Magnetic fields and childhood leukaemia – candidate mechanistic pathways

Circadian rhythm & melatonin disruption by extremely low frequency magnetic fields

Denis L Henshaw

Children with Cancer UK

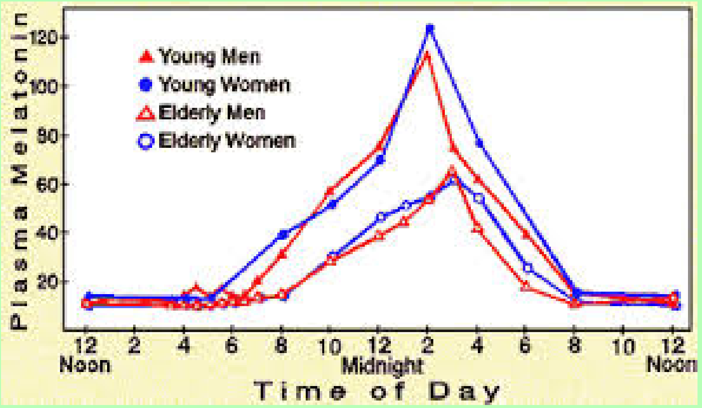
University of Bristol

www.electric-fields.com

CHILDREN with CANCER UK
Registered Charity No. 298405

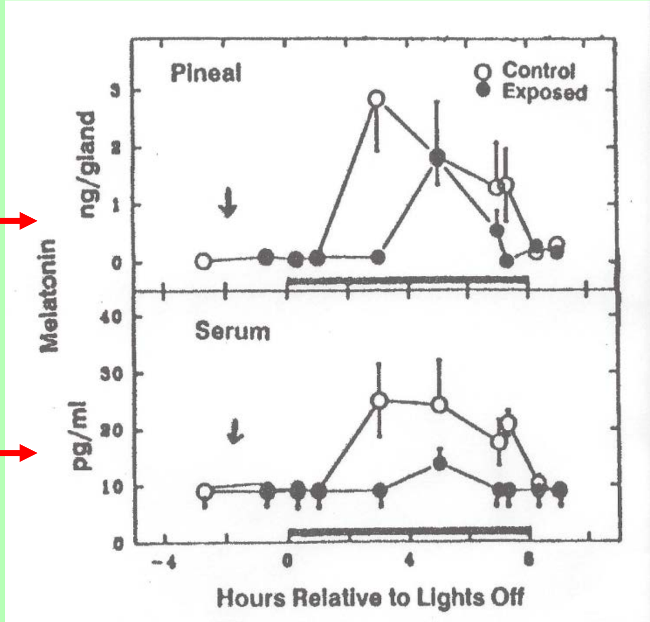
Disruption more important than suppression…..

Nocturnal production of melatonin



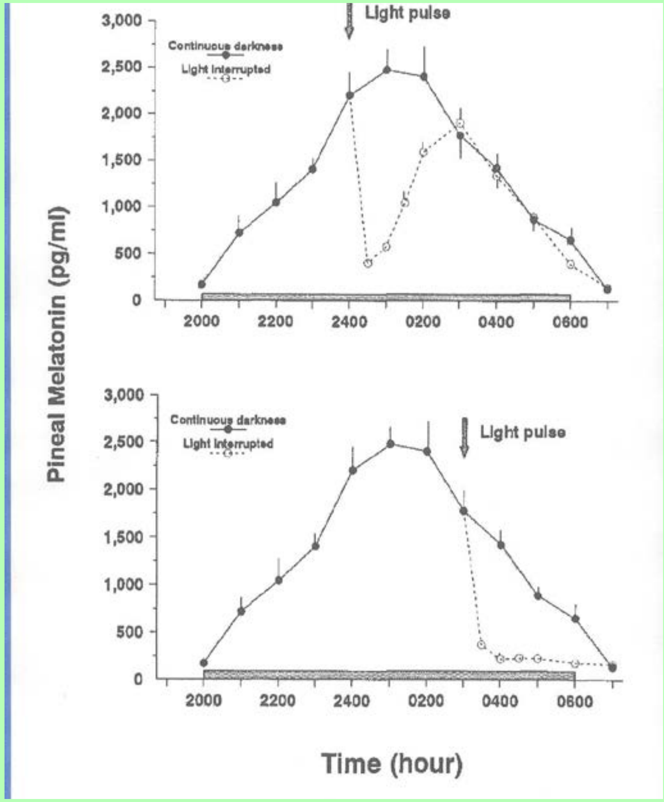
Phase delay in onset of nocturnal rise

Suppression of nocturnal rise



Slide from Reiter CwL Conference 2004

Effect of light-at-night on melatonin

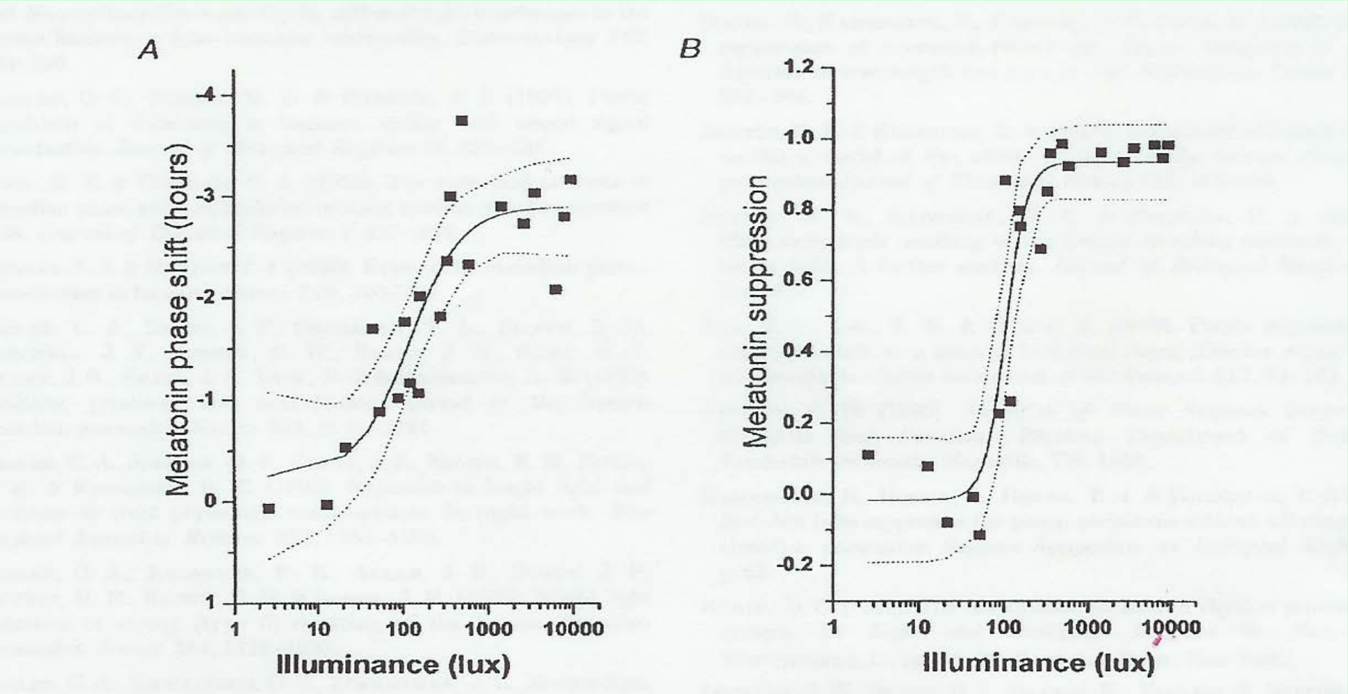


Constant exposure to LAN can fully suppress nocturnal melatonin
In contrast, MF exposures reduce melatonin by typically 7 – 14%

Slide from Reiter CwC Conference 2004

Dose response for light

Zeitzer *et al*. J Physiol (2000) 526, 695-702

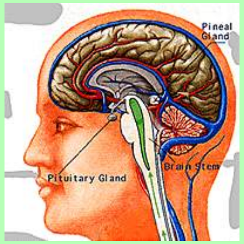


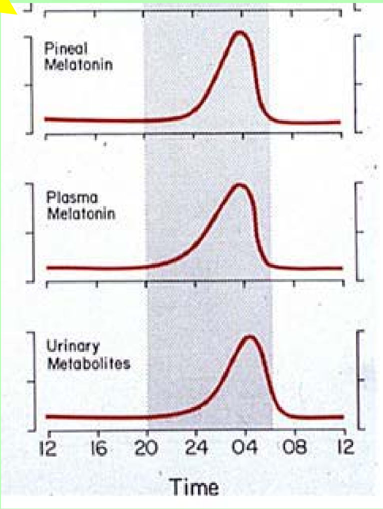
Melatonin

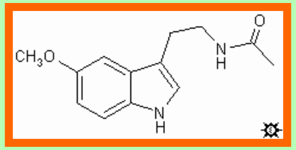
- Melatonin, a key component of circadian rhythms, is produced in the pineal gland mainly at night when light levels fall below ~200 lux

- Stevens (1987)[1] proposed that exposure to light-at-night and EMF may increase breast cancer risk, by melatonin disruption

- For many years it was assumed that nocturnal production in the pineal gland was the chief source of melatonin in man. However, melatonin has been found in multiple extrapineal tissues, including placenta, where it is also synthesised[2,3].



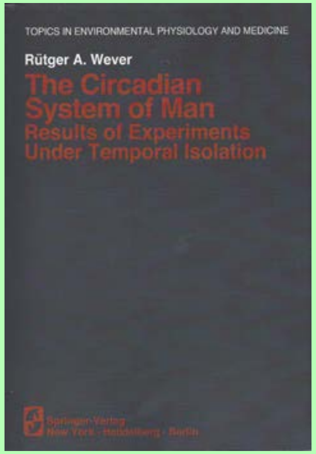




*N-acetyl-5-methoxytryptamine

[1]Stevens 1987. Am. J Epidemiol. 125:556-61.
[2]Dario Acuna-Castroviejo et al Cell. Mol. Life Sci. DOI 10.1007/s00018-014-1579-2
[3]Lanoix et al 2008 J. Pineal Res. 45:50–60

Electric fields also affect circadian rhythms in humans



Wever (1979)*: In a long series of experiments, human volunteers were exposed for several weeks to 10 Hz square wave **electric fields** of only **2.5 V/m**. The 24 h circadian rhythm was disrupted. Volunteers were immediately entrained to the external signal. Effect lasted for a few days, indicating E-fields acting as zeitgebers

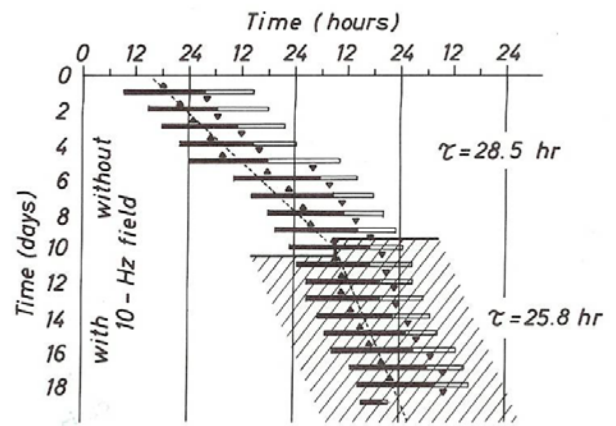


Figure 55. Autonomous rhythm of a subject (H.v.S., ♂ 23 y) living under constant conditions without time cues and protected from natural and artificial electromagnetic fields during the first section, but under the influence of an artificial electric AC field (i.e., 10 Hz square wave, 2.5 V/m) during the second section. *I*. Temporal courses of the rhythms of activity and rectal temperature, presented successively one period beneath the other. Indications are the same as in figure 16/I. Shaded area: field in operation. From Wever (1969b).

*Wever 1979. The circadian system of man. In: Results of Experiments Under Temporal Isolation. Schaefer KE, ed. Springer-Verlag, New York

Magnetic field disruption of melatonin, pineal cells, cryptochromes and circadian rhythms

- on pineal cells

  Small but detailed literature – action in synthesising melatonin disrupted. Some animals have MF compass in the pineal gland

- in animals

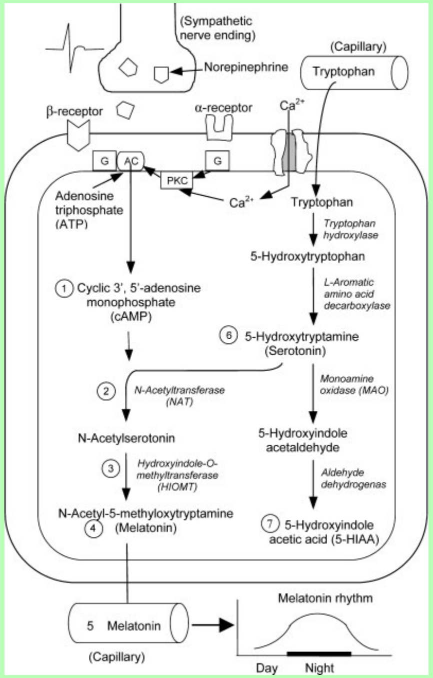
  Most effects observed with non-smooth AC MFs

  Strong findings in cows with "real" EMFs[1]

- in humans

  Not revealed in volunteer short exposures to pure AC MFs

  Seen in populations exposed to "real" EMFs[2] – down to 0.2 μT



Interactions of the post-ganglionic sympathetic neuron with the pinealocyte and the synthesis of melatonin. Each of the numbered sites has been reported to be influenced by magnetic Fields[1].

Circadian rhythms are controlled by Clock genes

– the Cry genes code the Cryptochrome[3] protein molecule in the eye, which in turn is involved in the regulation of circadian rhythms.

Cryptochrome acts as the magnetic compass in animals

[1]Burda et al 2009. ELF-MFs disrupt magnetic alignment of ruminants. PNAS 106:5708-13.

[2]Henshaw & Reiter 2005 BEMs Suppl 7:S86-S97

[3]Evolved ~2.5 bn years (Gu 1997 Mol Biol Evol 14:861-866)

How do you measure melatonin in the body?

1. Plasma melatonin - levels in blood at any one time can me measured from blood samples

2. Integrated measurements - urinary concentrations of the primary melatonin metabolite 6-sulfatoxymelatonin, using commercially available radioimmunoassay – a measure of total night-time melatonin

I will discuss two reviews on MF and melatonin

1. Henshaw DL, Reiter RJ. 2005. Do magnetic fields cause increased risk of childhood leukaemia via melatonin disruption? Bioelectromagnetics Supplement 7:S86-S97.

2. Touitou Y, Selmaoui B. 2012. The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. Dialogues Clin Neurosci. 14:381-399.

Henshaw & Reiter 2005 Bioelectromagnetics &:S86-S97

TABLE 1. Human Population Studies on Effects of Magnetic Fields (EMFs) on Pineal Melatonin Production

Study no.	No. of cases/controls	Type of EMF exposure	Location and time of year	Key observations
1. Wilson et al. [1990]	42 volunteers: 32 women; 10 men, volunteers acted as own controls	Volunteers used electric blankets for approximately 8 weeks (AC compared with DC)	Washington State, USA around winter solstice	No overall effect, but statistically significant 6-OHMS decrease (~25%) in seven individuals using blankets with 50% higher MFs (mean 0.42 μT) and which switched on and off at twice the rate of conventional blankets
EMF → 2. Pfluger and Minder [1996]	108 men: 66 engineers and 42 controls (train attendants & station managers with average exposure over 1 μT) both groups work shifts	Electric railway lines, average exposure: 20 μT in most exposed 1 μT in least exposed (E)[a]	Switzerland, early autumn, 1993	Lowered 6-OHMS daytime levels (factor of 0.81) in engineers compared to controls but no difference in nocturnal levels, evidence of a rebound of levels during leisure days
EMF → 3. Burch et al. [1998]	142 men 20–60 years, mean age 41 years; 29 generation workers; 56 distribution workers and 57 controls (utility maintenance & administration staff)	Electric utility workers highest exposure occurred in generation workers: geometric mean 0.22 μT (E)	Colorado, USA Morning 6-OHMS daily for 4 days	Association between residential MF exposure and lower nocturnal 6-OHMS levels, modest reductions in levels after work MF exposure, greatest reductions (35%) when work and home exposures combined
4. Wood et al. [1998]	30 adult males 18–49 years, subjects acted as their own controls	Laboratory generated, circularly polarized, 20 μT, 50 Hz magnetic fields, for three successive Friday night/Saturday mornings	February–September over a 2 year period 1994–1996	Exposure during a certain time window caused a mean 1 h delay in nightly melatonin onset in a subset of subjects, square wave fields produced more marked reduction in maximum melatonin levels compared with sinusoidal fields
EMF → 5. Burch et al. [1999a]	142 men as in Study 3	Electric utility workers highest exposure bin >0.135 μT (E)	Colorado, USA 1 year period	Reduction in 6-OHMS on the second and third days of occupational exposure to MF, bigger effects (up to 35% reduction) with low RCMS[b] values, negligible MF effects in subjects with high visible light exposure
6. Burch et al. [2000]	149 men mean age 44 years: 50 generation workers, 60 distribution workers, 39 controls (utility maintenance & administration staff)	Substations (3 phase–circularly polarized) Study compared ≥2 h with >2 h to geometric mean fields in the range 0.04–0.27 μT (E)	Colorado, USA January–September 1997	No effect due to 1 phase exposure, 6-OHMS reduction found due to exposure >2 h to 3 phase, low RCMS fields had greatest effect, up to 44% reduction in mean 6-OHMS between upper and lower exposure tertiles
7. Juutilainen et al. [2000]	60 women, mean age 44 years (workers) & 43 years (controls); 39 garment workers (8 of whom did not operate machines but were 'possibly exposed'), 21 controls	Sewing machine workers, eye level exposures >1 μT compared with 0.3–1 μT, likelihood of exposure to switched fields	Kuopio, Finland 3-week period around spring equinox	No week/weekend variations, but between 25% and 40% lower 6-OHMS levels in workers compared to controls, authors suggest effects on melatonin may require chronic exposures
8. Graham et al. [2000]	30 men 18–35 years, mean age 22 years (volunteers acted as their own controls)	Laboratory generated, circularly polarized, 28.3 μT, 60 Hz magnetic fields for 4 consecutive nights	Missouri, USA spring and summer	Compared with controls, repeated nightly exposure was associated with reduced consistency of 6-OHMS levels, results suggestive of cumulative effect

Henshaw & Reiter 2005 Bioelectromagnetics &:S86-S97

9. Davis et al. [2001]	203 women, 20–70 years	Night time residential 60 Hz magnetic fields, mean night time exposures were <0.2 μT	Washington State, USA two 72 hperiods at different seasons over 14 months	Higher bedroom MF associated with lower 6-OHMS levels during the same night, maximum 14% reduction in summer solstice for fourfold increase in mean MF above 0.04 μT
EMF → 10. Levallois et al. [2001]	221 women subjects and 195 women controls, mean age 45.5 years (subjects) & 45.8 years (controls)	Subjects <150 m from 735 kV Power Lines, controls >400 m away, exposure quartiles 1st versus 4th: <0.13 μT & ≥0.37 μT; <4.7 V/m & ≥ 12.2 V/m. (E)	Quebec City, Canada, 6-OHMS sampled over 2 consecutive days February–December 1998	Decrease in 6-OHMS levels in relation to age and body mass index, more pronounced in women living near the powerlines, Maximum 30% reduction between highest and lowest quartiles
EMF → 11. Burch et al. [2002]	Study 1: 149 as in Study 6; study 2: 77: 22 generation workers; 29 distribution workers; 23 controls	Cell telephone use in electric utility workers, arithmetic mean exposure to tertiles: 1st 0.05 μT; 3rd 0.5 μT (E)	Colorado, USA total overnight and post-work 6-OHMS on 3 consecutive workdays: Study 1, January–September '97; Study 2, April–June '98	Study 1—no effect, study 2—exposure-related 6-OHMS reductions in cell phone use >25 min per day, reduction (40%) between highest and lowest exposure tertiles, a combined effect of telephone use and occupational exposure to 60 Hz magnetic fields was observed
EMF → 12. Touitou et al. [2003]	15 men 31.5–46 years with exposures 0.1–2.6 μT compared with 15 men 34.5–47 years with exposures 0.004–0.092 μT	Chronic exposure in those who worked and lived near extra high voltage substations (E)	Paris, France autumn	No statistically significant differences in nocturnal plasma melatonin or the melatonin metabolite between the workers and controls
13. **Geomagnetic** Burch et al. [1999b]	132 male electric utility workers	Geomagnetic (GM) disturbances in conjunction with 60 Hz MF exposure, changes in GM fields >30 nT compared with ≤ 30 nT	Colorado, USA March '95–March '96	Lower 6-OHMS levels on days with high geomagnetic activity, effect enhanced when activity combined with high MF or low light levels, statistically significant 20% reduction between <and >30 nT disturbance
14. **Geomagnetic** Weydahl et al. [2001]	25 volunteers: 9 men, 16 women	Geomagnetic disturbances at latitude 70° N	Tromsø, Norway November–December '92–September '96	Statistically significant trend in reduced melatonin with indices of geomagnetic disturbance over 3 h above 80 nT, approximately, reduction (50%) in plasma melatonin for a 330 nT change in disturbance

[a](E) Indicates associated exposure to powerline electric fields, although field values generally not given.
[b]RCMS = Standardized rate of change metric: low values correspond to temporarily stable fields.

Conclusions from Henshaw & Reiter 2005

- Studies with comparatively small numbers of volunteers acutely exposed short-term to laboratory-generated smoothly-varying fields did not in general reveal signs of melatonin disruption.

- In contrast, studies with a comparatively large number of subjects exposed to an admixture of electric and magnetic neighbourhood fields tended to show melatonin disruption. Disruption with MFs as low as 0.2 μT was observed.

To explain these findings, we suggested:

I. In volunteer experiments, the relatively small numbers (e.g. <10) limit the ability statistically to resolve changes in melatonin secretion against the natural variations between individuals;

II. Volunteer exposures have tended to be for short periods compared with chronic exposures in real populations (the evidence in animals suggests that several days or weeks of exposure are required before effects on melatonin secretion become manifest);

III. Laboratory generated exposures may not contain features such as transients or rapid on/off changes in MFs which have been shown effective in demonstrating melatonin suppression in animals;

IV. Volunteer studies have not included exposure to electric fields which may also be a factor in melatonin disruption.

None of the studies reviewed had taken account of possible exposure to light-at-night

Conclusions from Henshaw & Reiter 2005 contd.

One, well conducted study no showed statistically significant evidence of MF melatonin disruption:

Touitou et al. 2003, *Magnetic fields and the melatonin hypothesis: A study of workers chronically exposed to 50-Hz magnetic fields*. Am J Physiol Regul Integr Comp Physiol 284:R1529–R1535.

30 subjects: 15 exposed; individual exposures ranged from 0.1 to 2.6 μT and 15 unexposed (controls): individual exposures ranged from 0.004 to 0.092 μT.

1. The comparison of subjects exposed to fields from 0.1 to 0.3 μT **(*n* = 6)** with controls **(*n* = 15)** did not show any significant difference between these two groups.
2. Neither did the subjects exposed to >0.3 μT **(n = 9)** (Fig. 2)

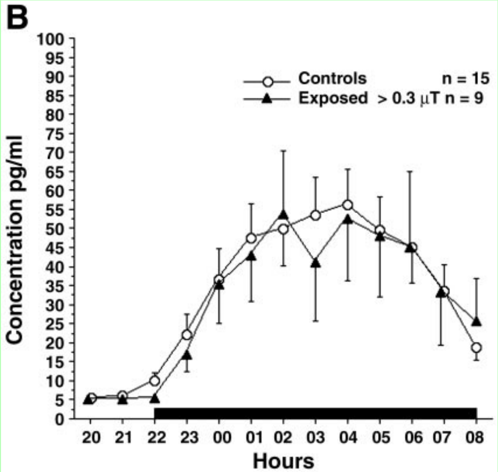


Fig. 2. Nocturnal plasma melatonin profiles chronically exposed to 50-Hz, >0.3 μT and control subjects.

Review by Touitou & Selmaoui 2012. Dialogues Clin Neurosci. 14:381-399:

"The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system"

Main conclusions [on melatonin suppression]:

- *Data from the literature reviewed here are contradictory.*
- *We have demonstrated a lack of effect of ELF-EMF on melatonin secretion in humans exposed to EMF (up to 20 years' exposure) which rebuts the melatonin hypothesis* **[Touitou et al. 2003]**.

under study (corticosterone for rats, cortisol for other mammals), exposure characteristics (short- and long-term), and timing and duration of exposure (1 to 6 months) in different animal species are detailed in *Table IV*.

Reference of the study	Subjects (N)	Sex	Age (years)	Exposure characteristics	Timing of exposure	Fluid or pineal	Sampling time	Effect on melatonin secretion
Pfluger and Minder, 1996[97]	108	M	NG	16 Hz- ~20 μT mean value in engine drivers	30 min – 4 h	Urinary aMT6s	Morning and evening samples	Decrease of aMT6s in evening; No evidence for a dose-response
Arnetz and Berg, 1996[98]	47	NG	NG	1 day exposure to video display unit (VDU)	1 day	Ser Mel	Morning and afternoon samples	Decrease but exposure not exclusively related to 50/60 Hz
Wood et al,1998[99]	44	M	18-49	50 Hz- 20 μT, sinusoidal or square wave field, intermittent	19 h-21 h	Pl Mel	20 min, 30 min, or hourly at night	Delay and decrease of Mel in subgroup
Burch et al, 1998[100]	142	M	20–60	60 Hz- 0.1–0.2 μT	Occupational exposure	Urinary aMT6s	Morning urine samples	No effect at work. urinary aMT6s decreased at home
Burch et al, 1999[101]	142	M	20-60	60 Hz- occupational exposure	Occupational exposure over a week	Urinary aMT6s	Overnight urine samples	Decrease in aMT6s excretion in workers exposed to more stable fields during work.
Burch et al, 2000[102]		M	NG	60 Hz- occupational exposure (electric utility workers), from 950 nT to 1.05 μT (exposure for < 2 h/day or > 2 h day)	3 consecutive days monitored	Urinary aMT6s	Overnight aMT6s	Decrease in aMT6s excretion in workers exposed for >2 h
Juutilainen et al, 2000[103]	60	F	mean age: ~44	50 Hz- 0.3–1 μT and > 1 μT and 0.15 μT	Occupational exposure	Urinary aMT6s	Nighttime and morning urine collection	aMT6s excretion lower in exposed workers compared with office workers
Davis et al, 2001[104]	203	F	20-74	60 Hz- domestic exposure. Half of the subjects had mean levels of <0.04 μT	residential 72 h	Urinary aMT6s	Nighttime samples	Decrease, primarily in subgroup using medication
Burch et al, 2002[105]	226 electric utility workers	M	**18-60**	60 Hz- occupational exposure	occupational exposure: measures on 3 consecutive work days	Urinary aMT6s	Overnight aMT6s	Decrease in aMT6s associated with mobile phone use
Davis et al. 2006[106]	115	F	20-40	60 Hz- 5 to 10 mG	At night for 5 consecutive nights	Urinary aMT6s	Overnight samples	Decrease
Burch et al, 2008[107]	153	M	Mean age = 44	0 Hz- 15 nT to 30 nT + 60 Hz	3 h, 24 h, 36 h	Urinary aMT6s	Overnight aMT6s	Decrease in aMT6s associated with elevated geomagnetic activity

Table IIIa. Magnetic field reports on a melatonin secretion decrease in humans. Mel, melatonin; aMT6s, 6 sulfatoxymelatonin; M, male; F: female; MF, magnetic field; NG, not given

11 Studies reporting decreased melatonin

Reference of the study	Subjects (N)	Sex	Age (years)	Exposure characteristics	Timing of exposure	Fluid	Sampling time	Effect of MF on melatonin secretion
Wilson et al, 1990[108]	42	F, M	NG	CPW electric blanket. 0.2-0.6 μT	8 weeks	Urinary aMT6s	Urine voidings	No effect
Schiffman et al, 1994[109]	9	M	22-34	0 Hz- Magnetic resonance imaging. 1.5 T	01 h	Pl Mel	Nighttime (2 samples)	No effect
Selmaoui et al, 1996[110]	32	M	20-30	50 Hz- 10 μT, to continuous or intermittent MF	23 h-08 h	Ser Mel and urinary aMT6s	Every 2 h during the daytime, hourly during the nighttime	No effect
Graham et al,1996[111]	33	M	19-34	60 Hz- 1 or 20 μT, intermittent	23 h-07 h	Pl Mel	Hourly at night	No effect
Graham et al, 1997[112]	40	M	18-35	60 Hz- 20 μT, continuous	23 h-07 h	Pl Mel	Hourly at night	No effect
Åkerstedt et al, 1999[113]	18	F, M	18-50	50 Hz- 1 μT	23 h-08 h	Pl Mel	At 23 h 02h30 h, 05 h, and 08 h	No effect
Graham et al,2000[114]	30	M	18–35	60 Hz- 28.3 μT	4 consecutive nights from 23 h – 07 h	Urinary aMT6s	Overnight urine samples	No effect
Crasson et al, 2001[115]	21	M	20-27	50 Hz- 100 μT, continuous or intermittent	30 min at 13 h30 and 16 h30	Ser Mel and Urinary aMT6s	Hourly from 20 h to 07 h	No effect
Graham et al, 2001[116]	24	M	19–34	60 Hz- 127 μT, continuous or intermittent	23 h – 07h	Ser Mel and Urinary aMT6s	Hourly from 24 to 07 h	No effect
Graham et al, 2001[117]	46	F, M	40-60	60 Hz-28.3 μT	23 h – 07 h	Urinary aMT6s	Morning urine samples	No effect
Griefahn et al, 2001[118]	7	M	16-22	16.7 Hz- 200 μT	18h – 02 h	Sal Mel	Hourly for 24 h	No effect
Haugsdal et al. 2001[119]	11	M	23-43	0 Hz- 2-7 mT, 9 h	22 h – 07 h	Urinary aMT6s	4 samples / 24 h	No effect
Hong et al, 2001[120]	9	M	23-37	50 Hz- 1–8 μT, electric 'sheet' over the body	11 weeks at night	Urinary aMT6s	5 times a day	No effect
Levallois et al, 2001[121]	416	F	20–74	50 Hz- between 0.1 and 0.3 μT	Residential exposure	Urinary aMT6s	Overnight urine samples	No effect except in subgroup of women with high BMI
Griefahn et al, 2002[122]	7	M	16–22	16.7 Hz, 0.2 mT	17 h-01 h	Sal Mel	Hourly for 24 h	No effect
Youngstedt et al, 2002[123]	242	F, M	50-81	60 Hz- Mean of one week exposure = 0.1 μT	Residential exposure within bed	Urinary aMT6s	Fractional urine	No Effect
Kurokawa et al, 2003[124]	10	M	20–37	50 Hz- 20 μT	20 h-08 h	Ser Mel	Hourly from 20 h to 08 h	No effect
Touitou et al, 2003[125]	30	M	31.5-46	50 Hz- mean fields of 0.1–2.6 μT	Occupational and residential exposure (1 to 20 years)	Ser Mel and urinary aMT6s	Hourly from 20 h to 08 h	No effect
Warman et al, 2003[126]	19	M	18-35	50 Hz- 200 or 300 μT	2- h exposure between 17 h and 23 h	Ser Mel	17 h and 10 h	No effect
Cocco et al, 2005[127]	51	F, M	Mean age 56.6	50 Hz- from 0.0045 μT to 0.148 μT	Residential	Urinary aMT6s	At 22 h and 08 h	No effect
Gobba et al. 2006[128]	59	F, M	Mean age 42 and 46	60 Hz- low exposed (≤0.2 μT) or higher exposed (>0.2 μT)	3 consecutive days recorded for workers	Urinary aMT6s	Morning urine	No effect
Juutilainen and Kumlin, 2006[129]	60	F	Mean age 40 to 53	50 Hz- from 0.1 to 2.5 μT	3 consecutive weeks	Urinary aMT6s	Morning urine	No effect Inconclusive results with light exposure
Clark et al, 2007[130]	127	F	12 to 81	60 Hz- 20 nT to 130 nT and RF 0.04 μW/cm² to 1.4 μW/cm²	Residential for 2.5 days	Urinary aMT6s	Overnight	No effect

Table IIIb. Continued

MRI exposed

22 Studies:
“no effect”

Observations:

These tables show 11 studies with MF effects in melatonin suppression

Average number of subjects = 150 (range 44 – 416)

And

22 studies which show “no effect”

Average number of subjects = 42 (range 7 – 242)

About three times fewer subjects in studies showing “no effect”

So, is there a resolving power issue?

Consider the natural subject-to-subject variation in morning urinary concentrations of 6-sulfatoxymelatonin

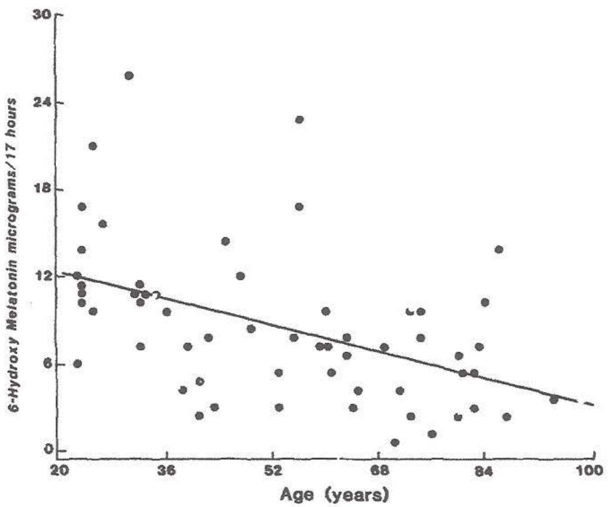


Fig. 1. Each point represents the mean of three overnight urinary 6-hydroxymelatonin determinations from a single subject. A "best fit" line is drawn through the data points showing an inverse relationship between 6-hydroxymelatonin excretion and age.

Sack et al 1986 J Pineal Res 3:379-388

Minimum number of subjects needed to resolve change in melatonin with 95% confidence:

% change	Number
10	116
20	26

Other observations on Touitou & Selmaoui 2012 from the 22 studies said to show "no effect"

- Touitou *et al* say "no effect" without further explanation
- 1. Wilson *et al* 1990, Graham *et al* 2001 and Juutilainen & Kumlin 2006 all do show effects in one of the exposure scenarios and in each case this is made clear in the abstract;
- 2. Crasson *et al* 2001 shows reduced melatonin with a p-value of 0.08. Cocca *et al* 2005 shows reduced melatonin with OR = 2.6, but this is not significant (95% CI = 0.4 to 15.7)
- 3. The title of the paper by Akerstedt *et al* 1999 is "A 50-Hz electromagnetic field impairs sleep" and this indeed is what they report. Griefahn *et al* 2002 report heart rate differences.
- 4. Youngstedt *et al* 2002 using 242 subjects with a mean age of 67.6 +/- 5.7 years found no melatonin reduction with electric bed sheets. However, the mean exposure was only 0.1 +/-0.014 μT and none of the studies have indicated melatonin reduction with fields below 0.2 μT. So, this is a consistent finding.
- 5. Schiffman *et al* 1994 is an MRI exposure and Clark *et al* 2007 mainly RF from radio transmitters. These are out of remit of the title of Touitou & Selmaoui 2012: "The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system".
- **Touitou et al. 2003** – based on 15 exposed and 15 controls has limited resolving power

Conclusion from Touitou et al. 2003

- Studies purporting to show “no effect” on melatonin suppression often find evidence of disruption in some scenarios
- Many studies have limited resolving power

Overall, the conclusions in Henshaw & Reiter 2005 remain unchanged

Overall conclusion

- Overall, studies of MF disruption of melatonin and circadian rhythms are inconsistent with no effect
- and are consistent with effects from chronic exposure to neighbourhood fields.

Acknowledgements

Children with Cancer UK

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