Electrosensibilité (EHS): résultats des recherches génétiques

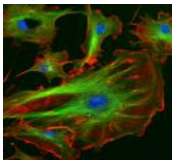
Patrick De Boever

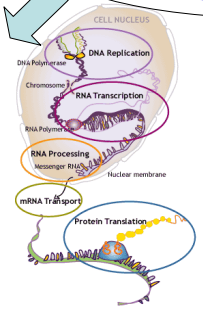
Effets biologiques des ELF

Population

Individu

Cellule





Molécule

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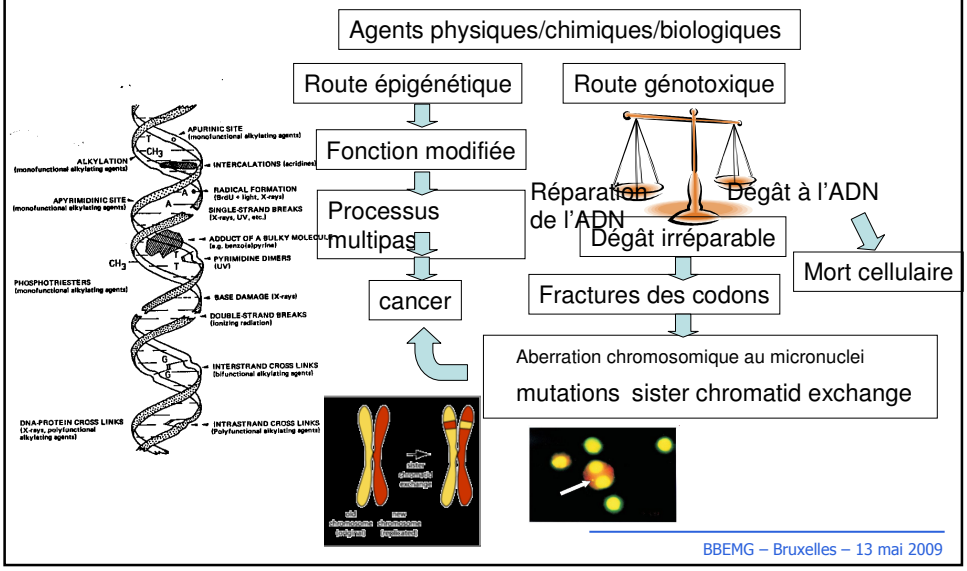
Impacts des ELF sur l'A.D.N.?

- McCann et al. 1993; Murphy et al. 1993; Moulder 1998; en McCann et al. 1998: pas d'effets génotoxiques
- ELF (30-300 Hz) à basse énergie
  - Pas de dégât direct sur l'A.D.N.
  - Des processus cellulaires sont modifiés
- Quelques résultats positifs + preuve épidémiologique
- IARC: "possible human carcinogen" Class 2B

Mécanisme de dégât à l'ADN



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Dégât à l'A.D.N.: test « comète »

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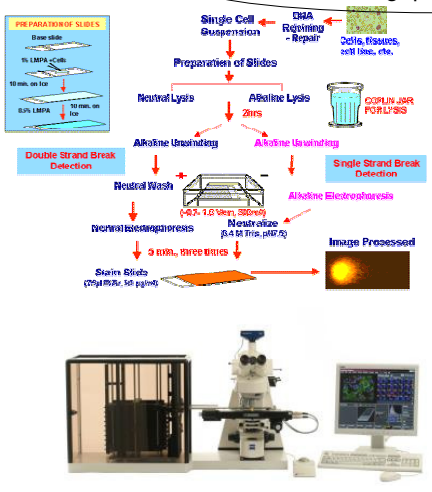
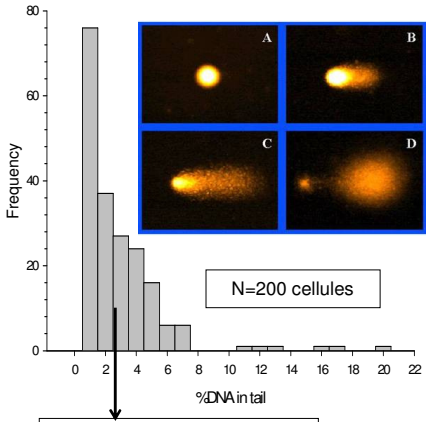
Echantillon biologique: contrôle/exposition





% moyen d'ADN dans la queue

Résultats REFLEX

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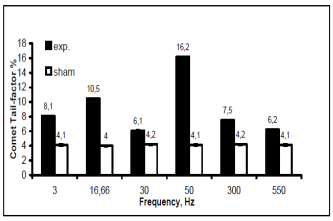

Figure 10. Alkaline Comet Assay tailfactors of ELF-EMF exposed and sham exposed fibroblasts (cell line ES-1, 15 hrs, 1000 µT, intermittent) after variation of exposure frequency (3-550 Hz).

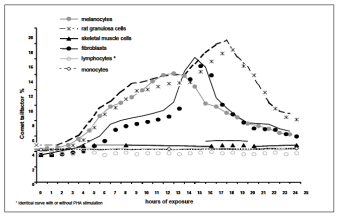

Figure 20. Alkaline Comet tailfactors of different human cell types (fibroblasts, melanocytes, monocytes, lymphocytes, skeletal muscle cells) and SV 40 transformed rat granuloa cells exposed to ELF-EMF (50 Hz sinusoidal, 1 mT, intermittent 5 min on/10 min off) for 1 to 24 hours.

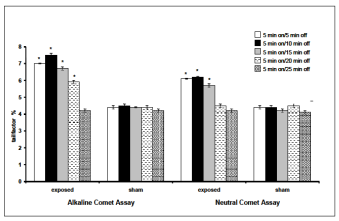

Figure 7. Alkaline and neutral Comet Assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24h,1000 µT, intermittent) - variation of off-time. * p<0.01 exposed versus sham-exposed

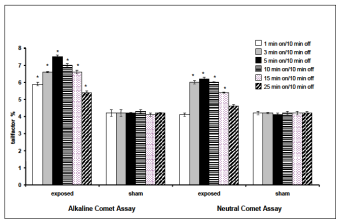

Figure 8. Alkaline and neutral Comet assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24 h, 1000 µT, intermittent) - variation of on-time. * p < 0.01 exposed versus sham-exposed

Conclusions REFLEX

- Effets génotoxiques dépendent de la fréquence
- Exposition discontinue 50 Hz - cause dégât de l'A.D.N.
- Différence entre types de cellules (déjà un effet à 35 µT)
- Importance de l'âge et fond génétique
- Dégât A.D.N. ↑ → fréquence micronucleus ↑
- Réparation A.D.N. était activée mais sans être error-free

Quelques réflexions

- *In vitro*, cellules individuelles
- A court-terme, doses élevées (1 mT)
- Mécanismes? Confirmation par d'autres techniques?

Controverse REFLEX

Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields.
Ivancsits S, Diem E, Jahn O, Rüdiger HW.
Mech Ageing Dev. 2003 Jul;124(7):847-50.

Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields.
Ivancsits S, Pilger A, Diem E, Jahn O, Rüdiger HW.
Mutat Res. 2005 Jun 6;583(2):184-8.

Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way.
Ivancsits S, Diem E, Jahn O, Rüdiger HW.
Int Arch Occup Environ Health. 2003 Jul;76(6):431-6. Epub 2003 Jun 12.

Comments on: "DNA strand breaks" by Diem et al. [Mutat. Res. 583 (2005) 178-183] and Ivancsits et al. [Mutat. Res. 583 (2005) 184-188].
Vijayalaxmi, McNamee JP, Scarfi MR.
Mutat Res. 2006 Jan 31;603(1):104-6; author reply 107-9. Epub 2005 Dec 27. No abstract available.

Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study.
Scarfí MR, Sannino A, Perrotta A, Sarti M, Mesirca P, Bersani F.
Radiat Res. 2005 Sep;164(3):270-6.
To confirm the main results reported in recent studies on the induction of genotoxic effects....our studies do not confirm the results reported previously for either comet induction or an increase in micronucleus frequency

Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analyses.
Stronati L, Testa A, Villani P, Marino C, Lovisolo GA, Conti D, Russo F, Fresegna AM, Cordelli E.
Bioelectromagnetics. 2004 Jan;25(1):41-8.

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Observations cytogénétiques Période 1990-2003

Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: a review and future research recommendations.

Vijayalaxmi, Obe G.

Bioelectromagnetics. 2005 Jul;26(5):412-30. Review.

- 63 publications uniques
  - 21: test comète
  - 44: CA, MN, SCE

First author	Year	Cells used	Expts/ donors	Frequency (Hz) fields	Flux density (mT)	Duration of exposure min/h/day	Endpoint (# cells examined)	Unexposed controls	Sham controls	Positive controls	Blind tests
Whole body exposure: animals											
Lai	1997a	Rat, whole brain	8–16 rats	60 Hz MF	0.1, 0.25, 0.5	2 h, sampled at 4 h	SSB/DSB (50)	Yes	Yes	?	Yes
Lai	1997b	Rat, whole brain	6–10 rats	60 Hz MF	0.5	2 h, sampled at 4 h	SSB/DSB (50)	Yes	Yes	?	Yes
Singh	1998	Rat, whole brain	8 rats	60 Hz MF	0.5	2 h, sampled at 4 h	SSB (50)	Yes	Yes	?	Yes
Svedenstal	1999a	Mice (*), brain cortex	6–18 mice	50 Hz EMF	0.008	11, 20, 22 days	DSB (10 ?)	?	Yes	?	?
Svedenstal	1999b	Mice (**), brain cortex	6 mice	50 Hz sinusoidal MF	0.5	2 h, 5–14 days, sampled at 0, 4 h	DSB (10 ?)	?	Yes	?	?
McNamee	2002	Mice, brain cerebellum	15 mice	60 Hz MF	1	2 h, sampled at 0, 2, 4, 24 h	SSB (50)	?	Yes	Yes	Yes
In vitro: human cells											
Fiorani	1992	Human tumor cells	? expts	50 Hz EF, MF, EMF	0.0002, 0.002, 0.02, 0.1, 0.2	1, 4, 6, 24 h	[illegible]	[illegible]	[illegible]	[illegible]	?
Ahuja	1997	Human blood lymphocytes	6 donors	50 Hz MF	2, 3, 5, 7, 10	1 h	[illegible]	[illegible]	[illegible]	[illegible]	?
Ahuja	1999	Human blood lymphocytes	6 donors	50 Hz MF	2, 3, 5, 7, 10	1 h	SSB (50)	Yes	?	?	?
Pucini	1999	Human neuronal cells	1 donor	Static MF	200	5–120 min	Mol alter (?)	Yes	?	?	?
Kindzelskii	2000	Human blood neutrophils	? donors	Pulsed EF, direct current	?	30, 45, 60 min	SSB (?)	?	Yes	Yes	?
Maes	2000	Human blood lymphocytes	21 donors	50 Hz MF	0.06 to 2.5	48–72 h	SSB (?)	?	?	?	?
Ivancsits	2002	Human skin fibroblasts	2 donors	50 Hz sinusoidal EMF	0.02 to 2	24 h	SSB/DSB (1000)	?	Yes	?	Yes
Ivancsits	2003a	Human skin fibroblasts	3 donors	50 Hz sinusoidal EMF	0.02 to 1	1–24 h	SSB/DSB (1000)	?	Yes	?	Yes
Ivancsits	2003b	Human skin fibroblasts	6 donors	50 Hz sinusoidal EMF	1	1–24 h	SSB/DSB (1000)	?	Yes	?	Yes

* outdoor, ** laboratory; ?, information not available.
EF, electric fields; MF, magnetic fields; EMF, electromagnetic fields.
SSB, single strand breaks where an alkaline buffer was used in the comet assay; DAB, double strand breaks where neutral buffer was used in the comet assay; Mol Alter, molecular alterations using polymerase chain reaction technology.

- exposition
- méthodologie

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Observations cytogénétiques Période 1990-2003…sommaire

Test system employed	Number of investigations Indicating: No increased damage	Increased damage	Inconclusive damage	Total number of studies
DNA single and double strand breaks and repair				
Whole body: animals	1	5	0	6
In vitro: human cells	3	6	0	9
In vitro: EMF + genotoxic mutagens	4	0	2	6
Chromosomal aberrations, micronuclei, and sister chromatid exchanges				
Whole body: animals and humans	6	0	2	8
In vitro: animal and human cells	9	2	6	17
In vitro: EMF + genotoxic mutagens				
Animal cells	1	1	4	6
Human cells	5	0	6	11
Total	29	14	20	63
%	46	22	32	

"Considering the weight of scientific evidence approach for gentoxicity investigations, as adopted by the IARC (2002), the preponderance *of data thus far available in the literature shows that EMF exposure per se is not genotoxic…*"

"However, reseach must continue to resolve the controversial data published in literature...The data from a well coordinated, multicenter collaborative study with adequate statistical power will be needed…"

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Méta-analyse de 87 publications Période 1990-2007

Genetic damage in mammalian somatic cells exposed to extremely low frequency electromagnetic fields: A meta-analysis of data from 87 publications (1990-2007).

Prihoda TJ.

Int J Radiat Biol. 2009 Mar;85(3):196-213.

PMID: 19296340 [PubMed - in process]

Year:	1990 – 2; 1991 – 4; 1992 – 1; 1993 – 7; 1994 – 3; 1995 – 6; 1996 – 2; 1997 – 9; 1998 – 4; 1999 – 7; 2000 – 5; 2001 – 7; 2002 – 5; 2003 – 7; 2004 – 5; 2005 – 8; 2006 – 2; 2007 – 3	87
Countries:	Austria – 5; Belgium – 2; Canada – 2; Egypt – 1; Finland – 2; France – 1; Germany – 6; India – 3; Italy – 24; Japan – 7; Jordan – 2; Mexico – 1; New Zealand – 1; Norway – 2; Poland – 3; South Korea – 1; Spain – 2; Sweden – 7; Turkey – 2; UK – 2; USA – 11.	87
ELF-EMF frequencies:	16 Hz – 1; 50 Hz – 65; 60 Hz – 16; 100 Hz – 1; 4400 Hz – 2; 32 and 50 Hz – 1; 50 and 60 Hz – 1	87
Flux density: 0.0– > 5.0 mT	1 Flux density – 54; 2 different flux densities – 6; 3 different flux densities – 14; 4 different flux densities – 1; 5 different flux densities – 3; 6 different flux densities – 1; 8 different flux densities – 1; Occupational – 7;	87
Studies:		
In vitro-Human; In vitro-Rodent; In vivo-Human; In vivo-Rodent	1 study – 78; 2 different studies – 8; 4 different studies – 1	87
In vitro-Human ± Mutagen; In vitro-Rodent ± Mutagen		
In vivo-Rodent ± Mutagen		
Genotoxicity end-points: DNA strand breaks; Chromosomal aberrations Micronuclei; Sister chromatic exchanges	1 end-point – 72; 2 different end–points – 11; 3 different end–points – 2; 4 different end–points – 2	87
Cell types (*):	1 cell type only – 79; 2 different cell types – 5; 3 different cell types – 2; 6 different cell types – 1	87

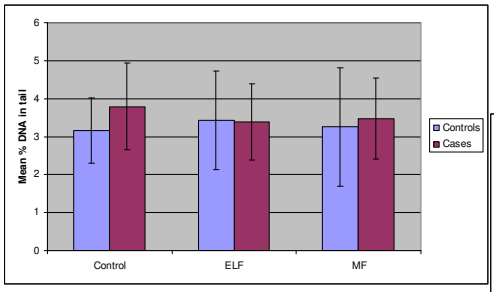
(*): Freshly collected and cultured human cells: Human amniotic cells; Human blood lymphocytes; Human blood neutrophils; Human glioma cells; Human lymphoblastoid cells; Human melanocytes; Human monocytes; Human skeletal muscle cells; Human skin fibroblasts; Human tumor cells. (*): Freshly collected and cultured rodent cells: Chinese hamster lung cells; Chinese hamster ovary cells; Mouse blood lymphocytes; Mouse bone marrow cells; Mouse brain cells; Mouse liver cells; Mouse red blood cells; Mouse skin cells; Rat brain cells; Rat blood lymphocytes; Rat bone marrow cells; Rat granulosa cells; Rat lung cells; Rat skin fibroblasts; Syrian hamster embryo cells.

Méta-analyse conclusions

- Test comète
  - Contrôle ELF biologiquement petit, mais significatif
  - Différence entre méthode de mesure et statistiques
  - Cycle cellulaire pour cellules à croissance continue
- CA, MN, SCE
  - Quelques différences statistiques
  - Hausse ≈ concentrations spontanées (databases)
- Nécessité de plusieurs points finaux pour évaluation génotoxique pertinente
- Public général ↔ Professionnels
- Directives: 5 kV/m (100 µT); 10 kV/m (1mT): pas d'effet génotoxique ct
- Co-exposition → Real-life
  - Peu d'expériences
  - Priorité selon OMS

Effets génotoxiques des ELF?

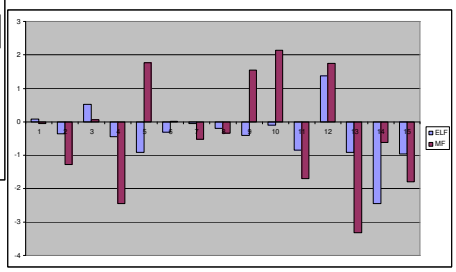
- Hypothèse: cellules sanguines de patients électrosensibles réagissent différemment sous un champ magnétique et/ou électrique comparées aux contrôles
- Différences avec test comète ?



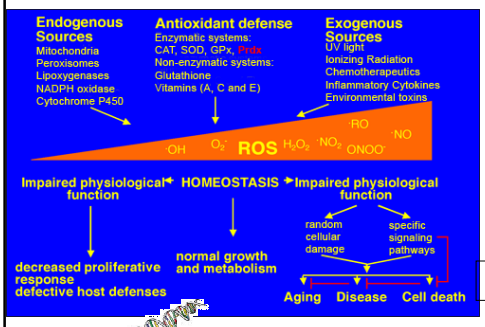
19 contrôles et 15 EHS

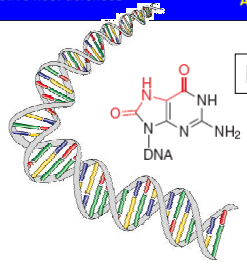
0.2 ml sang

1 h @25 mA or @800 µT



Radicaux & dégâts de l'A.D.N.





EMF

Changement en redox homéostase

ROS-antioxidants)

Amplification du système immunitaire

Activation cellulaire

Dégât A.D.N.

Transduction signal

Expression gène/protéine

Dérèglement cycle cellulaire

Apoptose

Manifestation réparation/dégât

Pathologie

Recherche *in vitro*

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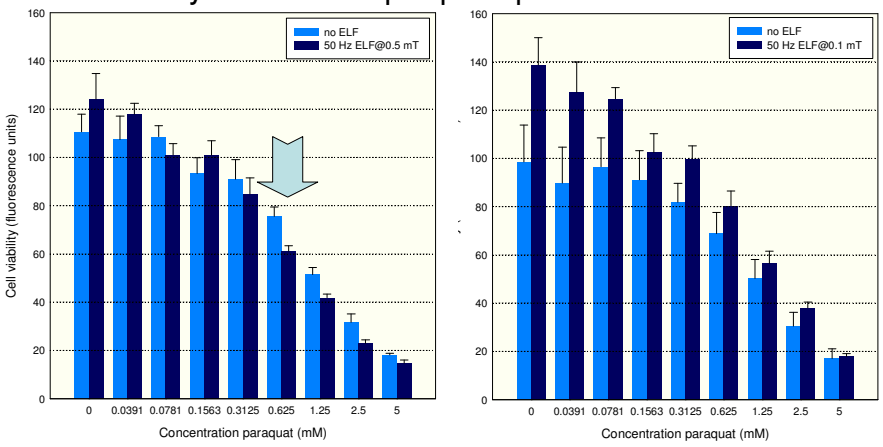
Lignée cellulaire monocyte (THP1): analyses cytotoxiques

Dose (0-1 mT)/temps (1h-24h) effet

Stress oxydatif stimulé par paraquat



Dégât oxydatif par ELF ?

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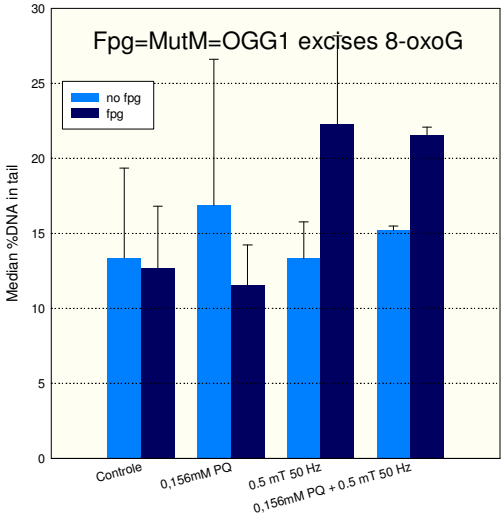
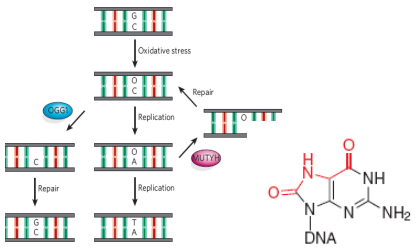
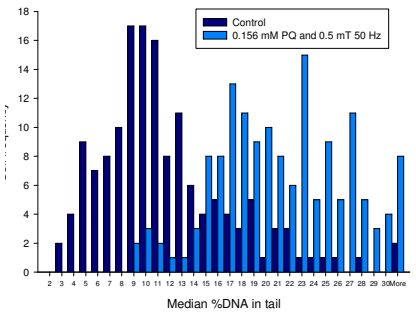
Lignée cellulaire monocyte (THP1)

ELF + stress oxydatif → test comète







Dégât oxydatif par ELF ?

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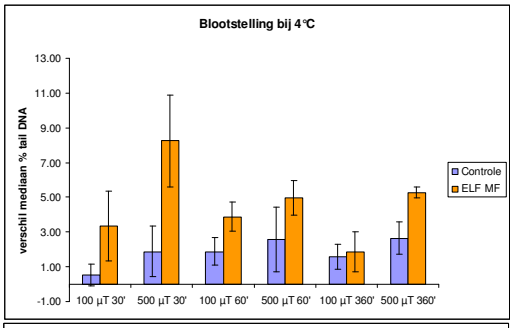
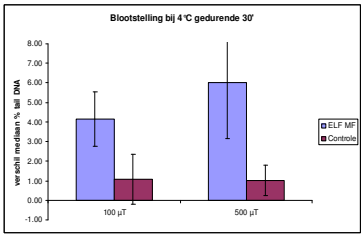
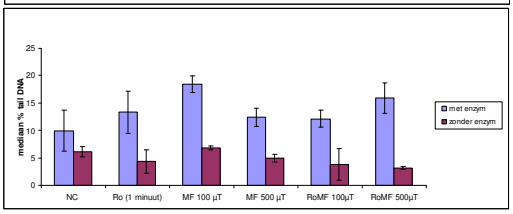
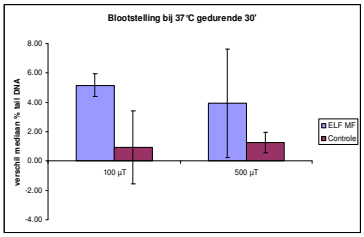
⇨ Lignée cellulaire ↔ matériau primaire (cellules mononucléaires périphériques)









« Take home messages »

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- Dégât génétique direct : improbable
  - Propriétés physiques
  - Doses
- Dégât indirect
  - Dégât oxydatif/processus cellulaires
  - Effets co-génotoxiques: priorité OMS
- Test comète peut démontrer dégât oxydatif de l'A.D.N.
  - Mécanisme d' activité
  - Implication
  - Interaction entre plusieurs disciplines est nécessaire!