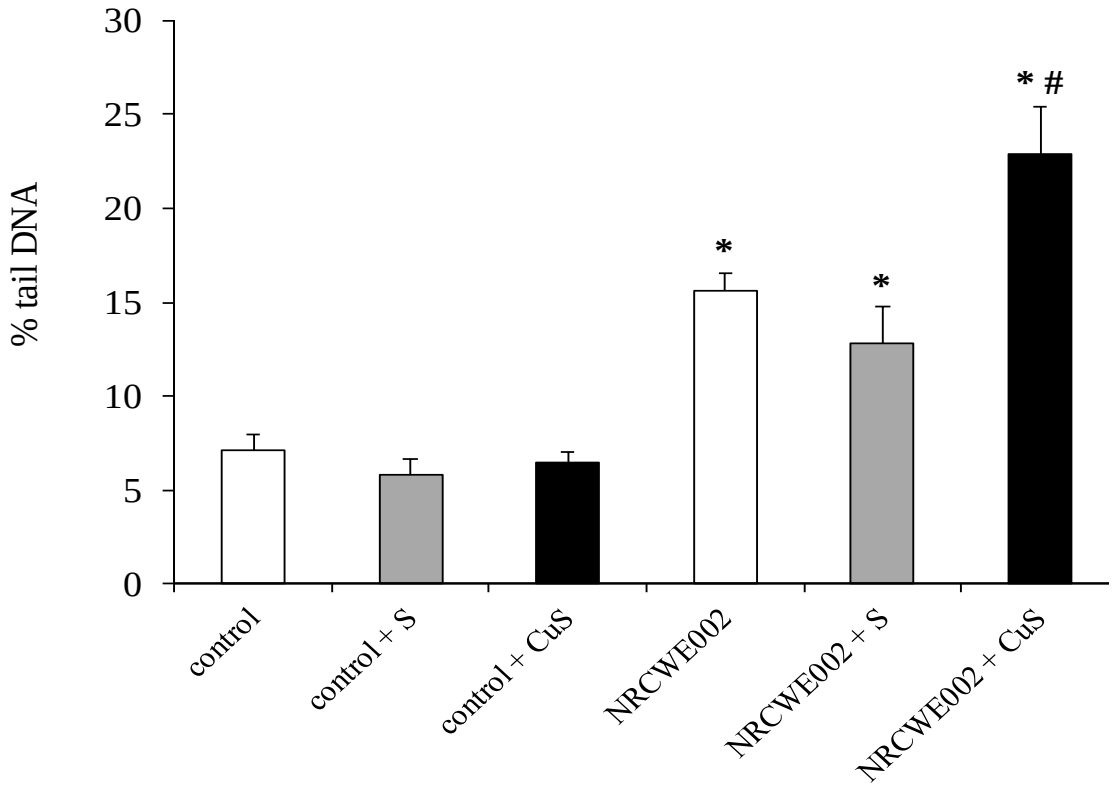
PM was sampled at a <u>remote background</u> (□) and three urban sites of varying traffic influence, i.e. <u>urban background</u> (▒), <u>roadside</u> (▓) and <u>road tunnel</u> (■)



Wessels et al (2010) Environ Sci Technol

= Not determined

DNA damage by TiO_2 NP in A549 lung epithelial cells Effects of serum and surfactant (submerged cultures)



DNA damage was evaluated by the comet assay following 4 h exposure to TiO_2 (NRCWE002) in the presence or absence of 2% serum (S, grey bars) or the surfactant Curosurf (CuS, black bars).

*p<0.05 vs corresponding control

#p<0.05 vs NRCWE002

Thongkam et al.. Mutagenesis 2017

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Bacteria (prokaryotic) versus mammalian cells (eukaryotic)

Cell wall

Introduction of *Rfa* mutation containing *Salmonella* tester strains: increased permeability for bulky chemicals

Particles ?

Can the Ames test provide an insight into nano-object mutagenicity? Investigating the interaction between nano-objects and bacteria.

Martin J. D. Clift, David O. Raemy, Zulqurnain Ali, Carola Endes, Andrea D. Lehmann, Christina Brandenberger, Alke Petri-Fink, Peter Wick, Wolfgang J. Parak, Peter Gehr, Roel P. F. Schins, Barbara Rothen-Rutishauser

Nanotoxicology, December 2013; 7(8):1373–1385
DOI: 10.3109/17435390.2012.741725

Toxicol Sci. 2017 Mar 1;156(1):149-166. doi: 10.1093/toxsci/kfw247.

From the Cover: An Investigation of the Genotoxicity and Interference of Gold Nanoparticles in Commonly Used In Vitro Mutagenicity and Genotoxicity Assays.

George JM[1], Magogotya M[1,2], Vetten MA[1,3], Buys AV[4], Gulumian M[1,3].

Author information

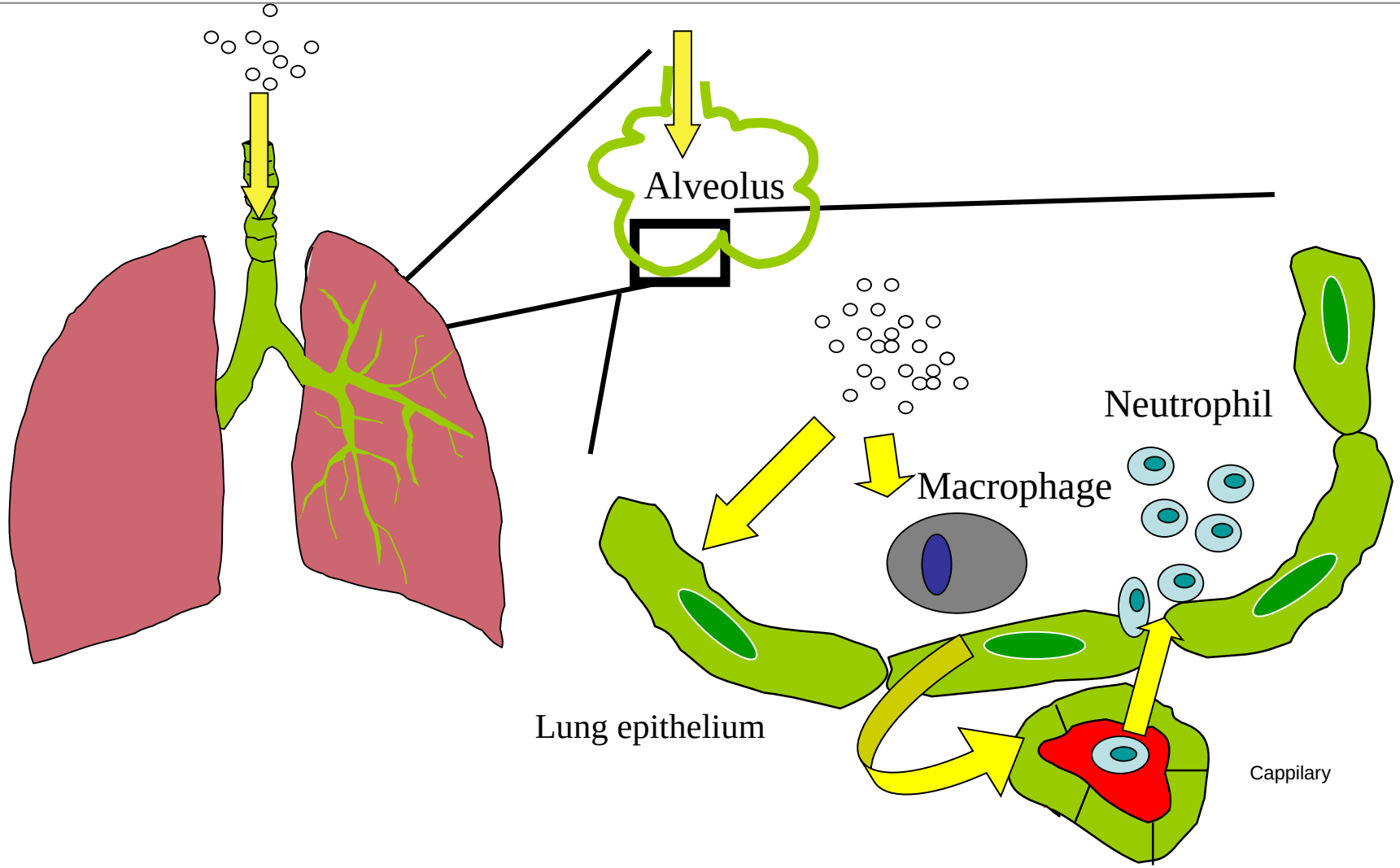
Abstract

The suitability of 4 in vitro assays, commonly used for mutagenicity and genotoxicity assessment, was investigated in relation to treatment with 14 nm citrate-stabilized gold **nanoparticles** (AuNPs). Specifically, the **Ames** test was conducted without metabolic activation, where no mutagenic effects were observed. High resolution transmission electron microscopy and Cytoviva dark-field image analysis showed that AuNPs did not enter the bacterial cells, thus confirming the unreliability of the **Ames** test for nanoparticle mutagenicity studies. .... ..

Bacteria (prokaryotic) versus mammalian cells (eukaryotic)

Intercellular crosstalk

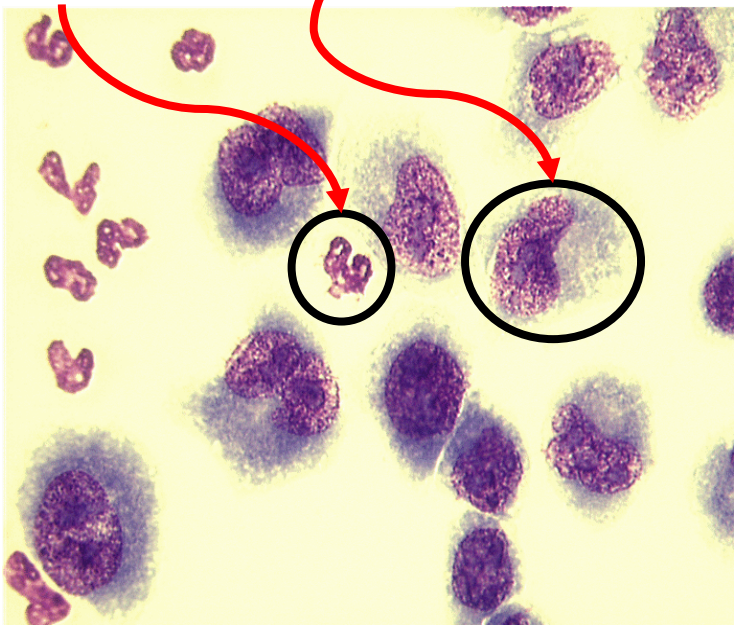
Particle deposition in the lung and inflammation

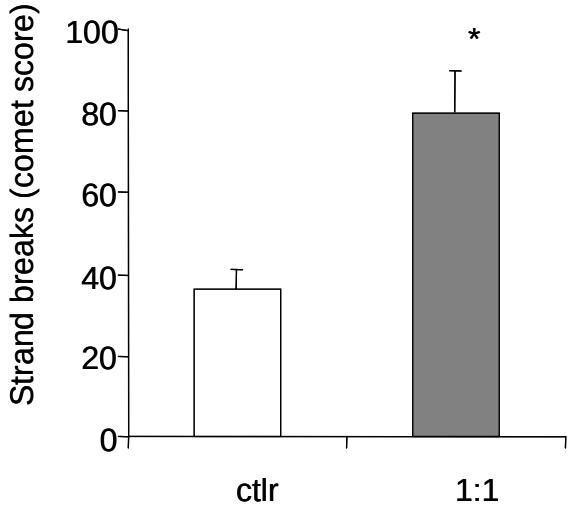


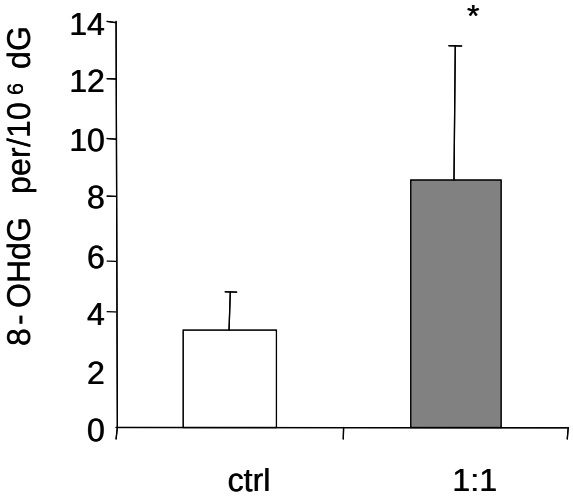
In vitro co-incubation studies: activated phagocytes (neutrophils) cause oxidative damage to the DNA of alveolar epithelial cells

Neutrophil

Alveolar epithelial cell







Inhibition of DNA damage by antioxidants (catalase, SOD)

Knaapen et al. 1999 Free Radic. Biol. Med.
Knaapen et al. 2002 Mol. Cel. Biochem.
Knaapen et al. 2006 Mutagenesis (review)

Mutation caused by human phagocytes

SA Weitzman, TP Stossel

Science 1981, Vol. 212, Issue 4494, pp. 546-547

DOI: 10.1126/science.6259738

→ Mutagenicity of of phagocytes in Ames test, mediated by formation of reactive oxygen species (ROS)

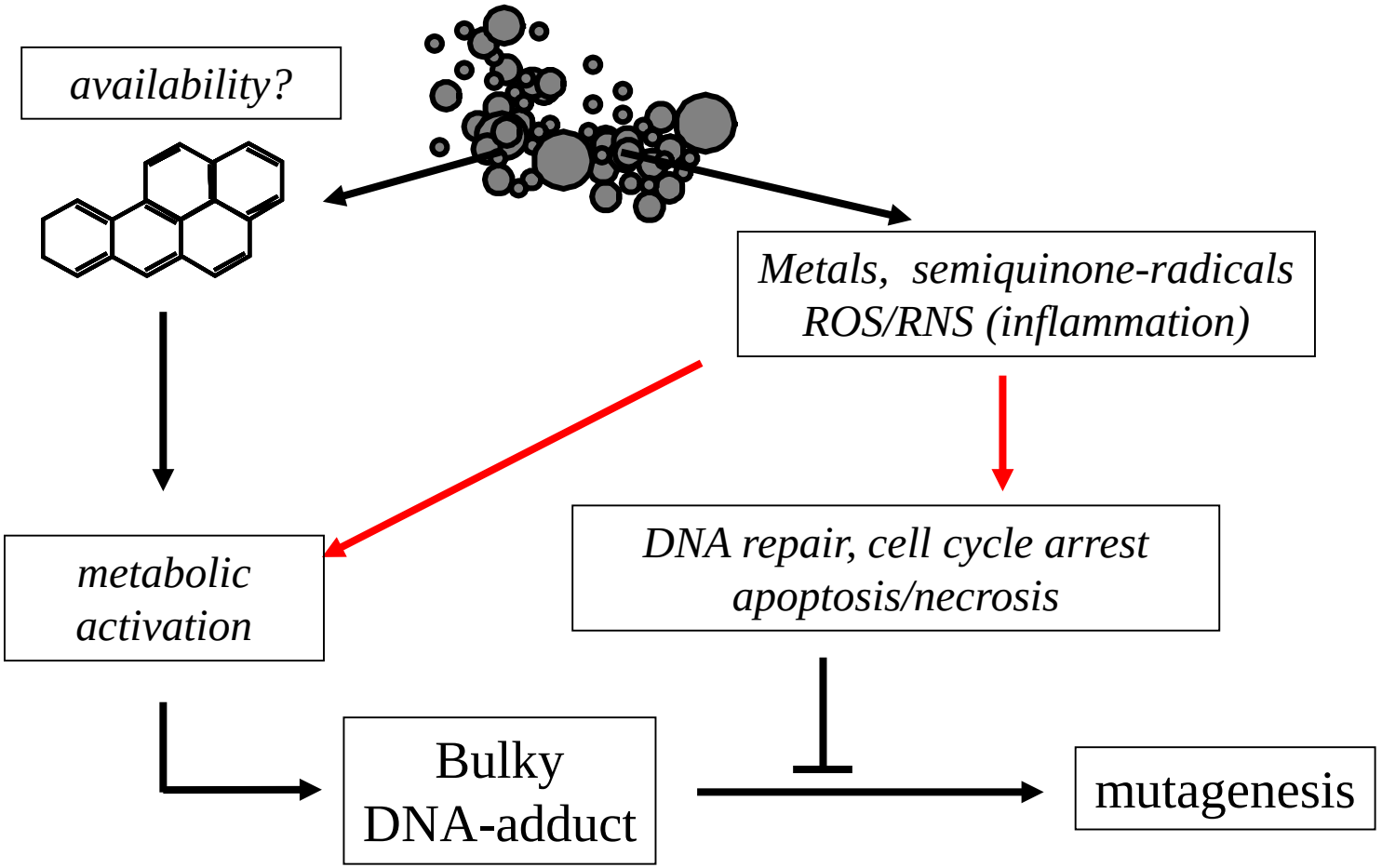
Evaluation of the Release of Mutagens and 1 -Nitropyrene From Diesel Particles in the Presence of Lung Macrophages in Culture

Leon C. King, Kenneth Loud, Silvestre B. Tejada, Mike J. Kohan, Joellen Lewtas

Environmental Mutagenesis 1983: 5577-588

→ Alveolar macrophages phagocytose diesel particles resulting in loss of mutagenic activity in Ames test

Mutagenesis of inhaled PAH-containing particles



Schins and Knaapen (2007) Inhal Toxicol

Ames Test - Summary & Conclusions

- Cheap and easy to perform. Demonstrated to identify the majority of chemical carcinogens. However, not all mutagens identified in Ames test are carcinogenic. Nota all carcinogens are mutagens → further testing needed

- Can include evaluation of metabolism dependent mutagens (rat/human liver homogenates). However, effects of mutagen-carrying (nano)particles may be overestimated when extraction methods are used.

- Does not take into account the complexity of particle-cell interactions seen in mammalian cells (cell wall, endocytosis, nucleus…).

- Does not take into account intercellular crosstalk mechanisms and effects which may increase/decrease mutagenic outcome:
  - → Mutagenicity of inflammatory mediators
  - → Modification of metabolism
  - → Detoxification of mutagen-carrying particles by macrophages.

Acknowledgements

Ad Knaapen
Anton Wessels
Catrin Albrecht
Christel Weishaupt
Damien van Berlo
Gabriele Wick
Gonca Çakmak Demircigil
Julia Kolling
Kirsten Gerloff
Paul Borm
Waluree Thongkam

Barbara Rothen-Rutishauser
Frederik-Jan Van Schooten
Günter Oberdörster
Martin Clift
Peter Gehr
Roger Godschalk
Roy Harrison
Wolfram Birmili