2. 6OHMS in early morning urine after creatinine correction may not correspond to the melatonin production and excretion during the night. Wilson et al. applied the same method in their study on the effect of the use of electrically heated blankets on melatonin and reported an effect. Bartsch et al. reported the use of early morning urine measuring 6OHMS. In this study, the correlation between total 6OHMS excretion and early morning urine was about 0.7 (personal communication), however, in a different laboratory test.

3. 6OHMS might be associated with sleep quality rather than with awakenings during the night.

4. Persons suffering from sleep disorders may have switched on the light. Their melatonin production may thus have been suppressed by the light of their lamp rather than by the Transmitter's electromagnetic field.

5. Other biophysical mechanisms may explain the relationship between the electromagnetic field and awakening during the night. There are probably many and especially many unknown mechanisms which could relate sleep disorders to EMF. The study of Wieser et al. suggests a reaction of epileptic patients to geostatic fields in the range of a few picoTesla which they relate to the presence of magnetite in the brain. To test this hypothesis, electronic probes have to be brought close to the temporal cortex, and brain biopsies are required. These are ethically unacceptable conditions in epidemiological studies.
   Fortunately Boldt and Bruderer (1994), two ornithologists, conducted a study on homing pigeons and their reaction to the electromagnetic field of the Schwarzenburg Shortwave Transmitter. It is assumed that pigeons orientate themselves by means of the geostatic field. This mechanism is believed to be transduced by the magnetite located in the brain. The pigeons' orientation was not influenced by the electromagnetic field of the Shortwave Transmitter. The authors considered the following options to explain the lack of an EMF-effect:

   (a) Pigeons' orientation by means of the geostatic field is not altered by radiowaves.
   (b) Unknown confounders hid the EMF influence.
   (c) The control pigeons were influenced by electromagnetic fields as well.
   (d) The disturbed orientation by means of the geostatic field was substituted by another orientation system.

6. The electromagnetic field is not strong enough to suppress melatonin levels. Melatonin levels may in principle explain sleep disorders partly,

but the sleep disorders reported by the surrounding population may have been caused by the Transmitter's mere presence. The association between awakening and the experimental transmission scheme may have been biased because the Transmitter's status could be checked by listening to Radio International Switzerland during the experimental period.

7. The frequency range of the Transmitter, in addition to its exposure range, does not suppress melatonin excretion

In the present situation considering the literature, the exposure measurements, and the study on dairy cows, we can state that

1. the IRPA limits were exceeded at no measuring site and in the present exposure and frequency range no biological effects had been reported previously.

2. no effect of EMF on early morning urine levels of 6OHMS could be established either in humans or in diary cows.

3. no melatonin-associated cancers had been reliably reported in the literature, which demonstrate an increase of cancer prevalences associated with electromagnetic fields in the range of shortwaves.

Conclusion

We conclude that the self-reported sleep disorders found increased in persons more strongly exposed to the EMF of the Schwarzenburg Radio Transmitter are not mediated by melatonin. This finding supports the hypothesis that the sleep disorders might be induced by the Transmitter's mere presence. A missing statistical association, however, should be treated with caution even if it may appear consistent with the range of exposure, the same finding in animals and the absence of an increased prevalence of life-threatening chronic diseases such as cancer.

We conclude that self-reported sleep disorder is found increased in persons more strongly exposed to the electro-magnetic fields of the Schwarzenburg Radio Transmitter. In two experimental situations with reduced or interrupted exposure, sleep disorder based on symptom diaries was significantly diminished. It is likely that this reflects a biological effect of EMF in the radio frequency range on sleep quality, although an alternative interpretation involving a psychosomatic effect among persons actively seeking information on the experimental changes of radio transmitter, cannot be excluded completely. To the extent that a biophysical effect of the Transmitter on sleep quality exists, we conclude that it is not mediated by melatonin. Further research is needed to better interpret our findings.

Bibliography

[1] Alvarez B, Dahlitz MJ, Vignau J, Parkes JD (1992): The delayed sleep phase syndrome: clinical and investigative findings in 14 subjects. J Neurol Neurosurg Psychiatry 55:665-670.

[2] Anders TF (1982): Biological rhythms in development. Psychosom Med 44:61-72.

[3] Arendt J (1994): Clinical perspectives for melatonin and its agonists (editorial). Biol Psychiatry 35:1-2.

[4] Arendt J, Labib MH, Bojkowski C, Hanson S, Marks V (1989): Rapid decrease in melatonin production during successful treatment of delayed puberty (letter). Lancet 1:1326.

[5] Arendt J, Aldhous M, Wright J (1988): Synchronisation of a disturbed sleep-wake cycle in a blind man by melatonin treatment (letter). Lancet 1:772-773.

[6] Arendt J, Bojkowski C, Franey C, Wright J, Marks V (1985): Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. J Clin Endocrinol Metab 60:1166-1173.

[7] Armstrong SM, McNulty OM, Guardiola Lemaitre B, Redman JR (1993): Successful use of S20098 and melatonin in an animal model of delayed sleep-phase syndrome (DSPS). Pharmacol Biochem Behav 46:45-49.

[8] Avoni P, Cortelli P, Montagna P, Tinuper P, Sforza E, Contin M, Parchi P, Pierangeli G, Maltoni P, Pavani A (1991): Circadian hormonal rhythms in two new cases of fatal familial insomnia. Acta Neurol 13:574-576.

[9] Bartsch C, Bartsch H, Schmidt A, Ilg S, Bichler KH, Fluchter SH (1992): Melatonin and 6-sulfatoxymelatonin circadian rhythms in serum and urine of primary prostate cancer patients: evidence for

reduced pineal activity and relevance of urinary determinations. Clin Chim Acta 209:153-167.

[10] Bearn J, Treasure J, Murphy M, Franey C, Arendt J, Wheeler M, Checkley SA (1988): A study of sulphatoxymelatonin excretion and gonadotrophin status during weight gain in anorexia nervosa. Br J Psychiatry 152:372-376.

[11] Beck Friis J, Kjellman BF, Aperia B, Unden F, von Rosen D, Ljunggren JG, Wetterberg L (1985): Serum melatonin in relation to clinical variables in patients with major depressive disorder and a hypothesis of a low melatonin syndrome. Acta Psychiatr Scand 71:319-330.

[12] Bojkowski CJ, Arendt J (1990): Factors influencing urinary 6-sulphatoxymelatonin, a major melatonin metabolite, in normal human subjects. Clin Endocrinol 33:435-444.

[13] Boldt A, Bruderer B (1994): (Initial orientation of homing pigeons under the influence of a short-wave radio transmitter. Orn Beob 91:111-123.

[14] Boyce PM (1985): 6-Sulphatoxy melatonin in melancholia. Am J Psychiatry 142:125-127.

[15] Brismar K, Hylander B, Eliasson K, Rossner S, Wetterberg L (1988): Melatonin secretion related to side-effects of beta-blockers from the central nervous system. Acta Med Scand 223:525-530.

[16] Carey VC, Zegger SL, Diggle PJ (1993): Modelling multivariate binary data with alternating logistic regressions. Biometrika 80:517-26.

[17] Cremer Bartels G, Krause K, Kuchle HJ (1983): Influence of low magnetic-field-strength variations on the retina and pineal gland of quail and humans. Graefes Arch Clin Exp Ophthalmol 220:248-252.

[18] Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS, Klein T, Rizzo JF (1995): Suppression of melatonin secretion in some blind patients by exposure to bright light (see comments). N Engl J Med 332:6-11.

[19] Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkes JD (1991): Delayed sleep phase syndrome response to melatonin. Lancet 337:1121-1124.

[20] Darcourt G, Souetre E, Pringuey D, Robert P, Feuillade P, Belugou JL (1992): (Disorders of circadian rhythms in depression). Encephale 18 Spec No 4:473-478.

[21] Diggle PJ, Liang KY, Zeger SL (1994): Analysis of Longitudinal Data, Oxford University Press.

[22] Folkard S, Arendt J, Clark M (1993): Can melatonin improve shift workers' tolerance of the night shift? Some preliminary findings. Chronobiol Int 10:315-320.

[23] Folkard S, Arendt J, Aldhous M, Kennett H (1990): Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. Neurosci Lett 113:193-198.

[24] Gonzalez R, Sanchez A, Ferguson JA, Balmer C, Daniel C, Cohn A, Robinson WA (1991): Melatonin therapy of advanced human malignant melanoma. Melanoma Res 1:237-243.

[25] Haimov I, Laudon M, Zisapel N, Souroujon M, Nof D, Shlitner A, Herer P, Tzischinsky O, Lavie P (1994): Sleep disorders and melatonin rhythms in elderly people. BMJ 309:167

[26] Hamilton JA, Gallant SA, Pinkel S (1988): Urinary 6-hydroxymelatonin in menstruating women. Biol Psychiatry 24:845-852.

[27] Hansen T, Bratlid T, Lingjarde O, Brenn T (1987): Midwinter insomnia in the subarctic region: evening levels of serum melatonin and cortisol before and after treatment with bright artificial light. Acta Psychiatr Scand 75:428-434.

[28] Harvalik ZV (1978): Anatomical localization of human detection of weak electromagnetic radiation: experiments with dowsers. Physiol Chem Phys 10:525-534.

[29] Hawkins L (1992): Seasonal affective disorders: the effects of light on human behaviour. Endeavour 16:122-127.

[30] Horne JA, Donlon J, Arendt J (1991): Green light attenuates melatonin output and sleepiness during sleep deprivation. Sleep 14:233-240.

[31] Hughes JT (1994): Electromagnetic fields and brain tumours: a commentary. Teratog Carcinog Mutagen 14:213-217.

[32] Jacobson JI (1992): Effect of weak magnetic fields on biological systems (letter). Int J Neurosci 67:125-126.

[33] James SP, Sack DA, Rosenthal NE, Mendelson WB (1990): Melatonin administration in insomnia. Neuropsychopharmacology 3:19-23.

[34] Jan JE, Espezel H, Appleton RE (1994): The treatment of sleep disorders with melatonin. Dev Med Child Neurol 36:97-107.

[35] Kanabrocki EL, Sothern RB, Scheving LE, Vesely DL, Tsai TH, Shelstad J, Cournoyer C, Greco J, Mermall H, Ferlin H (1990): Reference values for circadian rhythms of 98 variables in clinically healthy men in the fifth decade of life. Chronobiol Int 7:445-461.

[36] Kay RW (1994): Geomagnetic storms: association with incidence of depression as measured by hospital admission. Br J Psychiatry 164:403-409.

[37] Kennaway DJ, Van Dorp CF (1991): Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. Am J Physiol 260:R1137-44.

[38] Kennaway DJ, Royles P (1986): Circadian rhythms of 6-sulphatoxy melatonin, cortisol and electrolyte excretion at the summer and winter solstices in normal men and women. Acta Endocrinol 113:450-456.

[39] Kerenyi NA, Pandula E, Feuer G (1990): Why the incidence of cancer is increasing: the role of 'light pollution'. Med Hypotheses 33:75-78.

[40] Klein T, Martens H, Dijk DJ, Kronauer RE, Seely EW, Czeisler CA (1993): Circadian sleep regulation in the absence of light perception: chronic non-24-hour circadian rhythm sleep disorder in a blind man with a regular 24-hour sleep-wake schedule. Sleep 16:333-343.

[41] Laakso ML, Leinonen L, Hatonen T, Alila A, Heiskala H (1993): Melatonin, cortisol and body temperature rhythms in Lennox-Gastaut patients with or without circadian rhythm sleep disorders. J Neurol 240:410-416.

[42] Levitt AJ, Brown GM, Kennedy SH, Stern K (1991): Tryptophan treatment and melatonin response in a patient with seasonal affective disorder (letter). J Clin Psychopharmacol 11:74-75.

[43] Lewy AJ, Sack RL, Miller LS, Hoban TM, Singer CM, Samples JR, Krauss GL (1986): The use of plasma melatonin levels and light in the assessment and treatment of chronobiologic sleep and mood disorders. J Neural Transm Suppl 21:311-322.

[44] Lewy AJ, Sack RL, Singer CM (1985): Treating phase typed chronobiologic sleep and mood disorders using appropriately timed bright artificial light. Psychopharmacol Bull 21:368-372.

[45] Liang KY, and Zeger SL (1986): Longitudinal data analysis using generalized linear models. Biometrika 73:13-22.

[46] Liburdy RP, Sloma TR, Sokolic R, Yaswen P (1993): ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's

oncostatic action on ER+ breast cancer cell proliferation. J Pineal Res 14:89-97.

[47] Lingjaerde O, Bratlid T, Hansen T (1985): Insomnia during the "dark period" in northern Norway. An explorative, controlled trial with light treatment. Acta Psychiatr Scand 71:506-512.

[48] Lissoni P, Barni S, Tancini G, Ardizzoia A, Ricci G, Aldeghi R, Brivio F, Tisi E, Rovelli F, Rescaldani R (1994): A randomised study with subcutaneous low-dose interleukin 2 alone vs interleukin 2 plus the pineal neurohormone melatonin in advanced solid neoplasms other than renal cancer and melanoma. Br J Cancer 69:196-199.

[49] Lissoni P, Brivio F, Barni S, Tancini G, Cattaneo G, Archili C, Conti A, Maestroni GJ (1990): Neuroimmunotherapy of human cancer with interleukin-2 and the neurohormone melatonin: its efficacy in preventing hypotension. Anticancer Res 10:1759-1761.

[50] Loomis DP, Savitz DA, Ananth CV (1994): Breast cancer mortality among female electrical workers in the United States (see comments). J Natl Cancer Inst 86:921-925.

[51] Loscher W, Wahnschaffe U, Mevissen M, Lerchl A, Stamm A (1994): Effects of weak alternating magnetic fields on nocturnal melatonin production and mammary carcinogenesis in rats. Oncology 51:288-295.

[52] Loscher W, Mevissen M (1994): Animal studies on the role of 50/60-Hertz magnetic fields in carcinogenesis. Life Sci 54:1531-1543.

[53] Lovely RH (1988): Recent studies in the behavioral toxicology of ELF electric and magnetic fields. Prog Clin Biol Res 257:327-347.

[54] MacFarlane JG, Cleghorn JM, Brown GM, Streiner DL (1991): The effects of exogenous melatonin on the total sleep time and daytime alertness of chronic insomniacs: a preliminary study. Biol Psychiatry 30:371-376.

[55] Markey SP, Higa S, Shih M, Danforth DN, Tamarkin L (1985): The correlation between human plasma melatonin levels and urinary 6-hydroxymelatonin excretion. Clin Chim Acta 150:221-225.

[56] Matthews CD, Guerin MV, Wang X (1991): Human plasma melatonin and urinary 6-sulphatoxy melatonin: studies in natural annual photoperiod and in extended darkness. Clin Endocrinol 35:21-27.

[57] Maurizi CP (1990): The therapeutic potential for tryptophan and melatonin: possible roles in depression, sleep, Alzheimer's disease and abnormal aging. Med Hypotheses 31:233-242.

[58] McIntyre IM, Johns M, Norman TR, Armstrong SM (1990): A portable light source for bright light treatment. Sleep 13:272-275.

[59] Murialdo G, Fonzi S, Costelli P, Solinas GP, Parodi C, Marabini S, Fanciullacci M, Polleri A (1994): Urinary melatonin excretion throughout the ovarian cycle in menstrually related migraine (see comments). Cephalalgia 14:205-209.

[60] Murialdo G, Filippi U, Costelli P, Fonzi S, Bo P, Polleri A, Savoldi F (1991): Urine melatonin in alcoholic patients: a marker of alcohol abuse? J Endocrinol Invest 14:503-507.

[61] Nickelsen T, Samel A, Maass H, Vejvoda M, Wegmann H, Schoffling K (1991): Circadian patterns of salivary melatonin and urinary 6-sulfatoxymelatonin before and after a 9 hour time-shift. Adv Exp Med Biol 294:493-496.

[62] Nowak R, McMillen IC, Redman J, Short RV (1987): The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulphate excretion rates: two non- invasive techniques for monitoring human circadian rhythmicity. Clin Endocrinol 27:445-452.

[63] Persinger MA (1988): Increased geomagnetic activity and the occurrence of bereavement hallucinations: evidence for melatonin-mediated microseizuring in the temporal lobe? Neurosci Lett 88:271-274.

[64] Reiter RJ (1993): Electromagnetic fields and melatonin production. Biomed Pharmacother 47:439-444.

[65] Reiter RJ (1993): Static and extremely low frequency electromagnetic field exposure: reported effects on the circadian production of melatonin. J Cell Biochem 51:394-403.

[66] Reiter RJ (1992): Alterations of the circadian melatonin rhythm by the electromagnetic spectrum: a study in environmental toxicology. Regul Toxicol Pharmacol 15:226-244.

[67] Rojdmark S, Rossner S, Wetterberg L (1992): Effect of short-term fasting on nocturnal melatonin secretion in obesity. Metabolism 41:1106-1109.

[68] Sack RL, Lewy AJ, Blood ML, Stevenson J, Keith LD (1991): Melatonin administration to blind people: phase advances and entrainment. J Biol Rhythms 6:249-261.

[69] Sandyk R, Iacono RP (1994): Improvement by picoTesla range magnetic fields of perceptual-motor performance and visual memory in

a patient with chronic progressive multiple sclerosis. Int J Neurosci 78:53-66.

[70] Sandyk R, Derpapas K (1994): Successful treatment of respiratory dyskinesia with picoTesla range magnetic fields. Int J Neurosci 75:91-102.

[71] Sandyk R, Iacono RP (1994): Reversal of micrographia in Parkinson's disease by application of picoTesla range magnetic fields. Int J Neurosci 77:77-84.

[72] Sandyk R (1994): Improvement in word-fluency performance in Parkinson's disease by administration of electromagnetic fields. Int J Neurosci 77:23-46.

[73] Sandyk R, Awerbuch GI (1994): Pineal calcification and its relationship to the fatigue of multiple sclerosis. Int J Neurosci 74:95-103.

[74] Sandyk R (1994): Treatment of Parkinson's disease with magnetic fields reduces the requirement for antiparkinsonian medications. Int J Neurosci 74:191-201.

[75] Sandyk R, Iacono RP (1994): Multiple sclerosis: improvement of visuoperceptive functions by pico Tesla range magnetic fields. Int J Neurosci 74:177-189.

[76] Sandyk R (1994): Alzheimer's disease: improvement of visual memory and visuoconstructive performance by treatment with picotesla range magnetic fields. Int J Neurosci 76:185-225.

[77] Sandyk R, Derpapas K (1993): The effects of external picoTesla range magnetic fields on the EEG in Parkinson's disease. Int J Neurosci 70:85-96.

[78] Sandyk R, Derpapas K (1993): Further observations on the unique efficacy of picoTesla range magnetic fields in Parkinson's disease. Int J Neurosci 69:167-183.

[79] Sandyk R, Iacono RP (1993): Resolution of longstanding symptoms of multiple sclerosis by application of picoTesla range magnetic fields. Int J Neurosci 70:255-269.

[80] Sandyk R, Iacono RP (1993): Rapid improvement of visuoperceptive functions by picoTesla range magnetic fields in patients with Parkinson's disease. Int J Neurosci 70:233-254.

[81] Sandyk R, Derpapas K (1993): Magnetic fields normalize visual evoked potentials and brainstem auditory evoked potentials in multiple sclerosis. Int J Neurosci 68:241-253.

[82] Sandyk R (1993): Weak magnetic fields antagonize the effects of melatonin on blood glucose levels in Parkinson's disease. Int J Neurosci 68:85-91.

[83] Sandyk R, Awerbuch GI (1993): Nocturnal melatonin secretion in suicidal patients with multiple sclerosis. Int J Neurosci 71:173-182.

[84] Sandyk R (1992): Melatonin and maturation of REM sleep. Int J Neurosci 63:105-114.

[85] Sandyk R, Tsagas N, Anninos PA, Derpapas K (1992): Magnetic fields mimic the behavioral effects of REM sleep deprivation in humans. Int J Neurosci 65:61-68.

[86] Sandyk R (1992): The influence of the pineal gland on migraine and cluster headaches and effects of treatment with picoTesla magnetic fields. Int J Neurosci 67:145-171.

[87] Sandyk R (1992): Pineal calcification in relation to menopause in schizophrenia. Int J Neurosci 67:1-8.

[88] Sandyk R (1992): Successful treatment of multiple sclerosis with magnetic fields. Int J Neurosci 66:237-250.

[89] Sandyk R (1992): Magnetic fields in the therapy of parkinsonism. Int J Neurosci 66:209-235.

[90] Sandyk R (1992): Weak magnetic fields in the treatment of Parkinson's disease with the "on-off" phenomenon. Int J Neurosci 66:97-106.

[91] Sandyk R, Anninos PA, Tsagas N (1991): Magnetic fields and seasonality of affective illness: implications for therapy. Int J Neurosci 58:261-267.

[92] Sankila R, Joensuu H, Pukkala E, Toikkanen S (1993): Does the month of diagnosis affect survival of cancer patients? Br J Cancer 67:838-841.

[93] Savitz DA, Pearce N, Poole C (1993): Update on methodological issues in the epidemiology of electromagnetic fields and cancer. Epidemiol Rev 15:558-566.

[94] Schiffman JS, Lasch HM, Rollag MD, Flanders AE, Brainard GC, Burk DL, Jr. (1994): Effect of MR imaging on the normal human pineal body: measurement of plasma melatonin levels. J Magn Reson Imaging 4:7-11.

[95] Schmittbiel A, Gross MJ, Bujon Pinard P, Laxenaire M (1994): (Chronobiology and depression: the seasonal depressions. Clinical aspects, physiopathology and specific treatments). Ann Med Psychol 152:444-456.

[96] Skene DJ, Bojkowski CJ, Currie JE, Wright J, Boulter PS, Arendt J (1990): 6-sulphatoxymelatonin production in breast cancer patients. J Pineal Res 8:269-276.

[97] Souetre E, Salvati E, Belugou JL, Douillet P, Braccini T, Darcourt G (1987): Seasonality of suicides: environmental, sociological and biological covariations. J Affect Disord 13:215-225.

[98] Statistical Sciences, Inc. (1993), S-PLUS for Windows User's Manual, Version 3.1, Seattle, Statistical Sciences.

[99] Stevens RG (1993): Breast cancer and electric power. Biomed Pharmacother 47:435-438.

[100] Stevens RG (1993): Biologically based epidemiological studies of electric power and cancer. Environ Health Perspect 101 Suppl 4:93-100.

[101] Stevens RG, Davis S, Thomas DB, Anderson LE, Wilson BW (1992): Electric power, pineal function, and the risk of breast cancer (see comments). FASEB J 6:853-860.

[102] Stevens RG (1992): Re: "Use of electric blankets and risk of postmenopausal breast cancer" (letter; comment). Am J Epidemiol 135:834-835.

[103] Suzuki S, Dennerstein L, Greenwood KM, Armstrong SM, Sano T, Satohisa E (1993): Melatonin and hormonal changes in disturbed sleep during late pregnancy. J Pineal Res 15:191-198.

[104] Taubes G (1994): Electromagnetic fields. Breast cancer link claimed, criticized (news). Science 264:1658

[105] Tomoda A, Miike T, Uezono K, Kawasaki T (1994): A school refusal case with biological rhythm disturbance and melatonin therapy. Brain Dev 16:71-76.

[106] Tynes T (1993): Electromagnetic fields and male breast cancer (editorial). Biomed Pharmacother 47:425-427.

[107] Tzischinsky O, Shlitner A, Lavie P (1993): The association between the nocturnal sleep gate and nocturnal onset of urinary 6-sulfatoxymelatonin. J Biol Rhythms 8:199-209.

[108] Tzischinsky O, Pal I, Epstein R, Dagan Y, Lavie P (1992): The importance of timing in melatonin administration in a blind man. J Pineal Res 12:105-108.

[109] Vena JE, Graham S, Hellmann R, Swanson M, Brasure J (1991): Use of electric blankets and risk of postmenopausal breast cancer (see comments). Am J Epidemiol 134:180-185.

[110] von Knorring L, Almay BG, Haggendal J, Johansson F, Oreland L, Wetterberg L (1986): Discrimination of idiopathic pain syndromes from neurogenic pain syndromes and healthy volunteers by means of clinical rating, personality traits, monoamine metabolites in CSF, serum cortisol, platelet MAO and urinary melatonin. Eur Arch Psychiatry Neurol Sci 236:131-138.

[111] Waldenlind E, Ekbom K, Wetterberg L, Fanciullacci M, Marabini S, Sicuteri F, Polleri A, Murialdo G, Filippi U (1994): Lowered circannual urinary melatonin concentrations in episodic cluster headache (see comments). Cephalalgia 14:199-204.

[112] Waldhauser F, Saletu B, Trinchard Lugan I (1990): Sleep laboratory investigations on hypnotic properties of melatonin. Psychopharmacology 100:222-226.

[113] Waterman GS, Ryan ND, Perel JM, Dahl RE, Birmaher B, Williamson DE, Thomas CR, Puig Antich J (1992): Nocturnal urinary excretion of 6-hydroxymelatonin sulfate in prepubertal major depressive disorder. Biol Psychiatry 31:582-590.

[114] Weissbluth M, Weissbluth L (1992): Colic, sleep inertia, melatonin and circannual rhythms. Med Hypotheses 38:224-228.

[115] Wetterberg L, Aperia B, Gorelick DA, Gwirtzman HE, McGuire MT, Serafetinides EA, Yuwiler A (1992): Age, alcoholism and depression are associated with low levels of urinary melatonin. J Psychiatry Neurosci 17:215-224.

[116] Wetterberg L, Halberg F, Halberg E, Haus E, Kawasaki T, Ueno M, Uezono K, Cornelissen G, Matsuoka M, Omae T (1986): Circadian characteristics of urinary melatonin from clinically healthy young women at different civilization disease risks. Acta Med Scand 220:71-81.

[117] Wilson BW, Wright CW, Morris JE, Buschbom RL, Brown DP, Miller DL, Sommers Flannigan R, Anderson LE (1990): Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J Pineal Res 9:259-269.

[118] Wieser H. G. (1994): Magnetfelder und Epilepsiediagnostik, Internationale Tagung "Bio-Elektromagnetische Effekte", EMC Baden, Schweiz, 23. Juni 1994.

[119] Wilson BW, Stevens RG, Anderson LE (1989): Neuroendocrine mediated effects of electromagnetic-field exposure: possible role of the pineal gland. Life Sci 45:1319-1332.

[120] Wilson BW (1988): Chronic exposure to ELF fields may induce depression. Bioelectromagnetics 9:195-205.

[121] Wirz Justice A, Richter R (1979): Seasonality in biochemical determinations: a source of variance and a clue to the temporal incidence of affective illness. Psychiatry Res 1:53-60.

[122] Wood AW (1993): Possible health effects of 50/60Hz electric and magnetic fields: review of proposed mechanisms. Australas Phys Eng Sci Med 16:1-21.

[123] Zimmermann RC, McDougle CJ, Schumacher M, Olcese J, Heninger GR, Price LH (1993): Urinary 6-hydroxymelatonin sulfate as a measure of melatonin secretion during acute tryptophan depletion. Psychoneuroendocrinology 18:567-578.

Chapter 8

Influence of electromagnetic field exposure on melatonin concentrations in dairy cows

Stärk, K.D.C.; Krebs, Th.; Altpeter, E.; Manz, B.; Abelin, Th.; Griot, C.

Abstract:

Objective: to examine salivary melatonin levels in cows exposed/unexposed to shortwave electromagnetic fields.
Design: case-control study. Five exposed and 5 unexposed cows were observed during 10 nights. During 3 of these 10 nights the Shortwave Transmitter Schwarzenburg was switched off (off-phase). Salivary samples were tested by RIA for melatonin.
Results: There were no obvious cross-sectional (chronic) and time sequence (acute) effects upon switching off the Transmitter. An increase of peak values during the off-phase, suggested graphically, could not be confirmed in formal statistical inference, which was, however, limited by many missing values.
Conclusion: The electromagnetic fields of the Shortwave Transmitter Schwar zenburg could not be shown to induce changes of salivary melatonin levels in cows.

Keywords: saliva, melatonin, radiowaves, ae (adverse effects), robust statistics

8.1 Introduction

Possible negative health effects of electromagnetic field (EMF) exposure on animal health have been discussed in the literature (Mercer, 1985). One of

the aspects is the potential of EMF to influence melatonin concentrations in body fluids (Reiter, 1992, 1993a, 1993b; Grota et al., 1994). Melatonin is a hormone with complex physiological effects that are not yet completely understood (Reiter, 1991). It seems to act as a mediator of photoperiodic information to the endocrine system in all species. Melatonin is mainly produced in the pineal gland once the inhibitory effect of light is relieved (Reiter, 1992). Very short periods of light immediately reduce melatonin production (Vollrath et al., 1989). Receptors in the retina transmit signals via a neuronal pathway to the pineal gland where melatonin is produced from the precursor hormone serotonin and quickly released into the blood. Afterwards, it reaches all other body fluids (Reiter, 1991) and peripheral organs. Due to its nightly production and quick release, distinct diurnal cycles of melatonin serum concentrations have been observed in many species including the cow (Berthelot et al., 1990; Paterson and Foldes, 1994), peak production always occurring during the dark period. Receptors for melatonin have been described in the brain, the pituitary gland and various organs. In livestock, the most important physiological effect is the gonadotrophic influence on reproductive periods in seasonally reproductive species (Asher et al., 1993; Williams and Helliwell, 1993). But additional effects on other endocrine processes as well as gastrointestinal physiology have also been described (Reiter, 1991). Furthermore, melatonin seems to have oncostatic and immunoenhancing effects (Blask, 1990, cited by Reiter, 1992; Maestroni et al., 1989). Due to the complex effects of melatonin, all factors influencing its production might disturb body functions at various sites (Reiter, 1991). Applied as a supplement in livestock, melatonin has the potential to change and improve productivity (Paterson and Foldes, 1994; Haresign, 1992).

The objective of this study was to measure both the acute and the chronic effect of EMF generated by a shortwave radio transmitter on melatonin concentrations in the saliva of dairy cows and to discuss the possible consequences for animal health.

8.2 Methodology

8.2.1 Study Design

Melatonin levels in the saliva of EMF-exposed and unexposed dairy cows were measured during 10 days. The Shortwave Transmitter causing the exposure had a maximal power of 4 x 150 kW. During the period between day 3 and day 5, the transmitter was turned off. This intervention was known neither to the farmers, nor to the veterinarians collecting the samples or to the technicians analyzing them. The exposure was defined as the maximum EMF strength in the closest vicinity to EMF source whereas non-exposure was an EMF strength close to normal values measured throughout the country. Exposure was measured during the study period (see Chapter

4).

8.2.2 Farms and Animals

Melatonin measurements were performed on two farms, one being EMF exposed (A), the other one non-exposed (B). These farms were chosen from a list provided by local veterinarians. Farm A was the farm closest to the EMF source (500 m), while Farm B was 4 km from the source. Both farms were comparable as to their housing, feeding, and management types as well as to their production intensity. The herd sizes of farms A and B were 19 and 14 cows respectively. All animals were grazing outside on every single day of the study period.

From each farm 5 animals were selected for the study. In order to prevent possible confounding, the animals were chosen according to their breed, age, reproductive stage, and production level. They were all lactating Red-Holsteins, 3-4 years old and 118-242 days pregnant on the first day of the study.

8.2.3 Sampling Protocol

In order to measure the nightly peak of melatonin production it was necessary to collect samples at regular intervals during the night with the help of red light. Saliva samples were collected with saliva swaps (SALIVETTEN, Fa. Sarstedt, Germany) 7 times every two hours starting from 7.30 p.m. and stopping at 7.30 a.m. during 10 subsequent days from September 5 until September 15, 1993. Sunrise on the first day was at 06.49 a.m. and sunset at 7.59 p.m. (Swiss Institute of Meteorology Zürich). Saliva samples had been immediately stored on dry ice until they were centrifuged at 3500 rpm for 5 minutes and then stored again at -20 C.

8.2.4 Sample analysis

A new commercial radio immuno assay (RIA) was used (^{125}I-Melatonin, Immuno Biological Laboratories, Hamburg, Germany) [Manz et al. (1989)].

8.2.5 Statistical methods

Logarithmic ($\log_{10}$) values of the Melatonin concentrations of the exposed and non-exposed cows were compared. Due to a high number of missing values as well as due to outlying observations as a result of faulty laboratory tests, it was decided to use the median polish technique (Mosteller and Tukey, 1977; Hoaglin et al., 1983) for effect calculations. This procedure provides for each group an estimate of the overall median, a row vector with the median level of each animal and a column vector with the median time

series of the group. These median values are hardly influenced by outlying or missing laboratory values.

For the estimation of the chronic effect of the EMF exposure, the animals' median values of the two groups were compared by means of the Wilcox rank-sum test for two samples (Statistical Sciences (1993)).

With respect to the acute effect of the exposure, the analysis of the data was difficult. There were missing values even after the median polish procedure and moreover, the time series were unequally spaced. Therefore, it was decided to calculate the differences of the two group median time series and to analyze the resulting series by means of the runs test (Draper and Smith, 1981). The influence of the missing values on the runs test was investigated by simulations. The missing values were replaced at random by -1 or 1 with probability 0.5. This resulted in a complete series of signs. The runs test was computed on this series and it was checked whether a different conclusion would result from this hypothesis testing.

All statistical techniques used are characterized by a high robustness with respect to missing values and outliers.

8.3 Results

The average nightly EMF-exposures measured on the exposed and the non-exposed farms were 1.59 mA/m and 0.076 mA/m, respectively. The latter value corresponds to an average value in non-exposed rural areas in Switzerland. The maximum immission on the exposed farm reached 4.9 mA/m.

The melatonin concentrations measured were between 1.00 pg/ml and 750 pg/ml on the exposed and between 1.00 pg/ml and 316 pg/ml on the non-exposed farm.

8.3.1 Acute effect

Between the two groups 28 positive differences, 36 negative differences and 32 runs were observed after omitting the 6 missing values. Therefore no serial correlation between the time series of the two groups could be detected [runs test, p-value 0.5]. The simulations confirmed that even in the worst case no serial correlation would be detected.

The below diagram depicts a shift as well as an elevation of the profiles of the peak values shortly after the Transmitter was turned off. Statistical analysis of this finding did not reveal any significance (Figure 8.1).

8.3.2 Chronic effect

The overall median of the exposed cows was 17.7 pg/ml (sd = 1.25), while it was 19.0 pg/ml (sd = 1.32) for the non-exposed ones (Figure 8.2). The difference between the groups was not statistically significant.

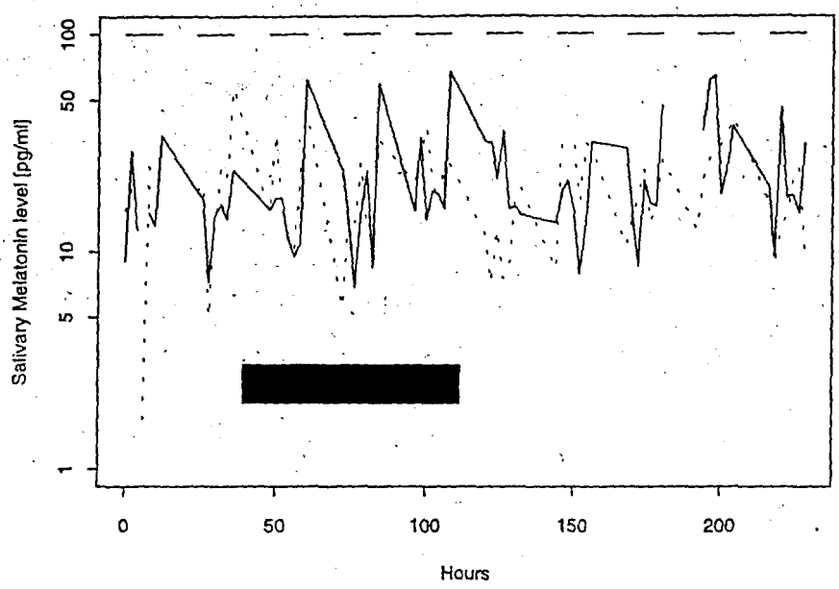

Figure 8.1: *Melatonin concentrations measured in the saliva of EMF-exposed (continuous line) and unexposed (dashed line) cows: acute effect of EMF exposure. The lines at 100 pg/ml indicate the dark phases when melatonin concentration was measured, black box is the period of no EMF exposure of both groups.*

8.4 Discussion and Conclusions

The influence of EMF exposure on melatonin levels in cows has been studied for the first time. The results of this study did not provide any evidence of a statistically significant acute or chronic effect of exposures with a maximum of 4.9 mA/m in the frequency range of shortwaves (3-30 MHz). Other studies using higher exposures and other animal species described a reduction of the duration and a blunting of the rise of nocturnal melatonin concentrations (Kato et al., 1994; Yellon, 1994; see also Chapter 6). As this was a pilot study, the sample size was restricted to only 5 animals per farm and consequently the power of the study was limited. The use of new sampling and analysis techniques was another reason for a certain loss of data.

Statistical analysis of the laboratory results did not reveal any influence of the radio transmitter on melatonin metabolism in dairy cows housed in its vicinity. These findings are consistent with the results of the part of this

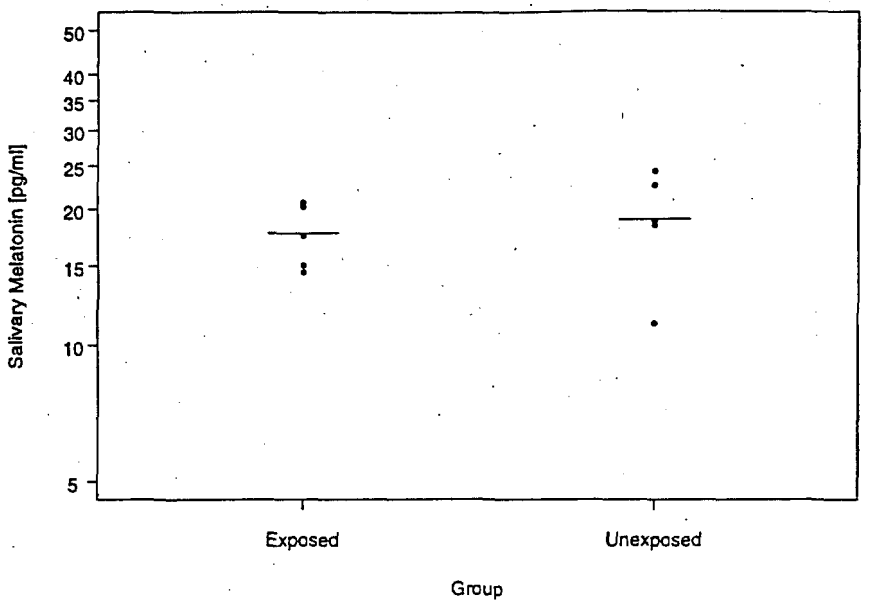

Figure 8.2: *Melatonin concentrations measured in the saliva of EMF-exposed and unexposed cows: chronic effect of EMF exposure.*

study dealing with humans.

Other studies concerned with the influence of EMF exposure on animal health in general rather than specifically on melatonin concentrations, also suggested similar conclusions. In pigs born and raised under the influence of 345 kV transmission lines no effect on reproductive performance was observed (Mahmoud and Zimmermann, 1984, cited by Mercer, 1985). Similarly, there was no effect on ovarian activity, oestrus signs, pregnancy or fetal viability in cows when exposed to 400 kV, 50 Hz transmission lines (Algers and Hultgren, 1986). Saliva sampling for melatonin testing in cows seems to be a promising technique because it is non-invasive and can be easily performed under dimmed light conditions. It can be also used for further investigations of the still poorly understood neuroactive effect of melatonin.

Conclusion

Our analysis could not confirm an EMF-effect on salivary melatonin levels in diary cows. However it has to be stressed that the sample size of 5 animals per group is insufficient to exclude a limited EMF-effect. Yet, due to the technical limitations of this study further research is necessary to conclusively rule out adverse health effects on dairy cows.

Bibliography

[1] Asher, G.W., Fisher, M.W., Fennessy, P.F., Suttie, J.M. and Webster, J.R. (1993): Manipulation of reproductive seasonality in farmed red deer (Cervus elaphus) and fallow deer (Dama dama) by strategic administration of exogenous melatonin. Anim. Reprod. Sci. 33, 267-287.

[2] Algers, B., Hultgren, J. (1987): Effects of long-term exposure to a 400-kV, 50-Hz transmission line on estrous and fertility in cows. Prev.Vet.Med. 5, 21-36.

[3] Berthelot, X., Laurentie, M., Ravault, J.P., Ferney, J. and Toutain, P.L. (1990): Circadian profile and production rate of melatonin in the cow. Domest. Anim. Endocrinol. 7, 315-322.

[4] Draper, N.R. and Smith, H. (1981): Applied regression analysis. 2nd ed. John Wiley & Sons, Inc., New York.

[5] Grota, L.J., Reiter, R.J., Keng, P. and Michaelson, S. (1994): Electric field exposure alters serum melatonin but not pineal melatoninsynthesis in male rats. Bioelectromagnetics 15, 427-437.

[6] Haresign, W. (1992): The effect of implantation of lowland ewes with melatonin on the time of mating and reproductive performance. Anim. Prod. 54, 31-39.

[7] Hoaglin, D.C., Mosteller, F. and Tukey, J.W. (1983): Understanding robust and exploratory data analysis. John Wiley & Sons, Inc., New York.

[8] Kato, M., Honma, K., Shigemitsu, T. and Shiga, Y. (1994): Circularly polarized 50-Hz magnetic field exposure reduces pineal gland and blood melatonin concentrations of Long-Evans rats. Neurosci. Lett.166, 59-62.

[9] Maestroni, G.J.M., Conti, A. and Pierpoali, W. (1989): Melatonin, stress and the immune system. Pineal Res. Rev. 7, 203-226.

[10] Manz B., Seidel A., Alexander H., Vollrath L., Wagner B., Zimmermann G., Wiedemann K., Pollow K. (1989), Development and Validation of

a Radioimmunoassay for Serum Melatonin. J.Clin.Chem.Clin.Biochem. 27, 797-802.

[11] Mercer, H.D. (1985): Biological effects of electric fields on agricultural animals. Vet. Hum. Toxicol. 27, 422-426.

[12] Mosteller, F. and Tukey, J.W. (1977): Data analysis and regression. Addison-Wesley Publishing Company, Reading, Massachusetts.

[13] Paterson, A.M. and Foldes, A. (1994): Melatonin and farm animals: Endogenous rhythms and exogenous applications. J. Pineal Res. 16, 167-177.

[14] Reiter, R.J. (1991): Melatonin: That ubiquitously acting pineal hormone. News Physiol. Sci. 6, 223-227.

[15] Reiter, R.J.(1992): Alterations of the circadian melatonin rhythm by the electromagnetic spectrum: A study in environmental toxicology. Regulatory Toxicology and Pharmacology 15, 226-244.

[16] Reiter, R.J. (1993a): Electromagnetic fields and melatonin production. Biomed. & Pharmacother. 47, 439-444.

[17] Reiter, R.J. (1993b): Static and extremely low frequency electromagnetic field exposure: Reported effects on the circadian production of melatonin. J. Cell. Biochem. 51, 394-403.

[18] Statistical Sciences (1993): S-Plus for Windows User's Manual, Version 3.1. Seattle: Statistical Sciences Inc.

[19] Vollrath, L., Seidel, A., Huesgen, A., Manz, B., Pollow, K. and Leiderer, P.(1989): One millisecond of light suffices to suppress nighttime melatonin synthesis in rats. Neurosci. Lett. 98, 297-298.

[20] Williams, L.M. and Helliwell, R.J.A. (1993): Melatonin and seasonality in the sheep. Anim. Reprod. Sci. 33, 159-182.

[21] Yellon, S.M. (1994): Acute 60 Hz magnetic field exposure effects on the melatonin rhythm in the pineal gland and circulation of the adult Djungarian hamster. J. Pineal Res. 16, 136-144.

Chapter 9

Blood pressure in the vicinity to a Shortwave Radio Transmitter

E.S. Altpeter, Th. Krebs, R. Blattmann, Th. Abelin

Abstract:

Objective: The purpose of the present study was to established an association between hypertension and EMF-exposure within the shortwave range.
Design: Case-control study. In 3 exposure zones (high, medium, low) study participants were asked to fill in a questionnaire. Their answers regarding blood pressure suggested an association between change of blood pressure and EMF-exposure. This was verified as follows:

All 58 persons who reported to suffer from hypertension, and 58 randomly selected persons who did not suffer from hypertension according to their answer in Study I were contacted. Blood pressure was measured on 3 occasions and the results compared with the answers in Study I. The diagnostic of a hypertensive value was compared with the routine measurements done by participants' physicians.
Results: 95 (82 %) of the contacted persons agreed to the study conditions (45 with and 50 without self-reported hypertension). Self-reported hypertension turned out to be rather reliable with a specificity of 82%, a sensitivity of 88% and a κ known as the 'interrater agreement' of 0.603. Reasons for the discrepancy between the answers of Study I and the results of measuring were: missing knowledge about one's own blood pressure in 10% (5/50),and an accidentally increased blood pressure at a check-up before blood donation or at a pharmacy under non-standardized conditions or, in 20 % (9/45), during pregnancy.
Conclusion: Self-reported hypertension is quite reliable and can be easily

verified by measuring blood pressure and contacting participants' physicians. However, no association between EMF-exposure and arterial hypertension could be established.

Keywords: hypertension, specificity, sensitivity, interrater agreement, *kappa*-statistics, radiowaves.

9.1 Introduction

The purpose of the Shortwave Radio Transmitter Schwarzenburg Study was to examine whether there was evidence that the Transmitter's electromagnetic field causes harm to the population living in its surroundings. One of the major problems in the field of public health is cardiovascular disease, and in particular hypertension. Literature offers very little in connection with blood pressure and exposure to electromagnetic fields. Since electromagnetic fields suppress melatonin by means of the noradrenergic axis (References see Chapter 7), it could be assumed that hypertension might be more frequent in the vicinity of the Transmitter.

9.2 Indirect indicators of blood pressure abnormalities in the Schwarzenburg health interview study (Study I)

9.2.1 Methodology

For details on sampling see Chapter on 'The Schwarzenburg health interview survey'. The study questionnaire included inquiries about sex, age, housing, occupation, education, reaction to electric phenomena, as well as about ideas concerning environmental protection, socioeconomic status and health.

Questions concerning health-related in particular to reason for actual medical treatment, smoking habits and knowledge about blood pressure. A part of the questionnaire section concerning the reasons for medical treatment inquired about diagnosis. The entered diagnoses were coded according to the ICD-9 code. Due to their small numbers, they were combined into generic terms, e.g. arterial hypertension, cardiovascular disease without hypertension, disease of the skeletomuscular system. Diseases that could not be classified in these terms were not further considered.

Furthermore, one question was worded as follows: " Do you consider your blood pressure normal, too high, too low or do you not know your blood pressure?"

	Zone A	*Zone B*	*Zone C*	*Total*
normal	58 (55%)	67 (56%)	133 (74%)	258 (64%)
too low	17 (16%)	22 (19%)	14 (8%)	53 (13%)
too high	18 (17%)	18 (15%)	22 (12%)	58 (14%)
no knowledge	12 (11%)	12 (10%)	11 (6%)	35 (9%)
Total	105 (100%)	119 (100%)	180 (100%)	404 (100%)

Table 9.1: Distribution of self-reported blood pressure

9.2.2 Results

Out of 684 contacted persons, 408 accepted the conditions of the health interview survey in 1992. The participation rates were 74% (109/408), 55% (120/408) and 56% (179/408) in Zones A, B and C, respectively. The interviews of 105 persons in Zone A (65 women and 40 men, age range 14 to 91 years), 119 persons in Zone B (62 women and 57 men, age range 15 to 81 years) and 180 persons in Zone C (106 women and 74 men, age range 14 to 84 years) could be further analyzed.

Table 9.1 summarizes the distribution of the self-reported blood pressure. The differences are statistically significant ($\chi^2, p = 0.013$). Fourteen per cent (15/105), 8% (10/119) and 8% (14/180) of the persons in Zone A, B and C reported that their physician diagnosed 'arterial hypertension'. This established a difference between Zone A and combined Zones B and C which is nearly statistically significant ($OR = 1.91$ 95% $C.I. : 0.91 < OR < 3.99$). Seven per cent, 6% and 7% from Zones A, B and C reported to suffer from 'cardiovascular disease' which is not statistically significant.

9.2.3 Discussion and preliminary conclusion

The purpose of the health interview study was to find out whether ill health reported by the population living in the neighbourhood of the Transmitter is more frequent and might thus be associated with its electromagnetic fields. Since self-reported changes of blood pressure seemed to be related to the electromagnetic field, and since there was a trend towards increased prevalence of arterial hypertension in the vicinity of the Transmitter, we concluded that further investigations were needed. Our interest concentrated on the reliability of the self-reported blood pressure, an estimate of number of unknown cases of hypertension and finally on an estimate of the odd's ratio corrected for under- respectively overreporting.