6.1.1 Insomnia

Insomnia is defined as a chronic inability to obtain an amount of sleep needed for optimal functioning and well-being [Hauri and Esther (1990)].

More popularly speaking, insomnia is the want of sleep to indicate any impairment in duration, depth or restorative properties of sleep. Insomnia may occur as a primary disorder - a disorder that has no obvious other cause -, as a secondary manifestation of psychiatric illness, anxiety, drug use, or medical conditions, e.g. paroxysmal nocturnal dyspnea, nocturnal asthma, cardiac asthma, and rheumatic or gastrointestinal illnesses well as in association with other disorders of sleep, e.g. sleep apnea. Poor sleep occurs in persons with a debilitating or painful illness which generates more pain and restlessness when muscles relax to leave painful areas unprotected.

Insomnias are classified into

1. psychophysiologic insomnia: Psychophysiologic insomnias are classified into situational and persistent insomnias. Situational insomnias are defined as lasting less than 3 weeks, usually with a suggested emotional cause. Symptoms include difficulty in falling asleep, frequent arousals, or persistent early morning awakening, each contributing to feelings of chronic fatigue and irritability. Such symptoms may become persistent or be worsened by worry about loss of sleep.

2. Insomnia associated with affective psychiatric disorders: The sleep abnormalities found in the affective disorders consist of the chronic or recurrent inability to maintain sleep through the expected sleep period. Patients complain about persistent restlessness at night and of a tired, apathetic feeling during the day. More frequent arousals throughout the night, failure to 'sleep deeply', and early morning awakenings complete the clinical picture.

3. Insomnia associated with the use of drugs and alcohol: The widely spread use and abuse of central nervous system depressants is well documented and may produce an insomnia syndrome.

4. Insomnia associated with adverse effects to medication, in particular in the elderly, e.g. antihypertensive therapy.

5. Insomnia associated with sleep-induced respiratory impairment.

6. Insomnia associated with sleep-related myoclonus e.g. restless leg syndrome.

6.1.2 Disorders of the sleep-wake schedule

Sleep is one of many daily rhythms synchronized with the earth's 24-h geophysical cycle. It is entrained by sunlight which is transmitted an electro-

magnetic wave. Without this entrainment, for example under laboratory conditions or in some blind people, the endogenous length of the 'free running' circadian periods for humans is approximately 25 hours. Under such time-isolated conditions the normal coupling of the rhythms of body temperature, plasma hormone levels and sleep cycle is lost, as the phase relationship between them becomes desynchronized. Such mechanisms are suggested for sleep disorders in blind people, in people suffering from jet lag or in inhabitants of arctic and antarctic areas.

This type of sleep disorder is associated with disturbances in melatonin levels and their rhythmicity (see Chapter on melatonin and sleep disorders). Melatonin reacts to electromagnetic fields, and is associated with insomnias, in particular in the elderly. If sleep disorders occur more frequently around the Transmitter and if they are sequentially associated in time with the varying electromagnetic field of the Transmitter, the hypothesis should also be tested that electromagnetic fields in the shortwave range and in the range of exposure which is produced by the Transmitter causes changes in melatonin levels and these in turn cause insomnia.

Roberts et al. 1985 suggested that sleep disorders might be caused by electromagnetic fields in the shortwave range. Wilson suggested that electromagnetic fields could cause depression which in turn causes insomnia. To the best of our knowledge neither has the study by Roberts has been repeated nor did we find a study that would have confirmed Wilson's hypothesis. It is, however, evident that a symptom such as insomnia has too many causes to be explained merely by EMF-exposure or by environmental stress alone.

6.2 Sleep disorder in The Schwarzenburg health interview survey

6.2.1 Methodology

Design:

In a cross-sectional study three population groups A, B and C with high, medium and low exposure were questioned about their health status in a personally structured interview and completed segments of standardized personality testing. Exposure measurements at 55 points around the transmitter during 24 hours confirmed this classification and permitted more differentiated analyses.

Sampling:

The population of the municipality of Wahlern was notified by a message in the local official bulletin (Amtsanzeiger Schwarzenburg, 3rd of April 1992).

The addresses of the potential study participants were supplied by the officials of the municipality of Wahlern.

The municipality of Wahlern was divided into exposed and unexposed areas (see Chapter 3). Then the families were contacted by letter. In the exposed area all 215 families received a personal letter. In the control area, a simple random sample of 379 families was contacted. Then an attempt was made to contact all participants by telephone and to obtain their participation. When a family agreed, as many family members as possible were asked to participate. For persons in the exposed group unwilling to participate , a letter was added.

Interview:

In the health survey interview all family members over 14 years participated. One member of the family was questioned about the children under 14 years. After the interview was conducted, the participants were asked to complete a standardized personality test (segments of the 'Freiburger Persönlichkeitsinventar').

The interview included inquiries about age, sex, living, housing, education, employment, general status of health, signs and symptoms of diseases, disturbances of electrical appliances of daily use, intention relating to environmental protection and participants' opinion about the hazards attributed to the Transmitter. The interviewer assessed the living standard of the family.

Exposure:

See Chapter on 'Exposure assessment' and 'The Schwarzenburg health interview survey' for details.

Statistical analysis:

In the health interview survey we asked the following questions:

1. Do you suffer from difficulties in falling asleep:

   (a) never (scored 1)
   (b) rarely (scored 2)
   (c) sometimes (scored 3)
   (d) frequently (scored 4)
   (e) ever (scored 5)
   (f) no answer (scored 9)

2. Do you suffer from difficulties in maintaining sleep:

(a) never (scored 1)
(b) rarely (scored 2)
(c) sometimes (scored 3)
(d) frequently (scored 4)
(e) ever (scored 5)
(f) no answer (scored 9)

In a similar manner we asked about sleep intensity. In the final analysis we made decision to use only these two questions. According to the definition of insomnia we considered all participants with a score of >3 as suffering from either difficulties in falling asleep or difficulties in maintaining sleep.

Median age was 45 years. On this basis we classified participants into a younger (≤ 45 y.) and older (≥ 45 y.) age group.

Educational level was defined by a score: no school education at all 0, compulsory school 1 point, and every further school or diploma degree added 1 point to the score.

In a first step, we established cross-tabulations of presence or absence of diseases by exposure zones. The association between disease and exposure was evaluated by means of the χ^2-test. Then the confounding by age, sex and social status on the association between exposure and disease was evaluated by estimating loglinear models by means of MIM 2.3β programmed and supplied by David Edwards.

6.2.2 Results

109 persons in Zone A (73.7%), 120 Persons in Zone B (55%) and 180 persons in Zone C (56.3%) participated in the health interview survey. Of hundred and five questionnaires of Zone A, 119 of Zone B and all questionnaires of Zone C could be further analyzed. The finally analyzed population showed the following characteristics:

- Zone A: 65 women, 40 men, aged 14 to 91.
- Zone B: 62 women, 57 men, aged 15 to 81.
- Zone C: 106 women, 74 men, aged 14 to 84.

There were no substantial socioeconomic differences between the three groups. Fourteen per cent of the study population suffered from difficulties in falling asleep, and 15% from difficulties in maintaining sleep. The frequency of difficulties in falling asleep was significantly higher in Zone A and B compared to Zone C ($\chi^2 = 16.1$, $df = 2$, $p < 0.001$). There was no substantial difference between Zone A and B. The proportions were 23% in Zone A, 17.6% in Zone B and 6.7% in Zone C. A more pronounced difference

was observed in difficulties of maintaining sleep during the night (32.4% in Zone A, 18.5% Zone B and 8.9% in Zone C, χ^2, p<0.001). We could confirm the findings of Dealberto (1992), that insomnias are more frequent among the elderly and in the low educational levels groups, but we did not find any differences with regard to sex. In particular, we could establish that the critical educational level was at the point of passing / not passing compulsory school. The association between insomnias and exposure remained constant even after correcting for age and educational level.

By means of loglinear we could show that difficulties in falling asleep are strongly associated with difficulties in maintenance of sleep during the night. Out of these two components of insomnia sleep maintenance during the night is more strongly associated with exposure than falling asleep. In another more complicated model difficulties in maintaining sleep became a central role for the relation between psychovegetative disorders and the Transmitter's EMF (see Chapter 5)

	Spare time spent:		
Zone	at home	not at home	Total
A	61 (58.1%)	44 (41.9%)	105
B	78 (65.6%)	41 (34.4%)	119
C	170 (94.4%)	10 (5.6%)	180
Total	309 (100%)	95 (100%)	404

Table 6.1: *Location of spending leisure time*

It is of interest to note that with high statistical significance ($\chi^2 = 59.9$, $df = 5$, $p < 0.001$) the population of Zones A and B used to spend their spare time more frequently elsewhere than at home (see Table 6.1).

6.2.3 Discussion and conclusion

We conclude that insomnias are associated with geographical closeness to the Transmitter. But is not clear whether this reflects an effect of exposure to electromagnetic fields in the shortwave range or of Transmitters mere presence. The association remains even after correction for various known confounders such as age and educational status. It is, however, not clear whether this effect is caused by a purely psychological effect or by the electromagnetic field itself. The fact that people in Zone A and B spend their spare time in an unexposed area rather than at home suggests that they have intuitively learned to avoid exposure.

Misperception of insomnia, however, is a known problem. In order to study the effect of misperception and to distinguish between purely psychological and a biological mechanism a health diary survey was done, during which electromagnetic exposure was modified experimentally.

6.3 Sleep disorders in the Schwarzenburg health diary surveys

6.3.1 Methodology

Design:

During the period between the 17th of August and the 30th of September 1992, a subsample of the study population of the health interview survey were asked to keep symptom and quality diary during three periods of 10 days. 200 persons in Zone A and 50 in Zone C were sampled at random and contacted. At the end of each 10-day period, the diaries were collected and new ones distributed. During this time, the Transmitter operated partly according to its regular transmission schedule, partly according to an experimental transmission schedule. Since shortwave transmissions reach their target by means of reflection from the ionosphere, there are two directions to reach it: one along the direct way, the alternative being by means of turning the beam by 180^0. This change of the beam's direction was made at random by Telecom. It was completely unknown to the exposed population and to the researchers. It should be pointed out that under the conditions of this design, it was impossible to check the Transmitter's functioning by means of a radio receiver.

The diary included standardized sections for difficulties in falling asleep, awakening during the night, nervosity and restlessness, vertigo, general illness, depressive mood, headache, back pain, joint and limb pains, difficulties in breathing, heart related complaints, digestive complaints, nose related complaints and others. In this section of the diary, the participants could also enter additional complaints.

Statistical analysis:

In order to answer the question of agreement of diary entries and self-reported insomnias the mean event rates per night were calculated. Since part of the population were farmers, we defined as 'night' when the event 'insomnia present' was recorded; this happened between 7 p.m. and 7 a.m. Since insomnias are defined as disorders characterized by difficulties in falling asleep and in maintaining sleep, we defined a person who responded to the two questions about insomnia with a score bigger than a fixed value a with $a \in \{1, ..., 4\}$ as an insomniac. Based on this, response-operator curves and their area under the curve as well as the maximum chance adjusted agreement κ were estimate.

In a first step we tried to analyze the diary with its binary outcome by means of a person-by- person logistic regression. This was a tremendous task and yielded no consistent or interpretable results.

Very recently a new method of generalized estimating equations [Liang and Zeger (1986)], has become available in form of a source code [V. Carey et al. (1994)]. This theory allows to adjust logistic regression for intraperson correlation even in the case of a binary outcome. This method allows also to adjust the association between exposure and symptom for its occurrence in the unexposed zone, which was not feasible by means of the person-by-person logistic regression. Since age was the only significant confounder in the health interview survey, only this variable was included in the models. As the source code of V. Carey allowed to estimate only two consecutive nights of a single 10-day diary, we had to assume that the effect was homogeneously distributed over the 3×10-day diary survey periods and that the regression estimates from the resulting 15 models were independent. To our judgement, this assumption was of minor importance. On the latter assumption, the 15 estimates can be combined by means of a weighted average. According to the theory of generalized estimating equations the regression estimates are distributed normally in the case of $n > 60$. In our data this requirement is fulfilled. According to Olkim (1994) mean and standard error for independent gaussian random variables $\beta, N \sim (\beta, se)$, are calculated as follows:

$$\widehat{\beta} = \sum_{i=1}^{n} a_i \cdot \beta_i$$
$$se = \sum_{i=1}^{n} a_i^2 \cdot se_i^2 \ ,$$

where a_i are the weights. This kind of weighting

$$a_i = \frac{se_i^{-2}}{\sum_{i=1}^{n} se_i^{-2}}$$

is turned out to be the most efficient way of combining evidence (Hedges and Olkim (1993)).

6.3.2 Results

In Zone A 88 persons received a diary for at least one ten-day period. The diaries of 70 persons could be included into the analyses. In Zone B, 101 persons received a diary for at least one period and the diaries of 70 persons could be included into the final analysis. In Zone C, 71 persons received at least one diary; the diaries of 47 persons could be analyzed. Participation is summarized in Table 6.2

Reliability of self-reported insomnias:

In table 6.3., AUC represents the unadjusted coefficient of agreement, k the chance-adjusted agreement. Self-reported insomnias and entries in the diary agree, although to a low degree. The relatively low degree of agreement is within the concept of misperception of insomnias. The chance-adjusted agreement between self-reporting in the interview survey and the diary survey ranged between 0.28 and 0.40 (see Table 6.3)..

Zone	Period 1		Period 2		Period 3	
	distributed	received	distributed	received	distributed	received
A	86	67 (77.9%)	78	61 (78.2%)	67	36 (53.7%)
B	97	66 (68.0%)	86	64 (74.4%)	77	63 (81.8%)
C	53	33 (62.3%)	50	46 (92.0%)	50	44 (88.0%)

Table 6.2: *Participation of the health diary survey*

Score	AUC	κ
Falling asleep (1)		
>1	0.676	0.28
>2	0.669	0.35
>3	0.675	0.22
>4	0.761	0.35
Maintaining sleep (2)		
>1	0.682	0.403
>2	0.681	0.340
>3	0.751	0.380
>4	0.764	0.420
(1) or (2)		
>1	0.730	0.403
>2	0.687	0.376
>3	0.704	0.386
>4	0.728	0.375

Table 6.3: *Reliability of self-reported insomnia*

Diary:

Since, due to limited capacity of the available hardware, the association between insomnia during the night and the EMF exposure could not be estimated in a single step, we decided to split the diary into 15 strata: we combined two consecutive nights. The results are summarized in Table 6.4. β represents the logistic regression parameter for exposure in the general estimating equations model and *se* its standard error. The risk of suffering from insomnia is more or less homogeneously distributed over the 30 nights. From night 23 to 28 the association estimate changes sign. This behavior cannot be explained. Data management errors could be excluded. It is of interest that during the last 10-day period the self-reporting habits changed: during the first two diary periods the persons reported insomnia from 7 p.m. to 7 a.m.. During the last 10-day period insomnia was reported from 6 p.m. to 6 a.m.. The pooled estimate β indicates a significant increasing risk of insomnia with increasing exposure. The effect, however, is small. On the average, the risk of insomnia was increased with a pooled odds ratio of 1.22.

Night	β	se
1 and 2	0.0176	0.157
3 and 4	0.1653	0.137
5 and 6	0.0138	0.137
7 and 8	0.2062	0.123
9 and 10	0.1878	0.130
11 and 12	0.6783	0.197
13 and 14	0.0955	0.221
15 and 16	0.0103	0.220
17 and 18	0.1865	0.200
19 and 20	0.7816	0.272
21 and 22	0.2250	0.166
23 and 24	-0.1588	0.192
25 and 26	-0.7059	0.228
27 and 28	-0.1991	0.182
29 and 30	0.2897	0.168
pooled	0.1222	0.044

Table 6.4: *Association between self-reported insomnia and EMF-exposure*

An increase of exposure from 1 mA/m to 10 mA/m is associated with an increased occurrence of insomnia of about 1.13 (95% C.I. $1.04 < OR < 1.23$).

6.3.3 Discussion and Conclusion

Self-reported insomnia is associated in time and space with the Transmitter's electromagnetic field. Self-reporting of sleep disorders, however, is not very reliable since misperception of the disorder leads to under- as well as overreporting.

The effect of EMF if really present, however, is not very strong (OR and 95% C.I.: $1.04 < 1.13 < 1.23$) and the effect is not really homogeneously distributed over the 3×10-day periods. Therefore, the pooled estimate has to be interpreted with caution, but it is actually the best estimate that is available.

As maximum exposure for the most exposed person was in the range of 25 - 30 mA/m, an odds ratio of about 1.5 for insomnia can be calculated. This would represent a significant increase of sleep disorders, but be less than needed to explain the frequency differences in sleep disorders found in the health interview study.

If we combine the evidence of spatial and time association and the habit to avoid the exposure zone during spare time, a biological mechanism between insomnia and EMF-exposure is to be suspected. A potential mechanism would be reaction of melatonin to EMF-exposure, as studied in Chapter 7.

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Chapter 7

Melatonin excretion and sleep disorders in the surroundings of a shortwave radio transmitter

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Abstract:

*Objective:*The purpose of the present study was to find a biophysical mechanism between sleep disorders and the electromagnetic fields of the Shortwave Radio Transmitter Schwarzenburg.
Design: Longitudinal study with follow-up of exposed and unexposed volunteers with and without sleep disorders. During 10 days with an experimental period of 3 days with the Transmitter turned off the volunteers completed a diary on awakening during the night and delivered their early morning urine for 6-Hydroxy-Melatonin-Sulfate (6OHMS) testing. The volunteers were not informed about the turning off of the Transmissions.
Results: Sixty-five volunteers aged between 16 and 83, 38 men and 27 women, accepted the study conditions. No association could be found between 6-OHMS levels and exposure. No variation of 6OHMS between days revealed an evidence of a reaction of 6OHMS levels on the experimental transmission scheme. However, we observed an exposure-dependent decrease of awakenings during the second night the broadcasting had been interrupted.
Conclusions: We conclude that sleep disorders in the surroundings of the Shortwave Radio Transmitter cannot be explained by the melatonin hypothesis. Results suggest recovery of sleep disturbance after experimental

cessation of exposure but neither a psychological effect nor a biophysical mechanism of the Transmitter can be positively demonstrated or excluded.

Keywords: repeated measurements, generalized estimating equations, sleep disorders, melatonin, early morning urine, epidemiological trial.

7.1 Introduction

As shown in the first phase of this study the population living in the surroundings of the Shortwave Transmitter Schwarzenburg suffers from sleep disorders. In order to find a plausible mechanism for a possible biophysical interaction between the Transmitter and the humans living in its vicinity we reviewed the literature.

7.1.1 Literature Review

Physiology of Melatonin: Most animals and healthy humans can distinguish between dark and light. The human photoreceptors are located in the eye. A photoreceptor is characterized by the ability to transform electromagnetic fields into a neural signal. The neuronal track to the cerebral cortex is well known. However, there are additional tracks to the interbrain and the middle brain. One of them guarantees the communication between the eye and the pineal gland which is located at the top of the interbrain and which served in early evolution of vertebrates as the third eye. One of the substances produced by the pineal gland is melatonin. Its production is suppressed by light.

There is some evidence that melatonin may be effective in regulating circardian rhythmicity in vertebrates [Underwood and Goldman (1987)]. Although in rodents pinealectomy does not affect the period of free running circadian rhythms [Cheung and McCormack (1982)], melatonin affects the period of the rate of re-entrainment following phase shifts of the light cycle and is effective in synchronizing disturbed cicardian rhythms in mammals, including man [Arendt et al. (1986), Cassone et al. (1986)]. The reason why pinealectomy has only a small or no influence at all on the period of free running circadian rhythms in rodents might be that melatonin is produced not only in the pineal gland but also in the retina and the gut.

The synchronizing effects of melatonin on disturbed circadian rhythms in mammals appear to be a consequence of these substances' action within the hypothalamus. Lesions of the rat suprachiasmatic nucleus, a neuronal center in the hypothalamus (part of the interbrain), prevents the entraining effects of melatonin [Cassone et al. (1986), Cassone (1990), Redman and Amstrong (1988)], it is therefore suggested that this effect is not explained by a neuronal signal but by the release of melatonin to the blood. A substance released into the blood and acting anywhere else in the body is called a

hormone.

The ability of melatonin to reset circadian systems and regulate neuroendocrine functions, e.g. the reproductive axis and interaction with dopamine, attracted the scientific interest in its use in the therapy and pathophysiology of jet lag [Arendt et al. (1986),], shift-work scheduling [Folkard et al. (1993)], sleep/wake cycle disturbances [Tomoda et al. (1994), Haimov et al. (1994), Jan et al. (1994), Suzuki et al. (1993), Armstrong et al. (1993), Laakso et al. (1993), Klein et al. (1993), Weissbluth and Weissbluth (1992), Tzischinsky et al. (1992), Sack et al. (1991), Dahlitz et al. (1991), Folkard et al. (1990)] and seasonal affective disorder [Kay (1994), Schmittbiel et al. (1994), Darcourt et al. (1992), Hawkins (1992), Levitt et al. (1991), Sandyk et al. (1991), McIntyre et al. (1990), Hallonquist et al. (1986)] as well as its reaction to electromagnetic fields in general. Due to its potential interactions with the immune response as well as its potential cytostatic effect, it has been widely investigated and its potential benefit disputed in oncology [Lissoni et al. (1994), Loomis et al. (1994), Liburdy et al. (1993), Sankila et al. (1993), Stevens (1993), Bartsch et al. (1992), Gonzalez et al. (1991), Vena et al. (1991), Kerenyi et al. (1990), Lissoni et al. (1990), Wetterberg et al. (1986)]. As there is some evidence that electromagnetic fields might be associated with increased risks of cancer it was suggested that the potential reaction of melatonin to electromagnetic fields might explain the biophysical interaction.

EMF and Melatonin:

The interaction between electromagnetic fields and melatonin levels seems to have not only an adverse but also a therapeutic effect.

As *adverse effects* of EMF, possibly associated with their biophysical interaction, brain tumors, breast cancer in males and femals and depression have been suggested. In various animal models these effects had been studied as well, but studies that show these effects have been disputed and questioned [Hughes (1994), Loscher (1994), Taubes et al. (1994), Beniashvili et al. (1993), Savitz et al. (1993), Stevens et al. (1993a, 1993b), Tynes et al. (1993), Wood (1993), Stevens et al. (1992), Vena et al. (1991), Wilson (1988)].

In various papers Sandyk suggests a *therapeutic effect* of electromagnetic fields mediated by the biophysical interaction of EMF and melatonin for the treatment of Parkinson's disease, Alzheimer's disease, chronic progressive multiple sclerosis, respiratory dyskinesia, migraine, cluster headache and seasonal affective illness [Sandyk and Sandyk et al. (1991 - 1994)].

In *conclusion,* it is widely accepted that melatonin is influenced by electromagnetic fields, provided these are strong enough. The impact on human health of these interactions, however, may not only be adverse but also therapeutic.

General Epidemiology of Melatonin:

The study of melatonin is a rather new field, and a little information about its epidemiology is available. The laboratory techniques are not yet standardized. It is known that the results vary from kit to kit (the laboratory sampling unit, a microtiter plate). They also vary strongly between individuals. Every person has his or her own overall mean level and rhythm; this rhythm, however, is assumed to be constant in every individual. Most investigations tend to test blood for melatonin levels, which is inconvenient in epidemiological trials. Bartsch et al. (1992) and the study by Wilson et al. suggest that early morning urine sampling and testing of 6OHMS are a good surrogate for melatonin levels.

Bojkowski and Arendt (1990) however studied the factors which influenced 6OHMS excretion in 130 normal volunteers by means of a direct radioimmunoassay in urines sampled during 24 hours. They investigated men and women within the age range of 2 to 80 years. In the group of young people (2-20 years) they found a decreasing 6OHMS excretion after body weight correction. In adults they observed the same decrease but weight correction seemed not to be necessary. The following variables had no influence on 6OHMS in that age group: body weight, body height, sex or presence of pineal calcification.

7.1.2 Study Question

The literature review and the data of Study I suggest that sleep disorders might be induced by the shortwave electromagnetic field of the Transmitter, and that this could be through influence of the pineal gland. We tested the following hypothetical path model:

If this model were accurate, sleep disorders should be more strongly associated with melatonin levels than with EMF-exposure.

We therefore tested the hypothesis that EMFs cause a depression in melatonin levels which in turn causes sleep disorders. If this model were accurate the sleep disorders should occur conditionally independently of exposure, provided the melatonin level was known.

Chronic effect

As light suppresses melatonin production it was suggested that chronic EMF exposure could lead to a suppression of the mean melatonin production. If this hypothesis were correct, a negative gradient of melatonin or its main metabolites between Zones A, B and C would be expected.

Acute effect

B. Wilson and others showed that low-frequency electromagnetic fields could influence melatonin levels, especially because after a period of 3 days of non-exposure a rise of melatonin levels was observed on the 4th day. We therefore tried to answer the question whether a 3-day period with the Transmitter switched off would be followed by a rise of melatonin levels.

7.2 Methodology

7.2.1 Design

The basic idea was to study a sample of 60 persons, 30 with and 30 without sleep disorders from three exposure Zones A, B and C during 10 days (for more details on the exposure classification see Chapter 3). Within this 10-day period, the Transmitter was switched off for 3 consecutive days, about which the study participants were not informed. During this period the volunteers kept a diary on sleep quality and delivered their early morning urine for the determination of 6-Hydroxy-Melatonin-Sulfate (6OHMS), a metabolite of melatonin.

We defined an early morning urine as the first urine sample taken after 4.00 a.m. This was necessary because most of the potential volunteers were farmers who usually get up early. The maximum delay for early morning urine was 8 a.m.

We assumed that 6OHMS levels in early morning urine, after adjustment by urinary creatinine, reflect the amount of melatonin production during the night.

Data collection started on the 5th of September, 1993. The last early morning urine was delivered on the 15th of September.

Sampling:

The database of Study I (Interview 1992) was stratified according to the three zones and presence or absence of sleep disorder. This resulted in six strata. Every stratum was sorted according to sex and age. A systematic random sample of 10 persons in each strata was taken as follows:

- An integer random number was generated defining the starting index number S in the stratum.
- Starting from S , every following index number with a constant index distance was selected until a sample of 10 was reached.
- The 10 sampled persons from Study I were contacted.

- When a person did not agree to the study conditions or could not be contacted, the sampling procedure was continued.

In total 102 persons were contacted out of whom 65 agreed to the study conditions. The distribution of sleep disorders from Study I is shown in Table 7.1. It is obviously difficult to obtain a balanced sample. In Zone C only 12 persons suffered from sleep disorders.

	Sleep disorder		
Zone	no	yes	Total
A	71	34	105
B	91	28	119
C	168	12	180
Total	330	74	404

Table 7.1: *Distribution of sleep disorders in interview survey Study I*

The number of contacted persons is shown in Table 7.2. It was problematic to get in touch with the participants, because the summer holidays were almost over and in some parts of the municipality of Wahlern (Schwarzenburg) the autumn holidays were overlapping with the experimental period.

	Sleep disorder		
Zone	no	yes	Total
A	24	18	42
B	19	15	34
C	15	11	26
Total	58	44	102

Table 7.2: Distribution of contacted persons

Participation and participation rates are summarized in Table 7.3. The final response rate was low. Contacted persons, especially in Zone *A*, were hard to convince of the need to participate. Some of them showed strong negative feelings. They believed that the only result of their petition was their filling-in forms, keeping diaries etc., which would bring about hardly any change.

EMF-exposure measurements and transmission scheme:

The survey started on the 5th of September, 1993, at 7.30 p.m. with the Transmitter following its regular broadcasting scheme. On the 7th of September at 11.00 a.m. the Transmitter was switched off, on the 10th of September, it went back into operation. The data collection ended on the morning of the 15th of September. During the 3-day period with the Transmitter off, background exposure measurements were carried out.

	Sleep disorder		
Zone	no	yes	Total
A	12/24 (50%)	12/18 (66%)	24/42 (57%)
B	12/19 (63%)	9/15 (60%)	21/34 (62%)
C	12/15 (80%)	8/11 (73%)	20/26 (77%)
Total	36/58 (62%)	29/44 (66%)	65/102 (64%)

Table 7.3: *Participation (perons participating /persons contacted)*

Unlike in Study I, exposure measurements were done in the bedrooms of the participants from Zones A and B. Only at two locations of Zone C indoor measurements were carried out. As for the other sites of Zone C, the background exposure measurements done during the off-period of the Transmitter were inserted.

Due to limitations of resources, it was not possible to measure all 65 sites simultaneously in the course of the 10 days. We therefore decided to measure the indoor exposure during the following 6 weeks. In principle the exposure should be measured with no persons at the measuring site. As this proved impossible we had to carry out the measurements with the volunteers sleeping in their beds. The measurements results uncertainty estimates of exposure assessment are summarized in Chapter 4.

Laboratory Test:

6OHMS was measured by an enzyme-linked-immuno-absorbent-assay (ELISA) developed by Repromed Hamburg. The melatoninsulfate ELISA-kit allows the quantitative measurement of 6OHMS in urine. The test is done on a plate with 96 respectively 92 wholes (microtiter plate) depending on the laboratory equipment. The assay procedure follows the basic principle of a competitive ELISA:

6OHMS adsorbed to the microtiter plates and 6OHMS in the sample compete for a fixed number of antibody-binding sites. The amount of complexes bound to the microtiter plate is inversely proportional to the concentration in the sample. After 16 - 24 hours of incubation, the non-fixed antigen-antibody complexes are removed by means of washing-out and the amount of fixed antibodies is determined by use of goat-anti-rabbit antibodies conjugated to peroxidase as a marker and tetramethylbenzidine (TMB) as a substrate. Quantification is achieved by comparing the enzyme activity of the sample under investigation with a standard response curve. The standards used are 6OHMS probes of $0.000, 0.010, 0.038, 0.135, 0.465, 1.650, 5.700$ and 20.000 ng/ml. For each assay these standards are measured twice. In addition, two internal quality controls are measured at least twice. If these controls are not within the limits of a tolerance range specified by the manufacturer Repromed, the results of the test set should be rejected. The

intra-assay variation is about 10%. The inter-assay variation is not specified but assumed to be larger than 10%.

It is impossible, on one microtiter plate with 96 respectively 92 holes, to measure 650 urine samples twice, which results in 1300 tests. In addition, not all wholes are available for the testing of the urine samples because the standard curve and the internal controls have to be determined by means of the same test set.

The price of the testing depends on the number of microtiter plates tested rather than on the number of samples. The options taken into consideration for testing 1300 samples were the following :

1. *Random assignment:* The urine samples of one person are assigned randomly to a microtiter plate and repeated on the same microtiter plate. This option allow for 36 respectively 38 samples to be determined simultaneously. 19 microtiter plates must be tested.

2. *Fixed day assignment:* All samples from the same day are tested on the same microtiter plate and repeated on another one. This assignment requires at least 20 microtiter plates.

3. *Fixed person assignment:* The 10 samples from the same person including the repetitions are measured on the same microtiter plate. This assignment requires 22 microtiter plates to be tested.

4. *Fixed day and person assignment:* The 10 samples of the same person are assigned to one microtiter plate, the replications to another one. This allows to test 7 persons on the same microtiter plate. When these 7 persons are uniformly distributed over the exposure range acute and chronic effects should be detectable. This assignment requires 19 microtiter plates.

The urine samples were stored at -20^0C as soon as they were picked up by our team. The samples were blinded, to the laboratory staff. The 6OHMS was improved and evaluated by U. Rogger and Prof. Dr. med. H. Gerber at the Central Chemical Laboratory of the University Hospital of Bern (Inselspital), Switzerland; creatinine was measured at the same laboratory. The definite 6OHMS tests were done at Repromed, Hamburg, according to the fixed day and person assignment as well as according to the fixed day assignment for the first 2 days.

7.2.2 Statistical analysis

Chronic effects:

In order to evaluate a possible chronic effect, the mean of the first two days of 6OHMS ($\log_{10}$ transformed) as an outcome was modelled by exposure (H-field [mA/m], $\log_{10}$ transformed), age, creatinine (mean of the first two days of the experiment after $\log_{10}$-transformation, after age correction) and insomnia (coded $present = 1$, $absent = -1$). Hypothesis testing was done on assumption of independent, identically distributed gaussian errors [Draper and Smith (1987)]

Acute effects:

In a first step mean profiles and their 95% confidence limits were calculated and plotted, which allowed an exploratory graphical data analysis. For this purpose the study population was classified according to the average exposure during the night into tertiles. This led to the definitions of low exposure (H-field: 0.073 - 0.33 mA/m), medium exposure (H-field: 0.33 - 1.5 mA/m) and high exposure (H-field: 1.5 - 7.0 mA/m).

We then tried to estimate a possible acute effect using generalized estimating equations [Liang and Zeger (1986)]. The continuous outcome $\log_{10}-6$OHMS was modelled by $\log_{10}-H-field$ exposure, $\log_{10}$ creatinine age-adjusted, age, self-reported sleep disorders, a day factor and the interaction between this day factor and the exposure. In order to account for the intraperson correlation we estimated our models while including an 'exchangeable' correlation structure for the errors. This means that the measurements from the first to the last day of the same person are assumed to be constant. This model is often termed a 'random effect' model [Diggle et al. (1994)]. The programming was done by means of Splus Version 3.2 under Windows 3.1 using the S code available from Statlib.

The association between awakenings during the night as outcome and melatonin levels as predictor was tested by means of the extension of generalized estimating equations for binary outcomes [Liang and Zeger (1986)] and programmed by V. Carey et al. (1993). The code was obtained from the public domain library Statlib.

7.3 Results

Sixty-five volunteers (38 men and 27 women) accepted the study requirements. They were between 16 and 83 years old. The exposure ranged between 0.073 and 7.00 mA/m (average during the night). For the purpose

of plotting the exposure was classified as low (H-field: 0.073-0.33 mA/m), medium (0.33 - 1.50 mA/m) and high exposure (1.5 - 7.00 mA/m).

7.3.1 Chronic effects

We could not show a chronic effect of EMF on 6OHMS levels - neither by the analysis of the 2-day averaging of the laboratory testing according to the *fixed day assignment option* nor by the *fixed person and day assignment option* (see Table 7.4). Creatinine ($Crea_{korr}$, $\log_{10}$-transformed) was age-adjusted. Age and creatinine as explanatory variables explained the 6OHMS

	fixed person and day assign.			*fixed day assign.*		
Variable	*Estimate* β	*se*	*p*	β	*se*	*p*
$H-field$	-0.035	0.043	0.43	<-0.001	0.039	1.00
$Crea_{korr}$	1.190	0.165	<0.01	1.055	0.148	<0.01
age	-0.757	0.185	<0.01	-0.902	0.157	<0.01
Sleep dis.	-0.038	0.033	0.265	-0.044	0.029	0.14

Table 7.4: Regression model with 6OHMS as outcome (see text for variable definition)

levels. Exposure ($H-field$ [$\log_{10}$(mA/m)]) and sleep disorder had no predictive value. The regression model could account for 56% respectively 62% of the variability of 6OHMS levels.

7.3.2 Acute effect

In a first step we performed an exploratory graphical analysis by estimating the group mean profiles and the pointwise 95% confidence limits. For this purpose the population was divided into six strata according to three exposure levels and self-reported sleep disorders. The mean profiles and pointwise 95% C.I. are shown in Figure 7.1. The plots suggested that the melatonin levels were constant during the time course and that there were no significant differences between the six groups. This hypothesis was confirmed by modelling the outcome 6OHMS ($\log_{10}$-transformation) with generalized estimating equations as follows:

1. age ($\log_{10}$-transformed, noted *age*)
2. age-adjusted creatinine levels (noted $Crea_{korr}$): calculated by estimating the residuals of the linear regression of age on creatinine (both $\log_{10}$-transformed, by the gaussian error extension of generalized estimating equations on the assumption of an 'exchangeable' error correlation structure).
3. exposure (H-field [mA/m], $\log_{10}$-transformed, noted $H-field$).

4. a factor with nine levels for the 10 days (noted *day* 1, ..., 9)

5. an interaction term between exposure and the factor *day* (noted *inter.*1, ..., 9).

6. a factor with two levels for disturbances of sleeping through the night (noted *sleep dis.*, absent=-1, present=1)

7. on the assumption of an 'exchangeable' error correlation structure (random effect model).

The results of this analysis are summarized in Table 7.5.

Variable	*Estimate* β	*se*	*z*
age	-0.610	0.144	-4.23
$Crea_{korr}$	1.030	0.063	16.30
$H - field$	0.001	0.034	0.04
day 1	-0.017	0.017	-1.03
day 2	0.008	0.010	0.81
day 3	-0.011	0.010	-1.21
day 4	0.006	0.007	0.95
day 5	0.001	0.005	0.24
day 6	-0.004	0.003	-1.12
day 7	-0.003	0.004	-0.68
day 8	0.001	0.003	0.36
day 9	0.001	0.003	0.30
inter. 1	-0.025	0.022	-1.16
inter. 2	-0.007	0.012	-0.62
inter. 3	0.0211	0.020	1.07
inter. 4	0.009	0.007	1.19
inter. 5	-0.003	0.006	-0.50
inter. 6	-0.001	0.005	-0.22
inter. 7	-0.002	0.003	-0.50
inter. 8	0.001	0.003	0.37
inter. 9	0.003	0.003	0.99
sleep. dis.	-0.095	0.060	-1.73
ρ	0.507		

Table 7.5: Regression model with 6OHMS as outcome (see text for variable definition)

None of the contrasts between days and none of the interaction terms between days and exposure reached a $|z|$−value larger than 1.96 which would correspond asymptotically to a level of significance of 5%. It has to be stressed that in generalized estimating equations hypothesis testing is still under development and the distributional theory about the $z-$ or $t-$values of the models is still under investigation. The slope parameters,

however, for the corrected creatinine and age could be properly identified ($\beta_{crea} = 1.03$, $se = 0.063$; $\beta_{age} = -0.609$, $se = 0.144$). The $z-$values were 16.3 and 4.22 respectively. The slope parameter for $Crea_{korr}$ $\beta_{crea} = 1$ indicated that 6OHMS and creatinine were principally measured in the same dimension e.g. pg/ml and μmol/l, respectively. Doubling one's age leads to a decrease of one's melatonin excretion by 2/3. The association between chronic sleep disorders and 6OHMS levels in urine did not marginally reach a statistical significance ($\beta = -0.095$, $se = 0.055$). The $z-$value was -1.73. Persons suffering from sleep disorders had 20% lower melatonin levels than the healthy volunteers. The chronic effect of the electromagnetic fields tended to zero ($\beta = 0.001$, $se = 0.035$). We performed similar analyses after changing the coding for the days as well as after age stratification, which did not influence the result materially.

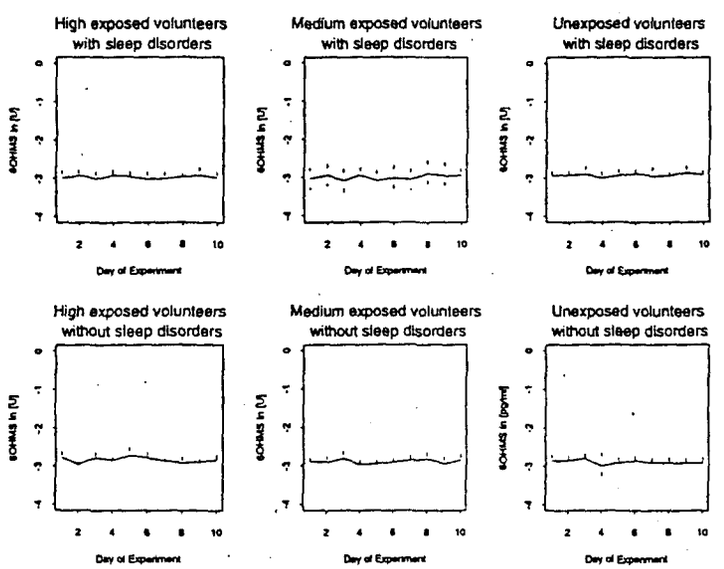

Figure 7.1: *Mean profiles of 6-OHMS after creatinine correction. The Transmitter was switched off between day 3 and 5 of the trial.*

7.3.3 Sleep disorders and 6OHMS

One part of the hypothesis was that if we knew the early morning urine level of 6OHMS after adjustment for creatinine, we could predict whether a person slept well during the night or not. We tested this hypothesis by means of a logistic regression model on the assumption of a common intraperson correlation, so-called exchangeable error correlation structure. The model was computed by means of generalized estimating equation. It

turned out that awakenings during night could not be predicted by 6OHMS [U] ($\beta = -0.102$, $se = 0.243$). There was, however, a trend suggesting that an increased urine melatonin level is associated with a higher probability of sleeping well during the night ($OR = 0.903$, 95% $C.I.$ 0.688; 1.455).

7.3.4 Sleep Diary and Electromagnetic Fields

Since the path model which tried to explain the sleep disorders by means of the 6OHMS levels did not fit, we tried to answer the question whether the experimental transmission scheme influenced the frequency of self-reported awakenings during the night. We tested this hypothesis by means of the generalized estimating equations' extension of logistic regression. The model was similar to the one fitted to the melatonin levels excluding creatinine as a predictor. The turning-off the transmitter was followed by a general decrease in awakenings during the night with a time lag of one day. This pattern was superimposed on an access decrease in awakenings during the night which could be associated with the exposure. The observed effect was biggest on the 2nd day with the Transmitter turned off. The cessation of an exposure of 1 mA/m to normal background of approximately 0.1 mA/m was associated with a decreased relative risk of awakening during the night on that day by 1/3 approximated by the Odd's ratio ($\beta = -1.09$, $se = 0.464$). Doubling the age increased the chance to wake up during the night 3 times ($\beta = 3.75$, $se = 1.20$). The information from Study I that a person suffered frequently from sleep disorders, was associated with a 3 times increased chance of awakening during the night ($\beta = 1.10$, $se = 0.447$). The chronic effect of EMF-exposure on awakening during the night did not reach statistical significance ($\beta = 0.736$, $se = 0.400$). Increasing the chronic exposure level from 0.1 mA/m to 1 mA/m leads to an odd's ratio of awakening of $OR = 2.1$ [95% $C.I.$ 0.951; 4.57]. Table 7.6 shows the wake-up frequencies by exposure zones and days. This simple crosstabulation neglects the fact that observations within one person are correlated and it is not sensitive enough to show the differences between zones one the 4th day of experiment.

7.4 Discussion and conclusions

The purpose of the present study was to find a biophysical mechanism between sleep disorders and electromagnetic fields generated by the Shortwave Transmitter Schwarzenburg. Especially the work of Haimov et al. (1994) suggested an association between sleep disorders and melatonin levels.

We failed to confirm such an association. Persons reporting sleep disorders, however, tend to have lower melatonin levels. Furthermore we failed to show an association between chronic exposure to EMF and could neither demonstrate a reaction of 6OHMS levels to an experimental transmission

Day	Low Exposure	Medium Exposure	High Exposure
1	13/25 (52%)	12/15 (80%)	17/22 (77%)
2	16/25 (64%)	11/15 (73%)	17/23 (74%)
3	14/25 (56%)	10/16 (63%)	20/24 (83%)
4*	16/25 (64%)	11/16 (69%)	13/24 (54%)
5	9/25 (36%)	8/16 (50%)	15/24 (63%)
6	10/25 (40%)	11/16 (69%)	16/24 (67%)
7	9/25 (36%)	6/16 (38%)	15/24 (63%)
8	15/25 (60%)	11/16 (69%)	14/24 (58%)
9	12/25 (48%)	8/16 (50%)	16/24 (67%)
10	13/24 (54%)	11/16 (69%)	14/24 (58%)

Table 7.6: *Wake-up frequencies during the night by exposure zones and days. No significant differences are obvious. This model, however, does not account for the intraperson correlation. The differences on the fourth day of experiment can only be shown by generalized estimating equations which in turn take into consideration the fact that observations within one person are correlated.*

scheme. This finding is not surprising when it is assumed that IRPA exposure limits are well chosen, because those were not exceeded on any site. The result is consistent with our finding in the study on dairy cows, which also failed to show an association between 6OHMS levels and EMF exposure in the frequency and intensity range of the Schwarzenburg Radio Transmitter. It also corresponds to normal prevalences of cancer in the area although this finding has to be considered with caution since the total number of persons living in the vicinity to the Transmitter is rather low.

A chronic effect of EMF on 6OHMS in morning urine could not be established. Since, as known from the literature, 6OHMS levels are correlated with creatinine and age, we consider the absence of such an effect as a reliable result.

The analysis of the diary survey of 1993, however, suggested that awakening during the night is associated with a modified transmission scheme, that persons suffering from sleep disorders according to a health interview conducted a year previously, awake more frequently than persons considering themselves healthy and that aged persons report awakening during the night more frequently. These findings support the findings of Study I.

The missing link between EMF-exposure and sleep disorders should be treated with caution. We considered the following possible explanations:

1. The use of early morning urine and its operational definition may have led to an increased variability in the outcome variable 6OHMS, which we could not control sufficiently by means of creatinine measurement. This is in fact one of the limitations of an epidemiological study.