Genetisch onderzoek naar elektrische overgevoeligheid

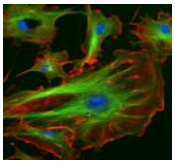
Patrick De Boever

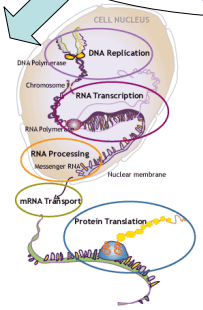
Biologisch effect van ELF

Populatie

Individu

Cel





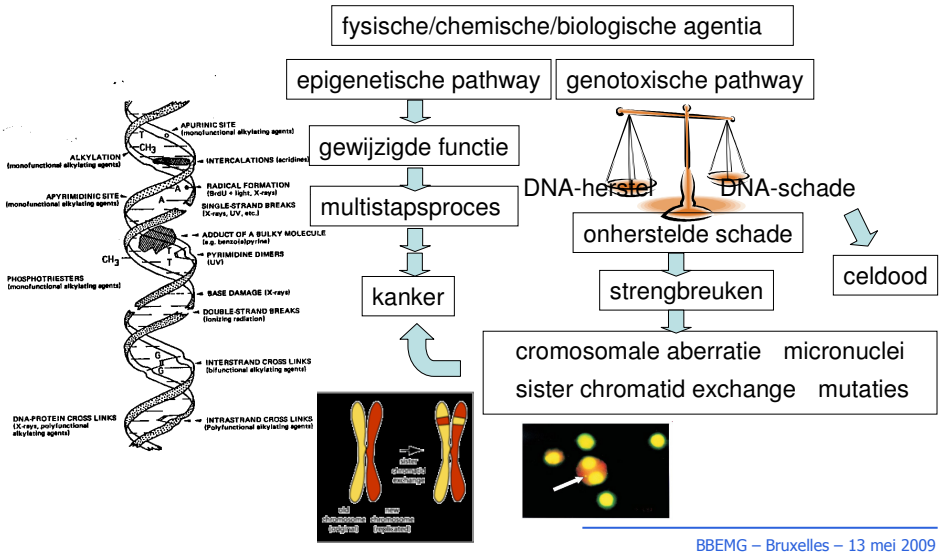
Molecule

BBEMG – Bruxelles – 13 mei 2009

Impact van ELF op DNA ?

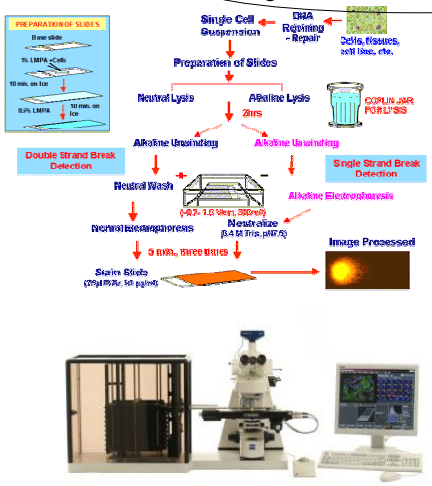
- McCann et al. 1993; Murphy et al. 1993; Moulder 1998; en McCann et al. 1998: geen genotoxische effecten
- ELF (30-300 Hz) lage energie-inhoud
  - Geen directe DNA-schade
  - Gewijzigde cellulaire processen
- Enkele positieve resultaten+epidemiologisch bewijs
- IARC: "possible human carcinogen" Class 2B

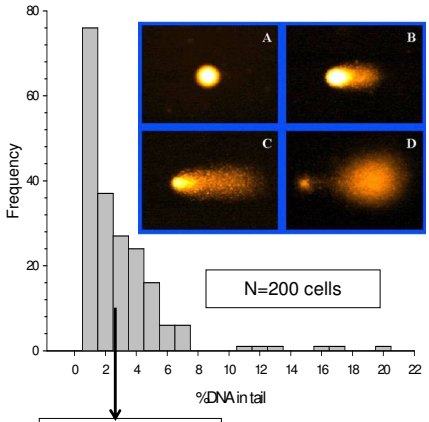
Mechanisme DNA-schade



DNA-schade: komeettest

Biologisch staal: controle/blootstelling





Resultaten uit REFLEX

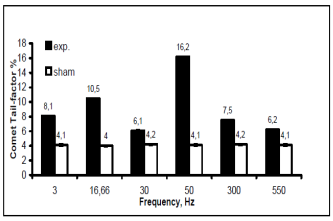


Figure 10. Alkaline Comet Assay tailfactors of ELF-EMF exposed and sham exposed fibroblasts (cell line ES-1, 15 hrs, 1000 µT, intermittent) after variation of exposure frequency (3-550 Hz).

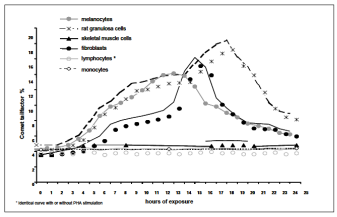


Figure 20. Alkaline Comet tailfactors of different human cell types (fibroblasts, melanocytes, monocytes, lymphocytes, skeletal muscle cells) and SV 40 transformed rat granuloa cells exposed to ELF-EMF (50 Hz sinusoidal, 1 mT, intermittent 5 min on/10 min off) for 1 to 24 hours.

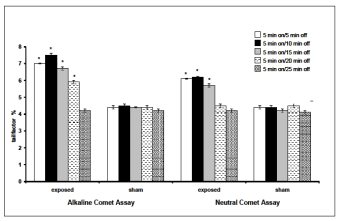


Figure 7. Alkaline and neutral Comet Assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24h,1000 µT, intermittent) - variation of off-time. * p<0.01 exposed versus sham-exposed

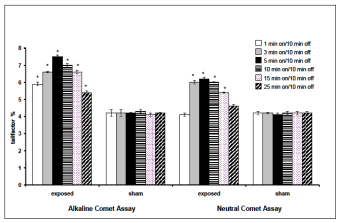


Figure 8. Alkaline and neutral Comet assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24 h, 1000 µT, intermittent) - variation of on-time. * p < 0.01 exposed versus sham-exposed

Conclusies uit REFLEX

- Genotoxische effecten zijn frequentie-afhankelijk
- Discontinue 50 Hz-blootstelling veroorzaakt DNA-schade
- Verschil tussen cel types (reeds een effect bij 35 µT)
- Leeftijd en genetische achtergrond zijn van belang
- DNA-schade ↑ → micronucleus frequentie ↑
- DNA-herstel werd geactiveerd maar was niet error-free

Enkele bedenkingen
- *In vitro*, individuele cellen
- Korte-termijn, hoge dosissen (1 mT)
- Mechanismse? Bevestiging via andere technieken?

REFLEX controverse

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.

Cytogenetische observaties periode 1990-2003

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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 unieke publicaties
  - 21: komeettest
  - 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/DSB (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	SSB (50)	Yes	Yes	Yes	?
Ahuja	1997	Human blood lymphocytes	6 donors	50 Hz MF	2, 3, 5, 7, 10	1 h	SSB (50)	Yes	?	?	?
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

-blootstelling

-methodologie

* 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.

Cytogenetische observaties periode 1990-2003…overzicht

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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…"

Meta-analyse van 87 publicaties periode 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.

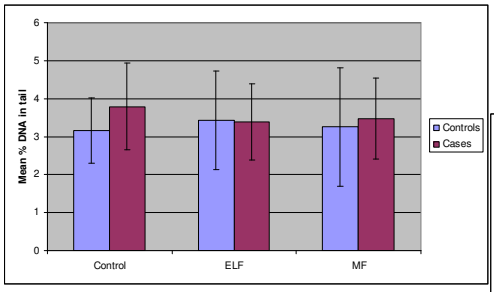
Meta-analyse conclusies

- Komeettest
  - ELF-controle biologisch klein, maar significant
  - Verschil in meetmethode en statistiek
  - Celcyclus voor continu groeiende cellen
- CA, MN, SCE
  - Enkele statistische verschillen
  - verhoging ≈ spontane concentraties (databases)

- Meerdere eindpunten nodig voor relevante genotox evaluatie
- Algemeen publiek ↔ Werknemers
- Richtlijnen: 5 kV/m (100 µT); 10 kV/m (1mT): geen genotox effect
- Co-blootstelling → Real-life
  - weinig experimenten
  - Prioriteit door WHO

ELF-genotoxische effecten?

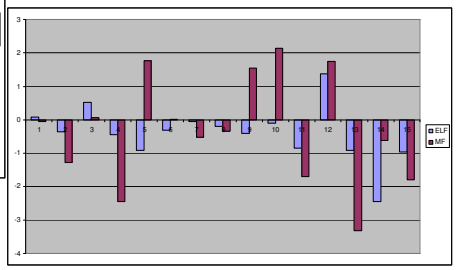
- Hypothese: bloedcellen van electrosensitieve patiënten reageren anders op magnetisch en/of electrisch veld in vergelijking met controles
- Verschillen met komeettest ?



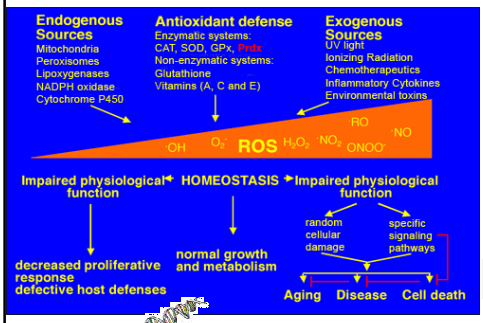
19 controls and 15 EHS cases

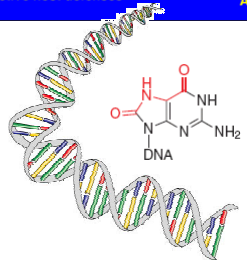
0.2 mL vol bloed

1 h @25 mA or @800 µT



Radicalen & DNA-schade





EMF

Verandering in redox homeostase

ROS-antioxidanten

Amplificatie van immuunsysteem

Celactivatie

DNA-schade

Herstel/schade manifestatie

Signaaltransductie

Gen/proteïne-expressie

Verstoring celcyclus

Apoptose

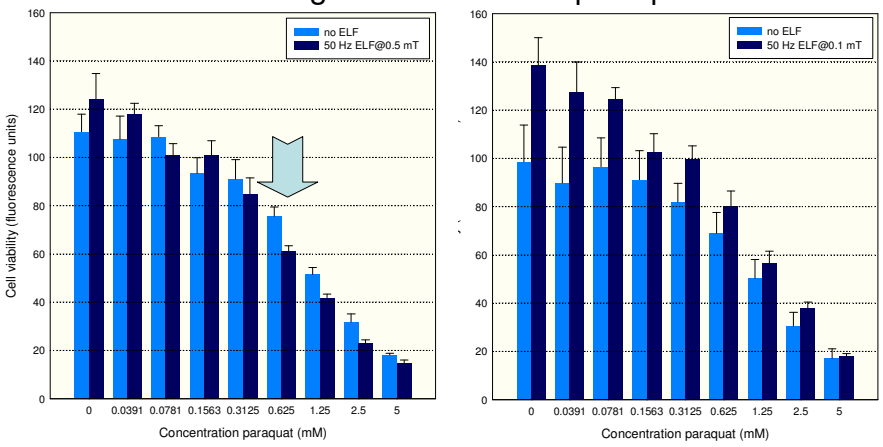
Pathologie

In vitro onderzoek

Monocyte cellijn (THP1): cytotox analyses

Dosis (0-1 mT)/tijd (1h-24h) effect

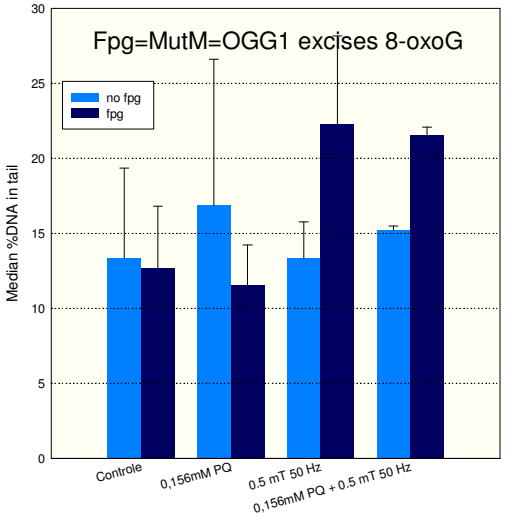
Oxidatieve stress gestimuleerd door paraquat

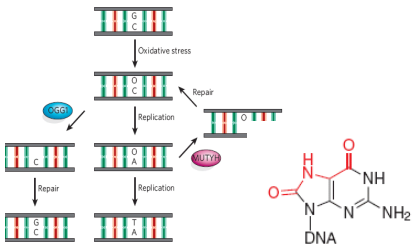


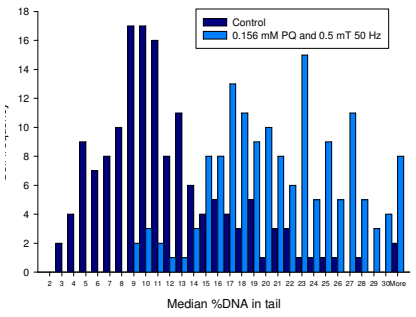
Oxidatieve schade door ELF?

Monocyte cellijn (THP1)

ELF+oxidatieve stress→komeettest







Oxidatieve schade door ELF?

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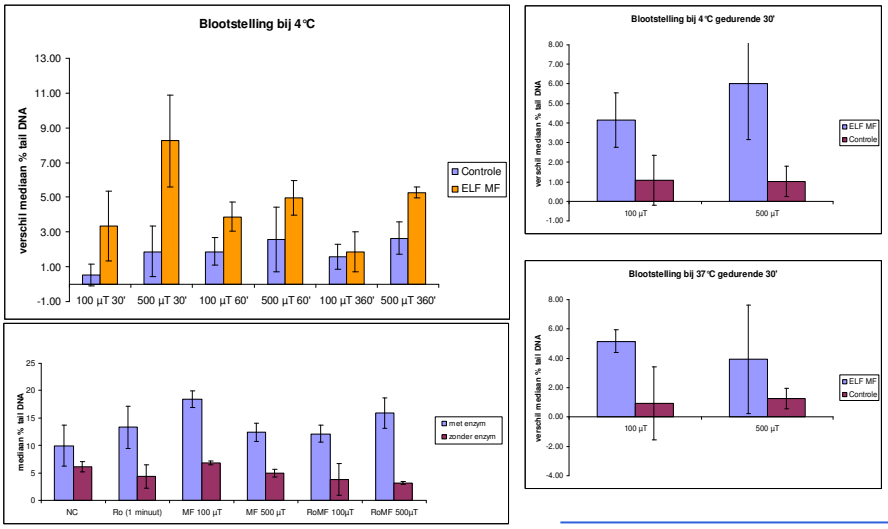
⇨ cellijn ↔ primair materiaal (perifere mononucleaire cellen)



Take home messages

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- Directe genetische schade onwaarschijnlijk
  - Fysische eigenschappen
  - Dosissen
- Indirecte schade
  - Oxidatieve schade/cellulaire processen
  - Co-genotoxische effecten: WHO prioriteit
- Komeettest kan oxidatieve DNA-schade aantonen
  - Werkingsmechanisme
  - Implicatie
  - Interactie tussen meerdere disciplines vereist !