Comet assay on mice testicular cells

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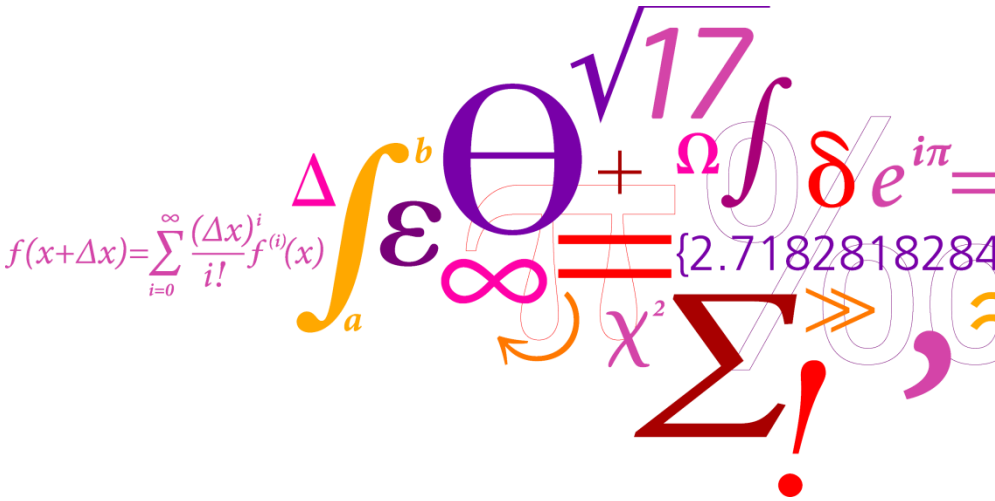

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Overall aim of the project:

- Extending and improving an existing quantitative structure-activity relationship (QSAR) computer model for in vivo Comet assay, developed at the National Food Institute, Technical University of Denmark.

- Twenty chemicals were tested in the *in vivo* Comet assay in the following tissues: liver, kidney, testicles, colon, urinary bladder and lungs.

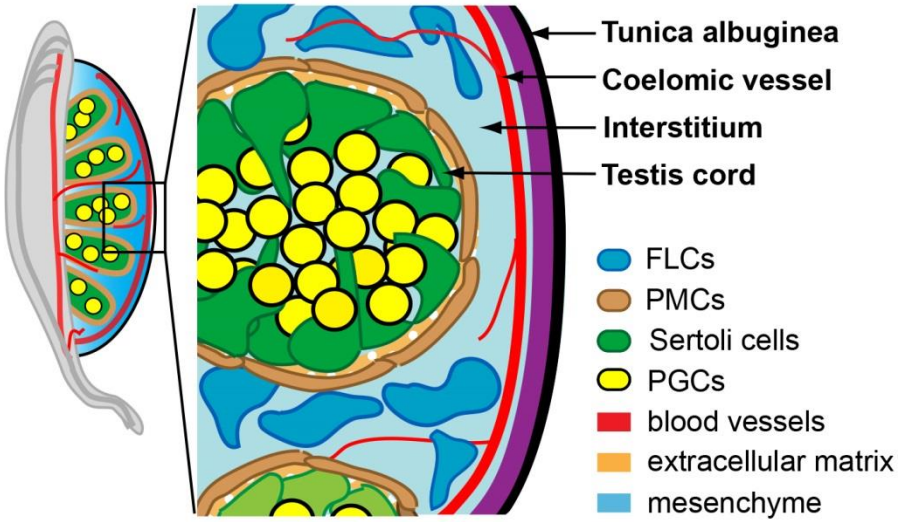
Aim of this presentation:

- To present results of mice testicular cells exposed to different chemicals.

- To present power curves for testicular cell data illustrating power as a function of sample size.

Testicular cells

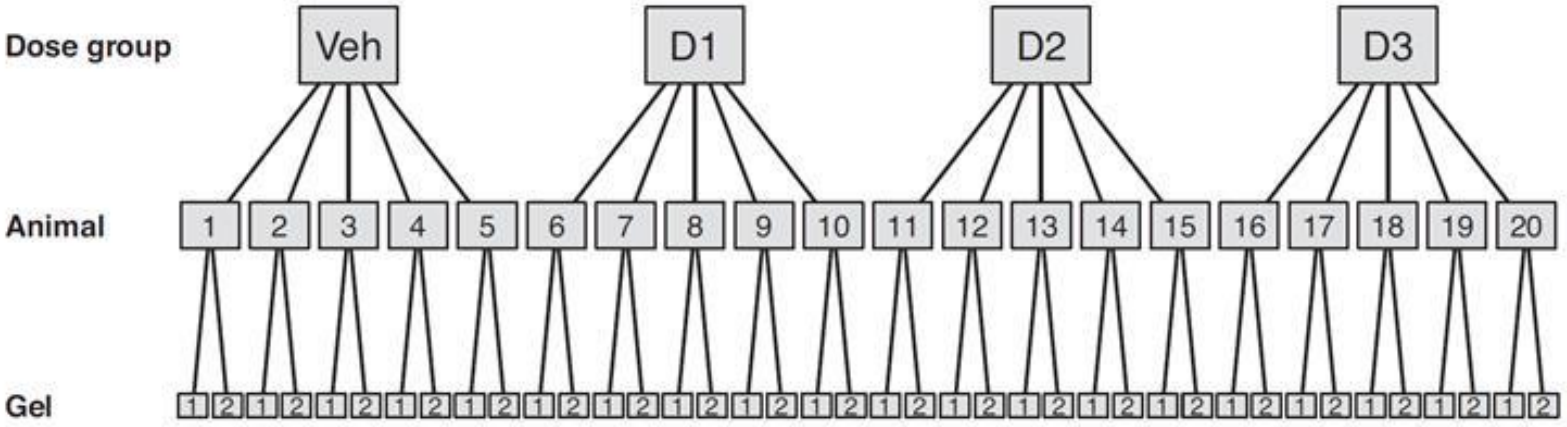
A testicle comprises different cell populations including somatic Sertoli cells, differentiating germ cells in various stages of spermatogenesis and spermiogenesis, Leydig cells, macrophages, and fibroblasts as well as blood vessels and lymphatic vessels containing different cell populations. Therefore,the DNA isolated from the testicular tissue origins from a mixture of different celltypes.



Svingen and Koopman, Genes & Development, 2013

Experimental design

The chemicals were strategically selected by selecting chemical groups not already covered by the model or chemicals that could strengthen the predictive statistics of specific structural fragments.



A nested structure of the study design.
Hansen et al. Mutation Research (2014).

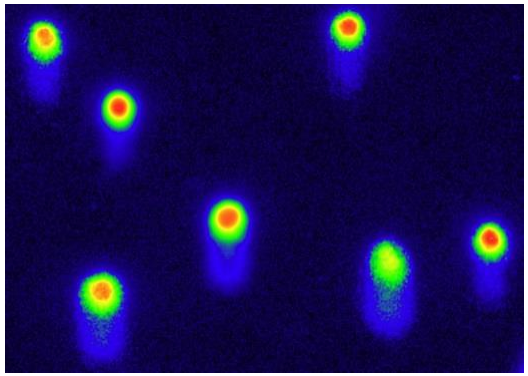
Vehicles: Water and corn oil.

Positive control chemical: EMS, 300 mg/kg bw/ day.

Exposure: Twice, 24 h apart, two to four hours after the second dosing the animals were anaesthetized in CO2/O2and decapitated. The testicles were taken out, the capsule removed and snap frozen.

Alkaline version of the Comet assay and statistical analysis

- For each CD-1 mice 200 cells were scored from two duplicate gels by the use of a fully automatic Comet assay scoring system, Pathfinder™ Cellscan Comet imaging system (IMSTAR, Paris, France).
- % tail DNA was used and the median values from the gels were used.
- Data were transformed (natural logarithm).
- A linear mixed-effects model was applied with dose as a fixed effect and animal and slides as random effects. Animal is nested within treatment and slide is nested within animal.
- Dunnett's test was subsequently applied to compare the three dose groups to the corresponding control group.
- In the linear mixed-effects model the hierarchical structure of data and the randomly selected animals are accounted for.
- Histopathology was performed on the samples that gave a positive result.



Results

CAS Nr, Doses (mg/kg bw/day)	% tail DNA of testicular cells				Pos. Control
110-26-9: 0, 50, 100, 180	**2.5±0.7**	**2.3±1.8**	**4.2±0.4**	**7.8±1.5*** **	**8.7±2.6 P<0.001**
512-56-1: 0, 125, 250, 500	**2.5±0.7**	**2.1±0.9**	**4.4±0.5***	**8.2±3.2*** **	
133-06-2: 0, 50, 138, 333	**2.3±0.4**	**2.2±0.9**	**2.3±0.6**	**2.0±0.3**	
154-93-8: 0, 2.5, 5, 10	**2.5±0.7**	**2.3±0.8**	**1.7±0.3**	**2.1±0.9**	
680-31-9: 0, 250, 500, 1000	**2.5±0.7**	**2.2±1.0**	**1.8±0.8**	**1.9±0.9**	
79-94-7: 0, 500, 1000, 2000	**2.7±1.7**	**2.5±1.3**	**2.6±1.6**	**1.9±0.5**	**7.6±1.7 P<0.01**
115-96-8: 0, 500, 1000, 1500	**2.7±1.7**	**4.7±4.0**	**4.2±3.2**	**7.3±3.3** **	
598-55-0: 0, 500, 1000, 2000	**2.4±1.1**	**4.8±1.0***	**4.9±1.6***	**3.4±0.7**	
123-31-9: 0, 75, 150, 300	**2.4±1.1**	**3.6±1.7**	**3.2±0.8**	**3.4±0.9**	
111873-33-7: 0, 500, 1000, 2000	**2.7±1.7**	**2.7±0.8**	**2.4±0.9**	**2.5±1.1**	

CAS 110-26-9, Water. Acrylamide; Building block for the polymer, polyacrylamide, contaminant in food products, crisp bread etc.
CAS 512-56-1: Water. Trimethyl phosphate; Intermediate for pesticides in closed systems or as a polymerization catalyst in industry.
CAS 133-06-2: Tween 40. CAPTAN; Fungicide
CAS 154-93-8: Water. Carmustine; Drug, treatment of tumors
CAS 680-31-9: Water. Hexamethylphosphoramide; Solvent for polymers, gases, and organometallic compounds
CAS 79-94-7: Corn oil. Tetrabrom BPA; Flame retardant
CAS 115-96-8: Corn oil. Tris(2-chloroethyl) phosphate; Flame retardant, plasticizer, and viscosity regulator
CAS 598-55-0: Water. Methyl carbamate; Intermediate in the textile industry
CAS 123-31-9: Water. Hydroquinone; Film and paper industry, drug
CAS 111873-33-7: Corn oil. PFOS; Fluorosurfactant, food contact materials

Results

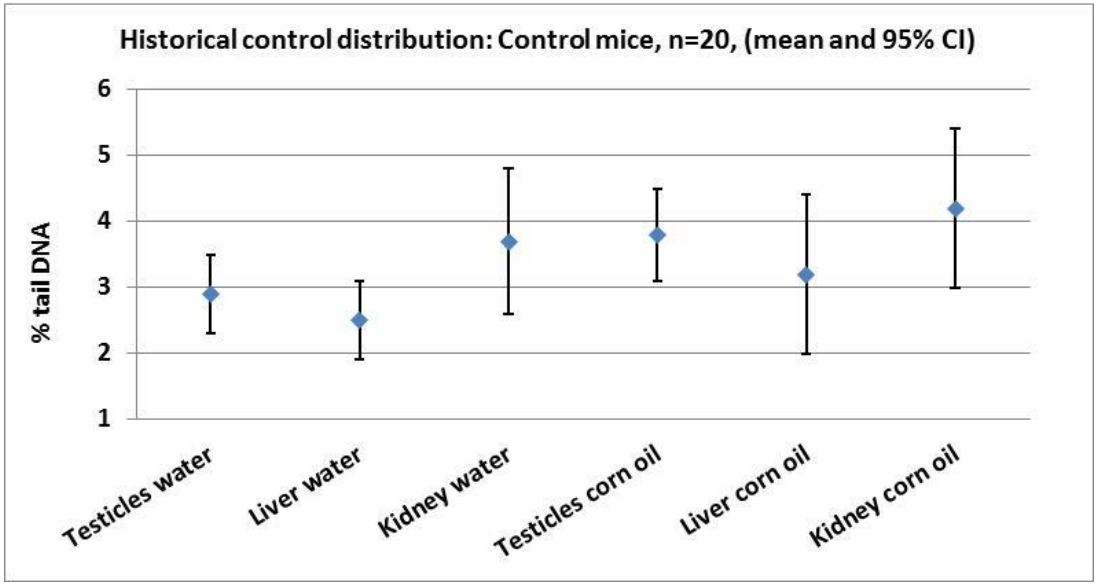
CAS Nr, Doses (mg/kg bw/day)	% tail DNA of testicular cells				Pos. Control
60965-26-6: 0, 45, 90, 180	**4.0±0.6**	**3.4±0.4**	**8.9±6.8**	**7.3±1.2****	**12.4±1.6 P<0.001**
43100-38-5: 0, 62.5, 125, 250	**4.0±0.6**	**2.8±0.4**	**4.0±1.5**	**7.6±1.0*****	
13674-87-8: 0, 225, 450, 900	**4.0±0.6**	**4.1±1.4**	**3.0±0.5**	**7.2±2.8***	
636-97-5: 0, 12.5, 25, 50	**2.5±0.6**	**2.2±0.6**	**2.5±0.6**	**2.6±0.8**	
85-28-9: 0, 500, 1000, 1500	**4.0±0.6**	**3.1±0.1**	**3.1±0.4**	**2.9±0.4**	
17804-35-2: 0, 375, 750, 1500	**3.3±0.6**	**4.5±0.6**	**3.7±1.0**	**3.8±0.7**	**10.9±1.4 P<0.001**
10605-21-7: 0, 500, 1000, 2000	**3.3±0.6**	**2.9±1.1**	**4.2±1.6**	**2.6±0.7**	
80-05-7: 0, 125, 250, 500	**3.3±0.6**	**8.0±2.9*****	**2.6±0.4**	**2.1±0.3**	
2451-62-9: 0, 25, 50, 100	**3.0±0.5**	**3.9±0.4**	**6.7±3.2***	**14.7±8.8*****	
88-72-2: 0, 125, 250, 500	**3.3±0.6**	**7.7±3.2*****	**8.9±1.1*****	**6.5±1.0****	

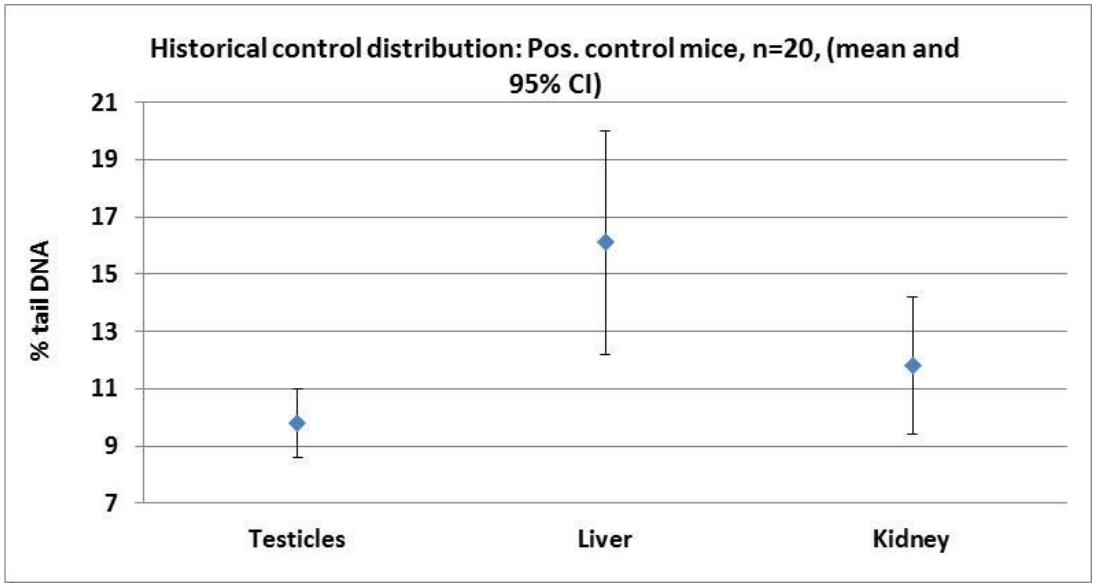
CAS 60965-26-6, Corn oil. 3',5'-Dimethoxyacetophenone; Component in acaricides that are used both in medicine and agriculture.
CAS 43100-38-5: Corn oil. 4-tert-Butylbenzoic hydrazide; Extraction processes.
CAS 13674-87- 8: Corn oil. Tris[2-chloro-1-(chloromethyl)ethyl]phosphate; Flame retardant, pesticides, plasticisers.
CAS 636-97-5: Water. 4-Nitrobenzhydrazide. Used in chemotherapeutic agents.
CAS 85-28-9: Corn oil. 4'-chloro-2-hydroxy-4-methoxybenzophenone. Organic photochemistry, organic synthesis, perfumery.
CAS 17804-35-2: Corn oil. Benomyl. Fungicide.
CAS 10605-21-7: Corn oil. Carbendazim. Fungicide and a metabolite of Benomyl.
CAS 80-05-7: Corn oil. Bisphenol A. Plasticiser.
CAS 88-72-2: Corn oil. 2-Nitrotoluene. production of pigments, antioxidants, agricultural chemicals, and photographic chemicals.
CAS 2451-62-9: Water. Tris(2,3-epoxypropyl) isocyanurate. Metal industry.

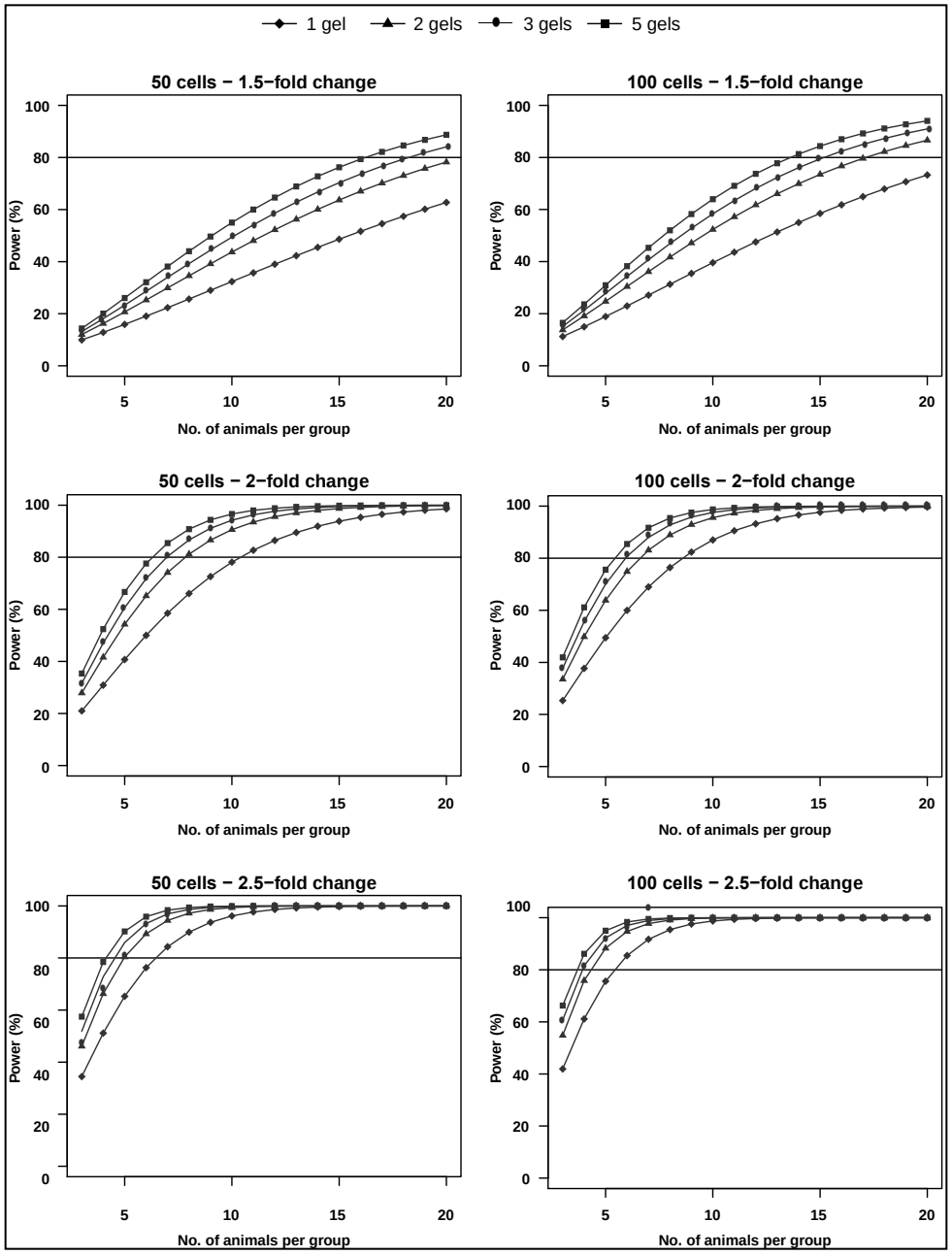
Results

CAS Nr.	Liver	Kidney	Testes	Urinary bladder	Colon	Lungs
2451-62-9	Yes	No	Yes			
88-72-2	Yes	Yes	Yes			
80-05-7	No	No	equivocal	No	No	No
10605-21-7	Yes	No	No			
17804-35-2	Yes	No	No			
85-28-9	No	No	No	No	No	No
636-97-5	No	Yes	No	No		
13674-87-8	Yes	No	Yes			
43100-38-5	Yes	No	Yes			
60965-26-6	No	Yes	Yes			
111873-33-7	No	No	No	No	No	No
123-31-9	No	Yes	No			
79-94-7	No	No	No	No	No	Yes
115-96-8	Yes	Yes	Yes			
598-55-0	No	Yes	Yes			
154-93-8	No	Yes	No			
133-06-2	Yes	No	No			
110-26-9	Yes	Yes	Yes			
680-31-9	No	Yes	No			
512-56-1	Yes	Yes	Yes			

Historical control distribution







Power curves outlining the number of animals per group and gels per animal required to detect certain fold changes with a power of 80%. The curves are based on the results of 11 chemicals.

Hansen et al. Mutation Research (2014).

Conclusions

- In control mice, the animal to animal variation for testicular cells was at the same level as liver cells and slightly lower than kidney cells.
- Effects could be detected at levels below 10% tail DNA.
- 20 chemicals were tested and ten showed an effect in testicular cells and all except Bisphenol A also gave an effect in either liver or kidney or both.
- Power curves of testicular cells indicated that more than 10 animals per group were needed for fold changes less than 2 and less than 10 animals per group were sufficient for fold changes of 2 or more.
- The largest increase in power was gained when using 2 gels per animal instead of 1 gel whereas less was gained using 3 gels instead of 2 gels and so on.
- Gain in power when 100 cells were scored per gel compared to 50 cells. To detect a 2.5 fold change at 80% power using two gels per animal, 5 mice were needed when 50 cells were scored per gel. Scoring 100 cells per gel, 4 mice were needed.

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