Metoder: Mutagenisitet

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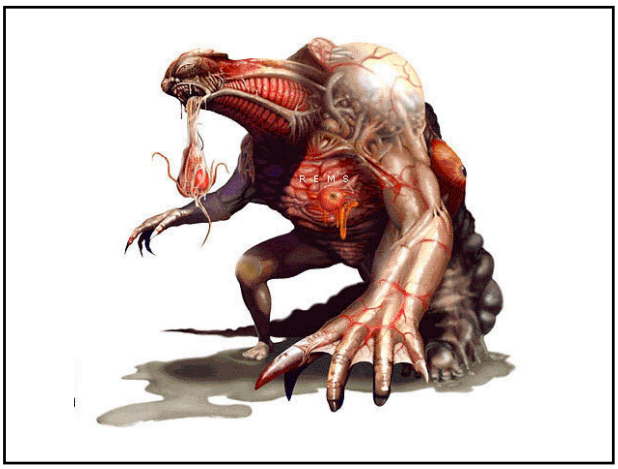
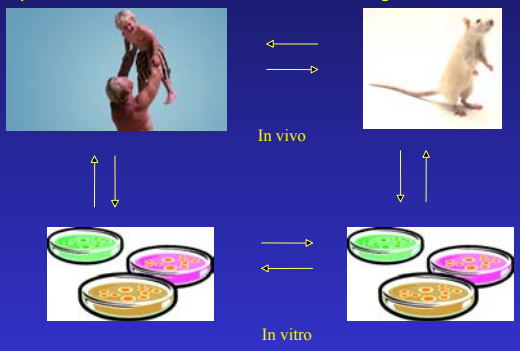


Testing for mutagens:
Important for cancer and possible germ cell mutations.
Extrapolation from animal to man and from high to low doses.



Elements in Risk Assessment of Chemicals

- Hazard identification
  - *What is the toxic potential of the chemical?*
- Hazard characterisation
  - *What is the relationship between the dose of the chemical and toxicity?*
- Exposure assessment
  - *What are the exposures to the chemical?*
- Risk characterisation
  - *What are the probabilities and consequences of toxic effects in the exposed population?*

Elements in Hazard Characterisation

- Establishment of **dose-response** relationship for critical effects
- Identification of the **most sensitive** species and strain
- Identification of potential **species differences** (qualitatively and quantitatively)
- Characterisation of the **mode of action**/- mechanism for critical effects
- Extrapolation from **high to low** dose and from experimental **animals to humans**

Mutasjoner i kjønnsceller

•Arvelige sykdommer (1.3%)

•Medfødte misdannelser
-3-6 % av alle svangerskap
-60 % sykdom med arvelig innslag

•Kromosomale mutasjoner
-5% av alle svangerskap
-6% av alle dødfødte
-30% av alle spontanaborter (85% av disse kan skylles nye mutasjoner

Categories of germ cell mutagens (German list)

•Category 1: Germ cell mutagens shown to increase the mutant frequency in the progeny of exposed humans.

•Category 2: Germ cell mutagens shown to increase the mutant frequency in the progeny of exposed mammals

Category 3: A.Substances shown to induce genetic damage in germ cells or animals, or are mutagenic in somatic cells and have been shown to reach the germ cells in their active form.

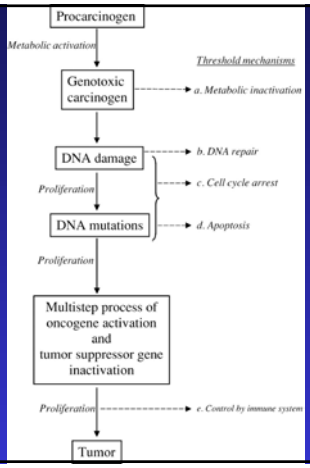
B.Substances suspected of being germ cells mutagens because of their genotoxic effects in mammalian somatic cells in vivo or, in exceptional cases, in the absence of in vivo data if they are clearly mutagenic in vitro and structurally related to in vivo mutagens

•Category 4: Not applicable (non genotoxic action)

•Category 5: Germ cell mutagens with a potency conceded to be so low that, provided the MAK value is observed, their contribution to genetic risk is expected not to be significant.

Establishment of the Dose-Response Relationship for Critical Effects

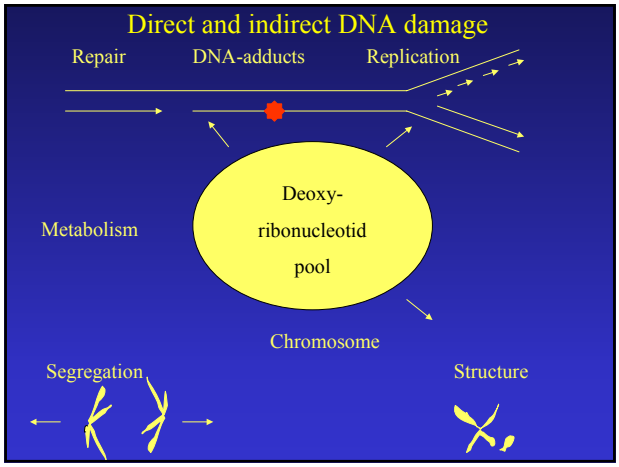
Non-Threshold versus Threshold Effects



DNA

- 1953 DNA dobbel-heliks struktur (Watson og Crick)
- 1972 Ustabilt (13 000 puriner og 55 000 SSB per somatisk celle per dag) og følsomt for kjemikalier og stråling
- 1973 DNA reparasjon (99.99999 % riktig reparasjon)
- Lengde 1.3 m, 10^{13} celler gir $1.3x10^{10}$ km eller 430x fram og tilbake til sola
- Vekt 5 pg
- 4 deoksyribonykleotider og deoksyribose sukker, 4 baser: A, G, C, T; T-A og C-G
- 64 kodon som koder for 20 ulike aa, 35 000 - 40 000 gener
- 130 reparasjonsgener

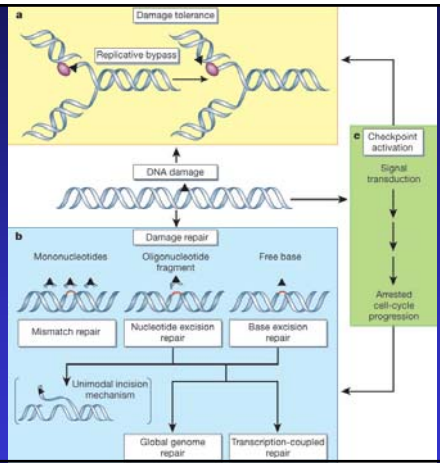
Direct and indirect DNA damage

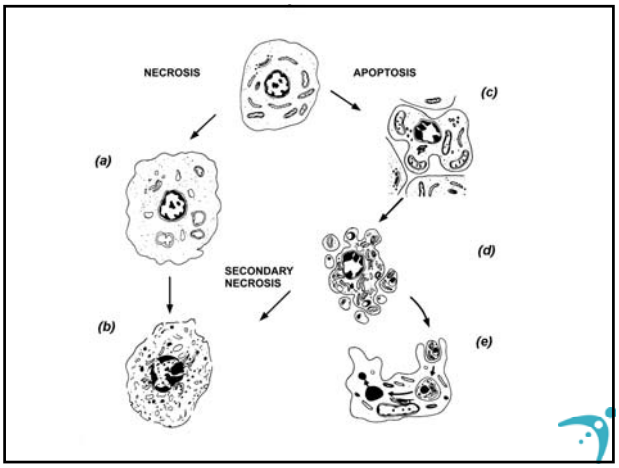


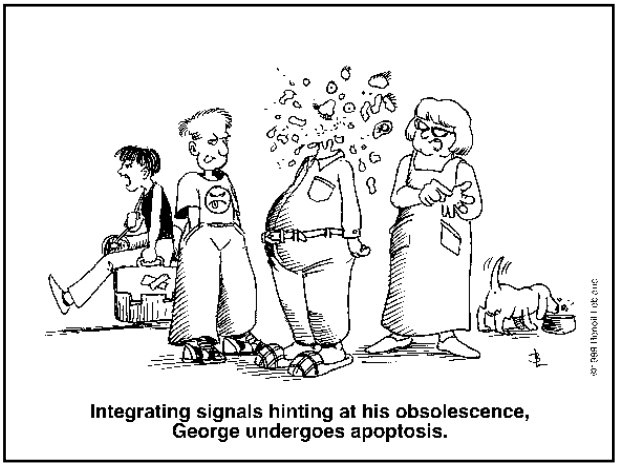
Responses to DNA damage

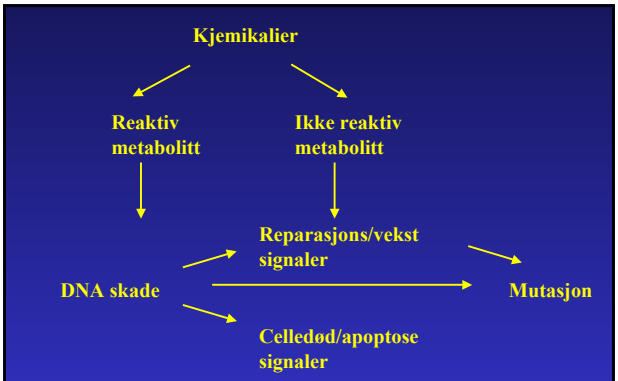
E. Friedberg

Nature, 421, 436, 2003









Betydningen av apoptose:

Balansen mellom reparasjon og celledød bestemmer antallet mutasjoner

Analysis of DNA-adducts

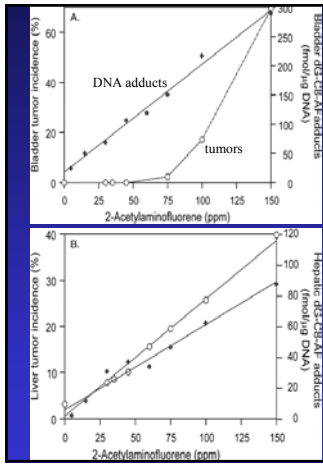
- Radiolabelled chemical
- Fluorescence
- HPLC/EC; LC-MS/MS; GC/MS; AMS
- ELISA
- ^{32}P-Postlabelling
- SCGE/with enzymes

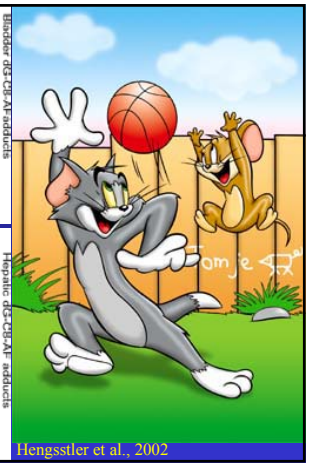
- *In vitro* and *in vivo*
- Tissue from various organs
- Identification of a adduct not always necessary

All the methods have advantage and disadvantage centred around sensitivity, cost, time, and interpretation of results

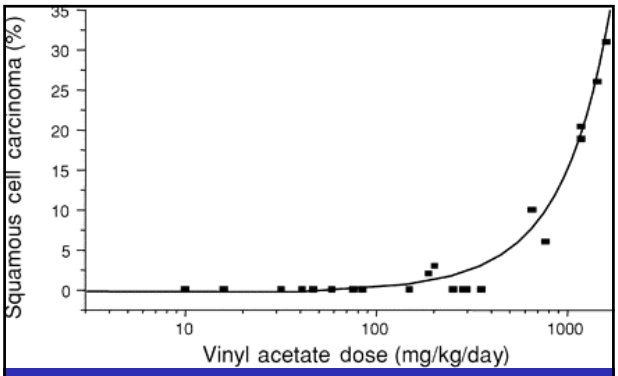
Correlation of hepatocarcinogenicity of chemicals with the covalent binding index (CBI=Damage to DNA/Dose)

Compound	CBI
Strong hepatocarcinogen	
Aflatoxin B1	17.000
Moderate Hepatocarcinogen	
2-Acetylaminofluorene	560
Vinylchloride	525
Weak hepatocarcinogen	
Uretane	29-90
Parcetamol	1.2
Non-hepatocarcinogens	
Saccharin	< 0.005





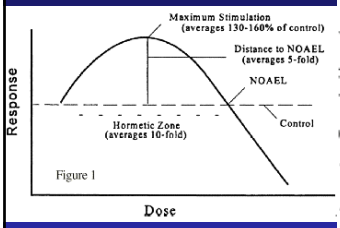
Hengsstler et al., 2002

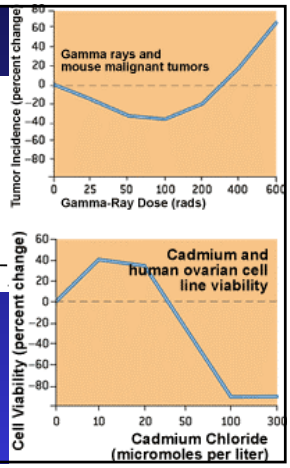


Challenging dogma: Thresholds for genotoxic Carcinogens?

Hengstler et al. Annu. Rev. Prhamacol. Toxicol. 2003,43: 485-520

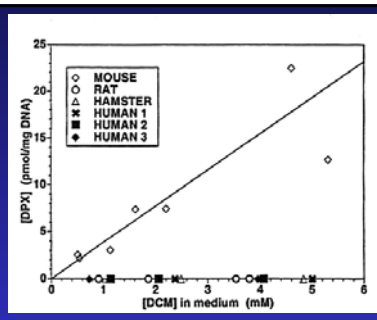
Hormesis?





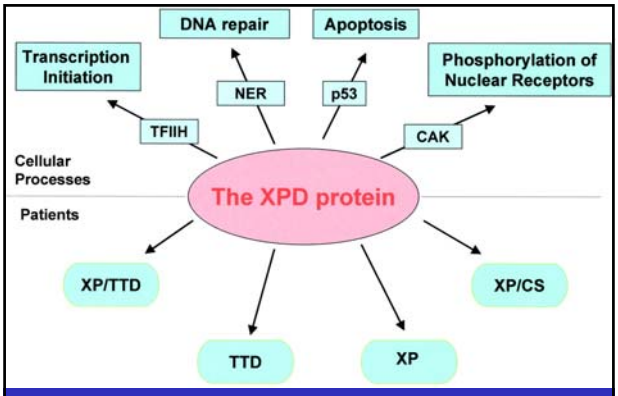
Identification of Potential Inter- and Intra- Species Differences

(Qualitatively and Quantitatively)



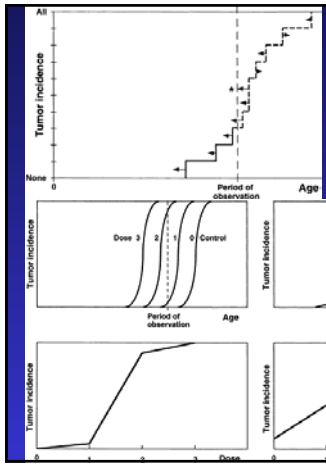
Species differences in dichloromethane DNA-protein crosslinks in isolated hepatocytes

Casanova et al., FAAT 37, 168, 1997



ERCC2/XPD gene polymorphism and cancer risk

Benhamou and Sarsin, Mutagenesis, 17, 463-469, 2002



Variasjon i individers følsomhet

- genetiskefaktorer
- livsstilfaktorer
- miljøfaktorer

Lutz, Mutat. Res., 2001, 482, 71-76

DNA damage and repair

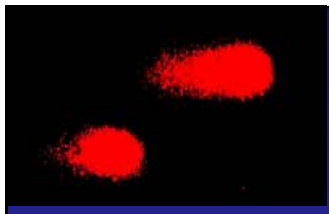
- Alkaline elution
- Comet assay/Single cell gel electrophoresis
- Unscheduled DNA synthesis

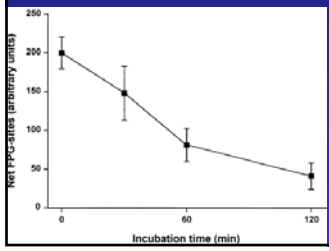
-*In vitro* and *in vivo*

-Tissue from various organs (UDS mostly the liver)

-Comet assay currently most popular

The comet assay





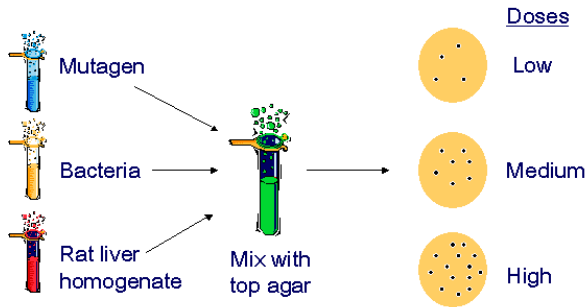
Cellular repair of 8-oxoguanine. The cells were exposed and incubated at 37°C to allow repair to occur and the remaining 8-oxoguanines were estimated, as FPG-sensitive sites, using the comet assay. Mean values from lymphocytes from six subjects are shown, with SD.
Collins and Harrington, 2002

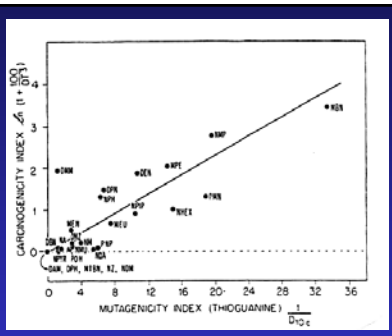
"Core" tests

- Bacteria mutation assays
- Mammalian gene mutation assay in cultered cells
- Chromosomal aberration assay *in vitro*
- UDS tests *in vitro* and *in vivo*
- Rodent erythrocyte micronucleus assay
- *In vivo* mammalian bone marrow chromosomal aberration test
- Mammalian germ cell tests

These tests are very important in hazard identification and characterisation

The Standard Ames Assay

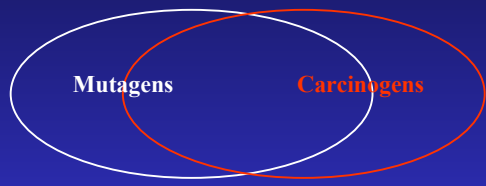




Correlation between *in vitro* mutagenicity and carcinogenicity for N-nitrosamines

Jones et al., Carcinogenesis 2, 1057, 1981

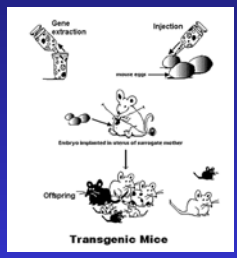
Lack of Correlation Between Salmonella Mutagenicity and Carcinogenicity of N-Nitroso Compounds

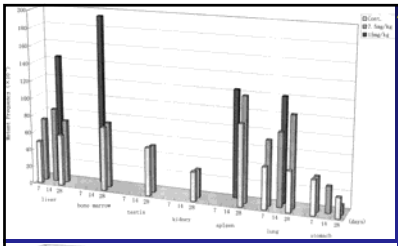


- **This may be due to the complexity of the metabolic activating process leading to formation of proximate carcinogens (*Lijinsky, Mol Toxicol 1, 107, 1987*).**
- **Cancer development are more than just a mutation formed in a bacterial test system *in vitro*.**
- **Similar arguments holds true for Germ cell mutations**

Up and coming tests

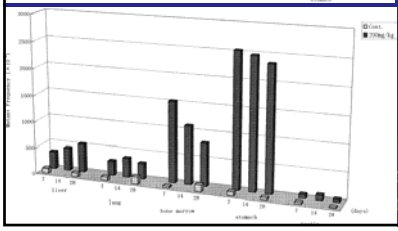
- *In vitro* micronucleic assay
- Photochemical genotoxicity
- Comet assay *in vitro* and *in vivo*
- DNA adduct determination
- Transgenic mutation assay





7.5 or 15 mg/kg 4-NQO i.p.

Transgenic Muta™Mous



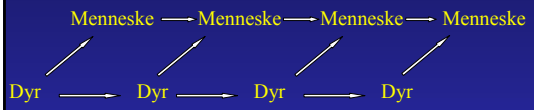
200 mg/kg 4-NQO p.o.

Nakajima et al. 1999
Mutat. Res., 444, 321-336

Comparison of positive results of *in vivo* transgenic mutation (TG) and rodent oncogenicity (Onco) assays at the site of first contact with the chemical, with *in vivo* rat liver UDS and mouse bone marrow/peripheral blood cytogenetics assays (Cyto)

Chemical	TG	UDS	Cyto	Onco
ß-Propiolactone	+	–	–	+
1-Chloromethylpyrene	+	ND	–	+
DMBA	+	–	+/–	+
BP	+	–	+	+
1,3-Butadiene	+	–	+	+
MNNG	+	–	+	+
4NQO	+	–	+	+
Urethane	+	–	+	+
Benzene	+	ND	+	+
MeIQ	+	ND	+/–	+
DMN	–	+	+	–

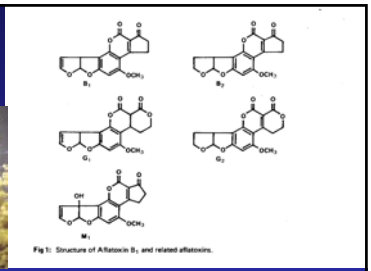
Metoder til påvisning av mutagene skader hos mennesket: Problemstillinger knyttet til ekstrapolering



Eksponering	*Måldose*	*Respons*	*Helseskade*
Påvisning av mutagener	Addukter	Markører på DNA skader	Kreft, Arvelige sykdommer, Missdannelser, Spontanaborter

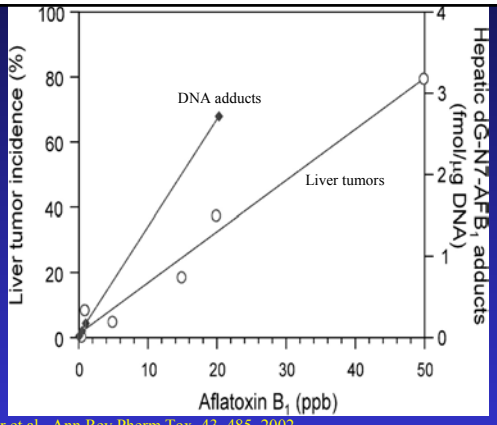
(Sobels, 1980)

Aflatoxin B1 (AFB1)



The most potent hepatocarcinogen known.
Liver is the usual target for both acute and chronic toxicity.

Metabolic activation of AFB1 to the 8,9-epoxide, leading to binding to GSH, DNA (N-7 guanine) and serum albumin



Hengsstler et al., Ann Rev Pherm Tox, 43, 485, 2002

Mutation spectrum in the tumor suppressor genet *p53* linking Aflatoxin B_1 exposure to human liver cancer

- In liver tumors from persons living in geographic areas (Asia, Africa and North America) where AFB_1 and hepatitis B virus (HBV) are cancer risk factors, the majority of *p53* mutations are in codon 249.
- Exposure of AFB_1 to human liver cells *in vitro* produces 249^{ser} (AGG to AGT) p53 mutants.

Expression of the 249^{ser} mutant p53 protein appear to provide a specific growth/survival advantage to liver cells (suppression of apoptosis).

(Hussain and Harris, Mutat. Res., 428, 23-32, 1999)

Aflatoxin B1 - cancer risk estimation

Interactions with virus:

Both experimental as well as epidemiological experiments shows a strong interaction with hepatitis B infection (possible also hepatitis C) with an increased sensitivity towards aflatoxin B1

HBsAg-:

0.01 (0.002-0.03) cancer cases/year per 100.000 pr ng aflatoxin/ kg bw. per day.
Lifetime risk 10^{-5} = 1.4ng/kg bw pr day

HBsAg+:

0.3 (0.05-0.5) cancer cases/year per 100.000 pr ng aflatoxin/ kg bw. per day
Lifetime risk 10^{-5} = 0.05 ng/kg bw pr day

Nordic TDI: 0.01 ng / kg bw Intake in Norway not estimated

Conclusions

- Mutagenicity assays are very important in **hazard identification and characterisation**
- **Carcinogenic effect** appear to be more critical than Germ cell mutagensis with regard to exposure to chemical mutagens
- Mutagenicity studies in experimental systems as well as on humanes can gives very important aids to the **carcinogenic risk estimation**
- Mutagenicity test systems are excellent models for characterisation of the **mode of action**/mechanism for critical effects
- Mutagenicity studies may give important information with regard to **species differences** in carcinogenicity and mutagenicity
- Mutagenicity studies will aid in the **extrapolation** from high to low dose and from experimental systems to humans
- Mutagenicity studies are also important when **characterising exposure**