SECTION 13

ELF MF – Melatonin Production – Alzheimer's Disease and Breast Cancer

2012 Updated Chapter

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Prepared for the BioInitiative Working Group

November 2012

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SECTION 1: UPDATE INTRODUCTION

It has been over 5 years since the publication of the initial BioInitiative in 2007. During that time the BioInitiative web site has been accessed by a considerable number of individuals worldwide: ***(Provide viewing figures.)*** Unfortunately, "pro-industry" representatives from industry itself, from government, and from academia have continued their campaign, despite all evidence to the contrary, against any possible serious ill effects of exposure to extremely low frequency (ELF) magnetic fields (MF) at levels experienced in occupational and residential settings. These pro-industry representatives simply argue that the evidence is insufficient because some epidemiologic studies are negative and some are positive and that there are no biologically confirmed causal pathways. As we showed in the earlier 2007 original BioInitiative publications, the negative studies have serious flaws while the positive studies do not have such flaws. In addition, we discussed two biological pathways related to Alzheimer's disease and breast cancer, which have plausibility based on scientific studies. A third suggested pathway is discussed in this update.

In this chapter update, we provide the following:

1. descriptions and evaluations of newly published epidemiologic studies relating occupational ELF MF exposure to the risk of (a) Alzheimer's disease (AD) and/or dementia, (b) breast cancer;
2. updates related to the three proposed or suggested pathways from ELF MF exposure to AD or dementia:
    a. increased peripheral and brain production of amyloid beta;
    b. decreased production of melatonin; and
    c. ELF MFs may cause chromosome instability, resulting in chromosome segregation errors and increased mutational loads;
3. a discussion of the potential increase in cellular production of amyloid beta (associated with the risk of AD) due to low melatonin production;
4. an update of the relationship between low melatonin production and the risk of breast cancer;

STRUCTURE OF THE UPDATED REPORT

New material is incorporated into the body of the Report. New and revised text and table additions are presented with a red text color.

EXECUTIVE SUMMARY

Melatonin Production

Melatonin is a hormone produced primarily by the pineal gland, located in the center of the brain. Melatonin is evolutionarily conserved and is found in nearly all organisms. It has numerous properties which indicate that it helps prevent both Alzheimer's disease and breast cancer. There is strong evidence from epidemiologic studies that high (≥ 10 milligauss or mG)* that long-term exposure to extremely low frequency (ELF, ≤ 60 Hz) magnetic fields (MF) is associated with a decrease in melatonin production(Section II.)

Alzheimer's Disease

Amyloid beta (Aβ) protein is generally considered the primary neurotoxic agent causally associated with Alzheimer's disease (AD). Aβ is produced by both brain and peripheral cells and can pass through the blood brain barrier.

1. There is longitudinal epidemiologic evidence that high peripheral blood levels of Aβ, particularly $A\beta_{1-42}$, is a risk factor for Alzheimer's disease (AD). (Section III.A.)
2. There is epidemiologic evidence that extremely low frequency (ELF, 50-60 Hz) magnetic field (MF) exposure up-regulates peripheral blood levels of Aβ. (Section III.A.)
3. There is evidence that melatonin can inhibit the development of AD and, thus, low melatonin may increase the risk of AD (Section III.B.)
4. There is strong epidemiologic evidence that significant (i.e., high), occupational ELF MF exposure can lead to the down-regulation of melatonin production. The precise components of the magnetic fields causing this down-regulation are unknown. Other factors which may influence the relationship between ELF MF exposure and melatonin production are unknown, but certain medications may play a role. (Section II.)
5. There is strong epidemiologic evidence that high occupational ELF MF exposure is a risk factor for AD, based on case-control studies which used expert diagnoses and a restrictive classification of ELF MF exposure. (Section III.C.)
6. There are no epidemiologic studies of AD and radiofrequency MF exposure, only one epidemiology study of non-acute radiofrequency MF exposure and melatonin. There are studies of "AD mice" and radiofrequency exposure (Sections III.D and II.) So, no conclusions concerning health consequences due to exposure are currently possible.

Breast Cancer

The only biological hypothesis which has been epidemiologically investigated to explain the relationship between ELF MF exposure and breast cancer is that high* ELF MF exposure can lower melatonin production, which in turn can lead to changes in the various biological systems which melatonin influences, including increased estrogen production and subsequent deleterious interactions with DNA, decreased antiproliferative activities, increased oxidative DNA damage, and immune response capabilities. Thus lowered melatonin production can be expected to lead to increased risk of breast cancer.

1. *In vitro* and animal studies have demonstrated that (i) melatonin is a potent scavenger of oxygen and nitrogen radicals that cause DNA damage, (ii) melatonin interferes with

estrogen's deleterious interactions with DNA, and (iii) melatonin inhibits the development of mammary tumors. (Section IV.A.)

2. A study published in 2009 (Davanipour et al.) evaluated guanine DNA/RNA damage in relation to melatonin production among 55 mother-father-adult daughter triples who were relatively healthy for their age. The lower melatonin production among the mothers was associated with higher guanine DNA damage. Lower melatonin production among the fathers was marginally associated with guanine damage in either DNA or RNA.
3. Human studies indicate that ELF MF exposure can decrease melatonin production. (Section II.)
3. Human studies have found that low melatonin production is a likely risk factor for breast cancer. (Section IV.B.)
4. Human studies have shown that light-at-night and night shift work reduce melatonin production and are both risk factors for breast cancer. (Section IV.D.)
5. Occupational studies indicate that high ELF MF exposure increases the risk of breast cancer. This is particularly true for a recent, large, and well-designed study from Poland (funded by the NCI, administered for the NCI by Westat, and conducted by Polish scientists).
6. A recent, large, and well-designed, Swedish case-control study used a new ELF MF job exposure matrix, developed by the same group, which is nearly completely at odds with earlier exposure classifications. The female occupation generally thought to be the one with the highest ELF MF exposure (seamstress) was considered to have medium-low exposure, while several lower ELF MF exposed occupations were considered high. The case-control study consequently found no risk associated with high ELF MF occupations as rated by the new matrix, but did find that seamstresses had a statistically elevated risk of breast cancer. This job exposure matrix is likely inappropriate in many important instances and needs to be thoroughly reviewed. (Section IV.E.)
7. Studies of residential ELF MF exposure and breast cancer have been generally negative. Measured residential ELF MF exposure may not be related to actual individual exposure. Residential exposure is most often low, is usually not measured in residences that may be related to the latency period of breast cancer, does not take into consideration point sources of strong magnetic fields which may be related to real exposure, and thus often does not relate to actual exposure. Residential exposure studies are therefore not considered to be of importance for the purposes of this report. (Section IV.F.)
8. Quality radiofrequency studies are lacking. (Section IV.G.)

<u>Seamstresses</u>

As a group, seamstresses have proven to constitute an important occupation for the demonstration of a relationship between ELF MF exposure and both Alzheimer's disease and breast cancer. Seamstresses who use industrial sewing machines have very high and relatively constant ELF MF exposure, particularly those seamstresses working in the apparel industry. This is because the motors of older AC machines are large and produce high levels of ELF MFs, and are on and producing such fields even when no sewing is being done. The AC/DC transformers of DC industrial machines always produce a high field even when the machine is turned off (but not unplugged). In addition, rooms, in which a large number of such machines are used, even have relatively high ambient ELF MF levels. Home sewing machines generally produce smaller ELF MFs, but even these weaker ELF MFs are substantial.

RECOMMENDATION Using the Precautionary Principal, mitigating exposure is a proper goal. Mean occupational exposures over 10 mG or intermittent exposures above 100 mG should be lowered to the extent possible. In situations where this is not feasible, the daily length of exposure should curtailed. Lowering ELF MF exposure can be done by improved placement of the source(s) of magnetic fields (e.g., electric motors in sewing machines, AC/DC converters), shielding, and redesign. It is clear that re-engineering products can greatly lessen ELF MF exposure, and possibly result in important innovations. It is noted that certain automotive models produce medium to high ELF MFs, as do steel-belted radial tires (Milham *et al.*, 1999).

I. INTRODUCTION

All of the studies discussed have based exposure classifications using magnetic field (MF) measurements, not electric field (EF) measurements. We separately discuss extremely low frequency (ELF, ≤ 60 Hz) MFs and radiofrequency (RF) MFs. Furthermore, the discussion is primarily limited to investigations related to ELF MF exposure as a possible risk factor for Alzheimer's disease (AD), female breast cancer (BC), and the possible biological pathways linking ELF MF exposure to AD and BC incidence, e.g., reduction in the production of melatonin.

Exposure Concerns

Epidemiologic investigations are sensitive to errors in exposure assessment and errors in case-control designation. This is particularly true for ELF MF exposure and for AD classification. With respect to occupational exposures, all job exposure matrices (JEM) are based on the measurement of a relatively small number of subjects in each job type. However, extensive measurements have been performed for workers in the electric utility industry and for seamstresses. Note, however, that the Swedish breast cancer study by Forssén *et al.* (2005) used only 5 essentially part-time seamstresses to determine exposure classification (Forssén *et al.* (2004).

The geometric mean ELF MF exposure over the time period of observation is generally used for classification. For ordinal classifications, individual subjects in jobs with mean ELF MF exposure measured close to a boundary value, e.g., between low and medium or between medium and high ELF MF exposure, will frequently be incorrectly classified. This misclassification will generally lead to bias in the estimated risk towards 1, i.e., no risk.

For residential exposures, which do not include living near high power lines, measurements of necessity need to be taken at the current residence. Measurements are usually taken in several rooms at various locations, sometimes with and without electrical equipment turned on, but rarely (if ever) with water lines turned on. Thus, individualized exposures, e.g., sitting near a fuse box, being near one or more AC/DC transformers, use of specific brands and models of home sewing machines, being near a microwave oven in operation, and a myriad of other point sources are missed. Previous residences are usually not available for measurements. Consequently, exposure classification is problematic for studies interested in risk associated with residential ELF MF exposure.

* Unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG or (relatively frequent) intermittent exposure above 100 mG,

while "medium" exposure is an average exposure of between 2 and 10 mG or (relatively frequent) intermittent exposure above 10 mG. "Long-term exposure" means exposure over a period of years. Often, other researchers use a cut-point of around 2-3 mG, or sometimes even less, as a "high" average. The reviews of each study presented here detail the specific cut-point(s) used.

** Also, unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG, while exposure means exposure over a period of years. **

Diagnostic Concerns

AD is difficult to correctly diagnose. Non-specialists frequently incorrectly diagnose a patient as having AD. Exposure assessment and case-control classification errors bias the odds ratio (OR) estimator, when based on dichotomous exposure classification, towards the null hypothesis. When based on three (3) or more classification groups, exposure assessment and case-control classification errors in the types of analyses used most likely also lead to bias towards the null hypothesis.

With respect to AD, unless the diagnosis is made by experts, there is a very large false positive rate. That is, community-based physicians often incorrectly diagnose dementia (versus depression, for example) and are particularly poor at determining the correct differential diagnosis of dementia. Most subjects with a diagnosis of dementia are simply assumed to have AD. This means that around 40% of all AD diagnoses by physicians who are not experts are incorrect. Diagnostic information on death certificates is even worse. Such a large error in caseness clearly biases the OR estimator towards the null hypothesis. (Many cases of AD go undiagnosed, especially early stage AD. However, this likely does not lead to a significant error rate in classification of controls.)

With respect to breast cancer, the sub-type of breast cancer is generally recorded, e.g., estrogen receptor positive (ER+) or negative (ER-), which may very well be important with respect to ELF MF exposure. However, sub-group analyses have not usually been performed.

Therefore, in reviewing published studies, particular emphasis is placed on these errors or caveats. Studies which assessed occupational exposures and those which assessed residential exposures are both discussed. Various algorithms for "ELF MF exposure" have been used, and these will also be discussed. Not all studies, exposure data, and exposure algorithms are of equal value.

For both AD and BC, a possible biological pathway of particular importance is down-regulation of melatonin production as a result of long-term ELF MF exposure. This is discussed in detail in this review.

A second possible biological pathway relates specifically to Alzheimer's disease. Long-term ELF MF exposure may increase the production of amyloid beta (Aβ), both in the brain and peripherally. Aβ, particularly the form with 42 amino acids ($A\beta_{1-42}$), is considered the primary neurotoxic compound causing AD. This pathway was proposed by Sobel and Davanipour (1996a). Recent epidemiologic studies have provided some degree of confirmation. A third

pathway has been proposed: genomic instability. Thus, ELF MF exposure may be a risk factor for AD through possibly three complementary biological pathways. (See Sections III.A. and III.B.)

There may certainly be other potential biological pathways that will be identified. For example, melatonin interacts with certain cytokines which appear to affect immune responses. This may be relevant to the early elimination of cells which are either pre-malignant or malignant, thus preventing the development of overt breast or other cancers. However, the two primary pathways outlined above can most easily be evaluated in human studies, both population-based studies and clinical trials.

There are also several epidemiologic studies of melatonin production among workers with long-term occupational exposure to magnetic fields and a single study of women with high (vs low) residential ELF MF exposure. These studies generally indicate that long-term ELF MF exposure can lead to lowered melatonin production.

II. ELF Magnetic Field EXPOSURE and MELATONIN ACTIVITY AND PRODUCTION

A. Melatonin Production

Conclusion: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high ELF MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production.

Eightly-five percent (85%) to 90% of pineal melatonin production is at night. Laboratory-based studies, using pure sinusoidal magnetic fields under experimental conditions have not found an effect on melatonin production (Graham *et al.*, 1996, 1997; *Brainard et al.*, 1999). However, several studies among subjects chronically exposed in occupational and residential environments have found an effect, while a few have not. The lack of an effect in laboratory settings may be because the ELF MF exposure was too "clean" or because the duration of exposure was not sufficiently long, e.g., days, weeks, months.

The evidence indicates that high and ELF MF exposures may lead to a decrease in melatonin production. Whether this decrease is reversible with a cessation of exposure is unknown. The extent of the decrease is hard to evaluate. It is also not yet possible to identify individual susceptibility to such a decrease in melatonin production.

Melatonin production is generally measured using its primary urinary metabolite, 6-sulphatoxymelatonin (aMT6s). Total overnight melatonin production is best estimated using complete overnight urine samples. Creatinine-adjusted aMT6s is slightly more correlated with cumulative melatonin estimates obtained from sequential overnight blood samples than is unadjusted aMT6s (Cook *et al.*, 2000; Graham *et al.*, 1998).

The human studies in occupational or residential environments which identified an effect are

summarized below.

Positive Studies

- Assessment in the Finnish Garment Industry As a follow-up component to a Finnish study of ELF MF exposures among garment factory workers, a small study of nighttime melatonin production was carried out (Juutilainen *et al.*, 1999). aMT6s excretion and creatinine were measured using complete overnight urine samples. Seamstresses (n=31), other garment workers (n=8), and non-exposed outside workers (n=21) participated. Observations were taken using complete overnight urine collections beginning on a Thursday night through the first morning void on Friday and on the subsequent Sunday night through the first morning void on Monday. There was very little variation between the two time period observations within each group, indicating that if there is an effect of ELF MF exposure, it does not disappear over the weekend, at least among seamstresses using older industrial alternating current machines. The average Thursday-Friday non-adjusted aMT6s excretion level and the average aMT6s excretion level adjusted for creatinine were both statistically significantly lower ($p < 0.05$) among the workers in the garment factory compared to the controls, even after controlling for other factors associated with a lowering of melatonin levels: creatinine-adjusted aMT6s - 16.4 vs 27.4 ng/mg; unadjusted aMT6s - 5.1 vs 10.0 ng. There was no indication of a dose-response relationship among the garment factory workers.

  In a follow-up study, Juutilainen and Kumlin analyzed the same data in conjunction with a dichotomization of a measure of light-at-night (LAN), obtained from items in the original study questionnaire concerning use of a bedroom light at night, street lights outside the bedroom windows, and use of curtains which do or do not let light filter through. There was a significant interaction between the dichotomized ELF MF exposure (high/low, i.e., cases vs controls) and LAN (yes/no). aMT6s was significantly lower for subjects with high ELF MF with or without LAN. In addition, aMT6s was significantly lower among subjects with high ELF MF and LAN exposure versus subjects with high ELF MF and no LAN exposure. Alternatively, aMT6s was essentially identical for subjects with low ELF MF exposure, regardless of the LAN status.

- Washington State Residential ELF MF Exposure and Melatonin Study Women, aged 20 to 74, were selected for a study of the relationship of bedroom 60 Hz magnetic field levels and melatonin production (Kaune *et al.*, 1997a,b; Davis *et al.*, 2001a). Approximately 200 women were recruited based on magnetic field exposure information from a case-control study of breast cancer (PI: S Davis). About 100 women were sought whose bedrooms were at the high end of magnetic field level in the original study and about 100 were sought who were at the low end. Concurrent measurements of light at night in the bedrooms of these women were also obtained using a specially modified EMDEX II system. Mean magnetic field levels in the two groups differed by less than 1 mG. Thus, compared to ELF MF exposures in many occupations, the women had quite low ELF MF exposures. However, there was an inverse association between bedroom magnetic field levels and urinary aMT6s adjusted for creatinine levels on the same night, after adjusting for time of year, age, alcohol consumption, and use of medications. The association was strongest at those times of the year with the longest length of daylight and in women who were using medications that themselves were expected to attenuate melatonin production,

e.g., beta and calcium channel blockers and psychotropic drugs.

- Crossover Trial of ELF MF Exposure at Night and Melatonin Production Davis *et al.* (2006) conducted a randomized crossover trial among 115 pre-menopausal women with regular periods between 25 and 35 days apart, a body mass index between 18 and 30 kg/m^2, not using hormonal contraceptives or other hormones for at least 30 days before the study period, no history of breast cancer, no history of chemotherapy or tamoxifen therapy, not having been pregnant or breast-feeding within the previous year, not working any night shifts, not taking supplemental melatonin, phytoestrogens or isoflavones, and not eating more than 5 servings of soy-based foods within any one week. ELF MF exposure or sham exposure was for 5 consecutive days. A random half of these women received ELF MF exposure and then sham exposure one month later. The other random half had the exposures reversed. Ovulation was determined in the first, second and third months. The initial exposure (ELF MF or sham) was in the second month during days 3-7 post-ovulation. The second exposure (sham or ELF MF) was during the same days in the third month. The charging base of an electric toothbrush which produced a steady magnetic field was used. It was placed under the subject's bed at the head level so that the subject's head received 5-10 mG exposure above baseline. Complete overnight urine samples were collected on the night of the last exposure (ELF MF or sham) in each of the two exposure periods. There were 2 subjects who did not ovulate during either exposure month and 13 who did not ovulate in one of the two months. Statistical adjustment was made for age, hours of darkness, body mass index, medication use, any alcohol consumption, and number of alcoholic beverages consumed. Because each subject was her own control, these adjustments probably did not affect the point estimates much. A regression analysis was undertaken. The 95% confidence interval (CI) of the regression slope was [-3.0 – +0.7] for all subjects and [-4.1 – -0.2] when the 15 subjects with "minor" protocol violations were eliminated from the analysis. These violations were (a) more than 40 days between the two assessments, (b) urine collections not on the same post-ovulation day, and (c) menstrual period started early. Only (b) appears to be really relevant because these subjects could have had less ELF MF exposure. However, this information is not provided. Separate analyses were conducted for "medication users" (n=14) and non-users (n=101). The slope point estimate for the users was numerically smaller (-3.1) than for the non- users (-1.0). The authors state that the study "found that nocturnal exposure to 60-Hz magnetic fields 5 to 10 mG greater than ambient levels in the bedroom is associated with decreased urinary concentrations of (aMT6s)". It should be noted that the p-value of the slope estimate in the primary analysis (all participants) was greater than 0.05. However, the 95% CI, [-3.0 – +0.7], was quite unbalanced, with 0 being much closer to the upper end of the CI than the lower end. Also, the 95% CI, when the 15 subjects with minor protocol violations are eliminated is entirely below 0, and thus the point estimate is statistically significant at the 0.05 level. The authors also state the following: "(t)he more pronounced effect of magnetic field exposure on melatonin levels seen in medication users and in those with an anovulatory cycle suggest {sic} that individuals who have decreased melatonin levels already may be more susceptible to the effects of magnetic field exposure in further decreasing melatonin levels." The justification for this statement is not based on statistical testing.

- Residential High Power Lines, ELF MF Exposure and aMT6s in the Quebec City Study Levallois *et al.* (2001) evaluated aMT6s among 221 women living near 735-kV power lines

compared to 195 age matched women who live far away from such lines. The subjects wore magnetic field dosimeters for 36 consecutive hours to measure their actual ELF MF exposure. The geometric mean 24-hour ELF MF exposure was 3.3 mG among women living near a high power line and 1.3 mG among those who did not live near a high power line. Similarly, geometric mean exposure during sleep was 2.9 mG versus 0.8 mG for the two groups. No direct effect of ELF MF exposure on creatinine-adjusted aMT6s was identified. However, living near a high power line and ELF MF exposure interacted with age and body mass index (BMI; kg/m^2). Living near a high power line was associated with a significant decline in creatinine-adjusted aMT6s among older subjects and subjects with higher BMI. There were similar significant decreases related to age and BMI for women in the lowest quartile versus highest quartile. All analyses were adjusted for age, BMI, alcohol consumption in the previous 24 hours, medication use in the previous 24 hours, light at night, and education.

- Assessment in the Electric Utility Industry Burch *et al.* (1996, 1998, 1999, 2000, 2002) have reported on the association between levels of occupational daytime magnetic field exposure, non-work ELF MF exposure, and the excretion of total overnight and daytime aMT6s among electric utility workers in several studies. These studies are among the largest to evaluate the relationships between ELF MF exposure and melatonin production in humans, and are the only studies to use personal exposure monitoring of both ELF MF and ambient light with a repeated measures design.

  - ✓ In their 1996 abstract, analyses were conducted for 35 of 142 electric utility workers enrolled in a larger study. ELF MF exposure was assessed continuously at 15 second intervals for three 24-hour periods, with logs kept to identify work, sleep and other non-work time periods. Ambient light intensity was also individually measured. Complete overnight urine samples and post-work spot urine samples were collected at the same times over the 3 days. There were statistically significant inverse relationships between nocturnal aMT6s levels and log- transformed worktime mean ELF MF exposure ($p=0.013$), geometric work-time mean ELF MF exposure ($p=0.024$), and cumulative work-time ELF MF exposure ($p=0.008$). There was no association, however, between sleep time and other time ELF MF exposure levels and aMT6s levels during the daytime or nighttime, even though average cumulative ELF MF levels were only somewhat higher during work: 18.3 mG-hours (work); 13.1 mG-hours (non-work); 12.6 mG-hours (sleep).

  - ✓ In their 1998 study, further results related to nocturnal aMT6s urinary excretion in relation to ELF MF exposure were presented, using all 142 electric utility workers. The ELF MF exposure metrics were geometric mean intensity, a rate-of-change metric (RCM), and the standardized rate-of-change metric (RCMS). RC was used as a measure of intermittence, while RCMS was used as a measure of the temporal stability of the serially recorded personal ELF MF exposures. Statistical adjustments were made for age, month, and personal ambient light exposure. 24-hour mean ELF MF exposure intensity, RCM, and RCMS were not associated with either nocturnal aMT6s or creatinine-adjusted aMT6s. However, there was an inverse relationship between residential RCMS and nocturnal aMT6s. The interaction between residential intensity and RCMS was inversely associated with total overnight urinary aMT6s excretion and with

creatinine-adjusted nocturnal aMT6s excretion. There was a "modest" reduction in nocturnal aMT6s with more temporally stable ELF MF exposures at work. The effect on nocturnal aMT6s was greatest when residential and workplace RCMS exposures were combined. The authors concluded that their study provides evidence that temporally stable ELF MF exposure (i.e., lower RCMS) are associated with decreased nocturnal urinary aMT6s levels. Given the strong correlation between cumulative overnight serum melatonin levels and both total overnight urinary aMT6s and creatinine-adjusted aMT6s levels, these results indicate a reduction in overnight melatonin production.

- ✓ In their 1999 study, data from the same 142 electric utility workers were further analyzed. Personal exposure to workplace geometric mean and RCMS were evaluated for their effect on post-work urinary aMT6s measurements. No association between creatinine-adjusted aMT6s and the geometric mean ELF MF exposure, before or after adjustment for age, calendar month and light exposure was found. However, ELF MF temporal stability was associated with a statistically significant reduction in adjusted mean post-work aMT6s concentrations on the second ($p=0.02$) and third ($p=0.03$) days of observation. Light exposure modified the ELF MF exposure effect. Overall, there was a significant ($p=0.02$) interaction between RCMS and ambient light exposure. Reductions in post-work aMT6s levels were associated with temporally stable ELF MF exposures among workers in the lowest quartile of ambient light exposure (mostly office workers), whereas there was no RCMS effect among workers with intermediate or elevated ambient light exposure.

- ✓ In their 2000 study, Burch *et al*. examined aMT6s levels among a completely different population of 149 electrical workers, 60 in substations, 50 in 3-phase environments, and 39 in other jobs, using the same data collection strategy as was used in the previous study, but with the added characterization of specific work environments. The rationale for this study was based on previous observations in experimental animals suggesting that non-linear field polarization was critical in the reduction of melatonin production. These types of fields were expected to be present within substations and in the vicinity of 3-phase electrical conductors. Other conductors (1-phase, linear polarization) were selected as a control condition because they had not previously been associated with an alteration of melatonin production in laboratory animal studies. Thus, participating workers recorded the times they spent in these environments over the 3-day data collection period. Comparisons were made separately for subjects working in substation or 3-phase environments, or among those working in 1-phase environments. Adjusted mean aMT6s levels were compared statistically among workers in the lowest and highest tertiles of ELF MF exposure, using either the geometric mean or the RCMS measurements. Among workers in either a substation or 3-phase environment for more than 2 hours, nocturnal aMT6s decreased 43% ($p=0.03$) when tertiles were based on geometric mean exposure and decreased 42% ($p=0.01$) when tertiles were based on RCMS. With RCMS tertiles, total overnight aMT6s excretion also decreased 42% ($p=0.03$) and post-work creatinine-adjusted aMT6s decreased 49% ($p=0.02$). With geometric mean tertiles, total overnight aMT6s excretion decreased 39% and post-work creatinine-adjusted aMT6s

decrease 34%. However, neither of these decreases was statistically significant. No ELF MF-related effects were observed among workers with less than 2 hours time spent in substation/3-phase environments. Similarly, no reduction in aMT6s levels were observed among workers in 1-phase environments.

- ✓ In 2002, Burch *et al.* studied two consecutive cohorts of electric utility workers using the same data collection strategy to evaluate the effects of cellular telephone use and personal 60 Hz ELF MF exposure on aMT6s excretion. The sample sizes were 149 for Cohort 1 (from the 2000 study) and 77 for Cohort 2. Total overnight and post-work urine samples and self-reported workplace cell phone use were obtained over three (3) consecutive workdays. ELF MF and ambient light exposure were also measured with specially adapted personal dosimeters. The outcome of interest was melatonin production as measured by aMT6s. The cut- point for high versus low cell phone use was 25 minutes per day. Only 5 worker- days of cell phone use more then 25 minutes were reported in Cohort 1 versus 13 worker-days in Cohort 2. No differences in aMT6s production were found in Cohort 1. However, for Cohort 2 there were significant linear trends of decreasing overnight aMT6s and creatinine-adjusted aMT6s levels with increasing cell phone use. There was also a marginally significant increasing trend in post-work creatinine-adjusted aMT6s with increasing cell phone use. Finally, there was a combined effect of cell phone use and ELF MF exposure on aMT6s excretion: among workers in the highest tertile of ELF MF exposure, those who used a cell phone for more than 10 minutes had the lowest overnight aMT6s and creatinine-adjusted aMT6s levels compared to those with lower ELF MF exposure or cell phone use. All analyses used a repeated measures method and were adjusted for age, month of participation, and light exposure.

- Swiss Railway Worker Study Pfluger and Minder (1996) studied 66 railway engineers operating 16.7 Hz electric powered locomotives and 42 "controls". Mean ELF MF exposure at the thorax for the engineers was above 150 mG and approximately 10 mG for the controls. Thus most controls also had high ELF MF exposure, certainly compared to residential and most occupational ELF MF exposures. Morning and early evening (post-work) urine samples were used to measure aMT6s. Evening aMT6s values were significantly lower following work periods (early, normal or late shifts) compared to leisure periods for the engineers, but not for the controls. Also, morning samples did not differ between leisure and work mornings. This indicates that there was at least somewhat of a recovery from the work-time ELF MF exposures. Evening aMT6s values did not differ between work time and leisure time for either engineers or controls. However, there was a rebound in morning aMT6s between a work period and leisure period. Pfluger and Minder did not report the results of a comparison of nighttime aMT6s levels between engineers and controls.

- Video Display Unit Studies Non-panel video display screens, e.g., computer monitors, produce significant ELF MF exposure despite improvements over the last decade or so. Arnetz and Berg (1996) studied 47 Swedish office workers who used video display units (VDU) in their work in the 1980s. Circulating melatonin levels significantly decreased during work, but not during a day of "leisure" in the same environment.

Nighttime melatonin production was not observed. In 2003, Santini *et al.* conducted a similar, but quite small, study of 13 young female office workers, 6 of whom worked for at least 4 hours per day in front of a video screen. Overnight urine samples were used to measure aMT6s. The aMT6s values of the exposed workers was 54% lower ($p<0.01$) compared to the non- exposed workers.

Negative Studies

- Italian Study of Workers Gobba *et al.* (2006) recruited 59 workers, 55.9% of whom were women, for a study of melatonin production and ELF MF exposure. Actually more workers were recruited, but urine samples for only those subjects who did not get up to urinate during sleep time were assayed. Creatinine-adjusted aMT6s was measured using a Friday morning urine sample and the following Monday morning urine sample. Mean age was 44.4 years (standard deviation, 9.2). Exposure during worktime was measured over a three-day period. The logarithm of the time weighted average (TWA) and the percent of time above 2 mG were used as the measures of exposure. 2 mG was the cut-point between low and high exposure. 52.5% were in the low exposed group; a larger percentage of men than women were in the low exposed group. Occupations included clothing production (n=26), utility companies (14), teachers (6), engineering industry (5), and miscellaneous (8). There were no significant differences in creatinine-adjusted aMT6s values based on the logarithm of the TWA or percent of observations above 2 mG.

- Occupational ELF MF Exposures among 30 Males Subjects in France Touitou *et al.* (2003) studied 15 men exposed to occupational magnetic fields for between 1 and 20 years and age-matched15 controls. All subjects were free of acute or chronic diseases, had regular sleep habits, did not do night work, took no transmeridian airplane flights during the preceding 2 months, took no drugs, were nonsmokers, and used alcohol and coffee in moderate amounts. Furthermore, they did not use electric razors or hair dryers during the study or in the 24 hours prior to blood sampling. All of the 15 ELF MF exposed men worked in high voltage electrical substations. They also lived near substations. None of the controls had an occupation associated with ELF MF exposure. Exposed subjects had a mean exposure of 6.4 mG during work and 8.2 mG during other times. For the control subjects, the mean exposure was 0.04 mG, both during the day and at other times. Blood samples were taken hourly from 8:00 pm until 8:00 am in a standard manner. All urine between these times was collected. Melatonin concentration (pg/ml) was measured in each blood sample. The study was done in the autumn. The 12 hour melatonin blood concentration curves for the exposed and non-exposed subjects are almost identical. The creatinine-adjusted aMT6s levels are also nearly identical. No analyses were conducted based on length of time in the occupation.

B. **Melatonin Activity and ELF MF**

Conclusion: New research indicates that ELF MF exposure, in vitro, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor.

Girgert et al. (2010) studied the effects of 12 mG 50 Hz ELF MF exposure on signal transduction of MT1 in parental MCF-7 cells and MCF-7 cells transfected with the MT1 gene. MT1 is a high-affinity melatonin receptor and is responsible for many of melatonin's activities. 12 mG is an

exposure experienced by individuals in many occupations, e.g., seamstresses and welders. Melatonin, as discussed in this chapter, has many important properties related to cancer prevention and growth, particularly breast cancer, and to the delay or prevention of AD. For proliferation tests, the MT1-negative and MT1-transfected cells were placed in a medium with and without an estradiol solution – estradiol concentrations ranged from 10^{-12} to 10^{-10} moles. $4x10^{-9}$ moles of melatonin were used in a parallel series of estradiol concentrations to evaluate the effect of melatonin. Cell proliferation assays demonstrated that (i) melatonin inhibited cell growth and (ii) 12 mG ELF MF exposure nearly eliminated the effect of melatonin on cell growth. Furthermore, melatonin's growth inhibitory effect was more prominent in the MCF cells transfected with the MT1 receptor than in the cells which were not transfected.

> Girgert et al. (2010) note that several studies designed to evaluate the effects of melatonin in breast cancer cells were negative. They measured the ELF MF produced by various cell incubators and found several that generated approximately 12 mG. They suggest that negative findings may be due to the use of incubators which produce these relatively high fields.

III. ALZHEIMER'S DISEASE

A. Possible Biologic Pathways from ELF MF Exposure to Alzheimer's Disease

A.1. Over-Production of Peripheral Amyloid Beta Caused by ELF MF Exposure

> *<u>Conclusion</u>: There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta.*

Sobel and Davanipour (1996a) have published a biologically plausible hypothesis relating ELF MF exposure to AD, based on the unrelated work of many researchers in several different fields. The hypothesized process involves increased peripheral or brain production of amyloid beta (Aβ) as a result of ELF MF exposure, and subsequent transportation of peripheral Aβ across the blood brain barrier. Figure 1 provides a schematic outline of the hypothesis. Each step in the proposed pathway is supported by *in vitro* studies.

Two versions of the amyloid beta protein have been identified. They are identical, except one is longer, 42 versus 40 amino acids. These are specified, respectively, by $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$. $A\beta_{1\text{-}42}$ is considered the more neurotoxic of the two.

This hypothesis has not yet been fully tested. However, two recent studies of elderly subjects and electrical workers, respectively, have provided important initial support. The Mayeux *et al.* (1999, 2003) papers demonstrate that higher levels peripheral $A\beta_{1\text{-}42}$ are a risk factor for AD. The Noonal et al. (2002a) paper demonstrates that ELF MF exposure can increase the peripheral levels of $A\beta_{1\text{-}42}$ and that contemporaneous blood levels of melatonin are inversely associated with peripheral levels of $A\beta_{1\text{-}42}$.

- Mayeux *et al.* (1999, 2003, 2011) conducted a population-based, longitudinal study of

elderly subjects who were cognitively normal at baseline and found that higher peripheral blood levels of $A\beta_{1-42}$ were prognostic of subsequent development of AD. The 2003 paper had a longer follow-up period and 282 additional subjects (169 vs 451).

In the first paper, 105 subjects, cognitively normal at baseline, were followed for an average of 3.6 years. The mean age at baseline was 74.3 +/- 5.3 years. Sixty-four (64) subjects developed AD. Table 1 provides the baseline and follow-up means for age, education, $A\beta_{1-42}$, $A\beta_{1-40}$, and the ratio $A\beta_{1-42}/ A\beta_{1-40}$. The subjects who developed AD were older at baseline, had nearly two years less education, and higher $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$. All mean differences were significant at the p=0.001 level, except for the ratio, which was significant at the p=0.05 level.

For $A\beta_{1-42}$, the OR for AD, based on the actual $A\beta_{1-42}$ values, was 1.0114, p = 0.006. Thus, for example, the OR for an individual with an $A\beta_{1-42}$ value 10 pg/ml above the cutpoint for the 1st quartile (24.6 pg/ml) is estimated to be $(1.0114)^{10} = 1.12$, an increase of 12%; for an individual with an $A\beta_{1-42}$ value 40 points above this cutpoint, the estimated increase in risk is 57%. A similar analysis for $A\beta_{1-40}$ did not yield a significant result.

Subjects were then divided into quartiles based on their $A\beta_{1-42}$ values. For $A\beta_{1-42}$ there was a highly significant (p=0.004) trend across quartiles. The adjusted odds ratios (OR) for the 2nd – 4th quartiles were 2.9, 3.6, and 4.0, using logistic regression. The latter two were statistically significant at the 0.05 level. The ranges for the 3rd and 4th quartiles were 45.9 – 85.0 pg/ml and > 85.0 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was not provided; however, the 95% confidence interval (CI) was [0.9 – 6.8]. Perhaps because the per unit analysis was not significant for $A\beta_{1-40}$, an analysis using quartiles was not reported.

In the second paper (Mayeux *et al*., 2003), follow-up of patients was up to 10 years and there were 451 patients who were cognitively normal at baseline, versus 169 in the initial paper. Table 2 contains the same information for this study as is provided in Table 1 for the initial study. Eighty-six (86) of the 451 subjects developed AD. Presumably, the additional subjects had had their peripheral amyloid beta assayed after the submission of the original paper. Again, the $A\beta_{1-42}$ values were divided into quartiles, based on the 451 subjects who were cognitively normal at their last follow-up. The adjusted relative risk (RR) estimates for the 2nd – 4th quartiles were 1.3, 1.9, and 2.4, using Cox survival analysis. The latter two were statistically significant at the 0.05 and 0.006 levels, respectively. The ranges for the 3rd and 4th quartiles were 60.2 – 84.15 pg/ml and ≥ 84.15 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was again not provided; however, the 95% confidence interval (CI) was [0.6 – 2.1].

The mean levels of $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-42}/A\beta_{1-40}$ at baseline in the second paper were 133.9 pg/ml, 62.2 pg/ml, and 0.50. In the initial paper, the comparable figures were 120.5 pg/ml, 63.2 pg/ml, and 0.57. The means for $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ are quite similar in the two studies. However, the means for $A\beta_{1-40}$ are quite different, so there were most likely several subjects who were not in the initial report, and who had $A\beta_{1-40}$ assays which were very high. These subjects were evidently almost all in the cognitively normal group. This is because in the AD groups, the $A\beta_{1-40}$ means were 134.7 and 136.2 pg/ml. However, in the cognitively normal group, the means were

111.8 and 133.3 pg/ml. Thus, the additional 260 subjects with did not develop AD (365-105=260) had an average $A\beta_{1-40}$ of 142.0 pg/ml. Such a large difference is left unexplained in the Mayeux *et al*. (2003) paper.

Mayeux *et al*. (1999) comment that "cerebral deposition of $A\beta_{1-42}$ is unlikely to result directly from increased plasma $A\beta_{1-42}$". However, studies by Zlokovic and colleagues provide a basis for concluding that, in fact, peripheral $A\beta_{1-42}$ is likely to cross the blood brain barrier, perhaps chaperoned by apolipoprotein E (ApoE), particularly the ε4 isoform (see Sobel & Davanipour, 1996a). Currently, the relative amounts of peripheral and cerebral $A\beta_{1-42}$ or $A\beta_{1-40}$ which aggregate are unknown.

Two newly developed PET scan techniques, however, provide the ability to investigate the relative amounts in humans (Klunk *et al*., 2004; Ziolko *et al*., 2006; Small *et al*., 2006). It is also straightforward to use labeled amyloid beta to determine the rate at which peripheral amyloid beta is transported to the brain, at least in animal models and perhaps also in humans.

In 2011, Mayeux and Schupf further discussed their and other researchers findings and their hypothesis that a high blood level of $A\beta_{1-42}$ is a risk factor for late onset AD, but the $A\beta_{1-42}$ blood levels decline with advancing dementia. Similarly, blood levels of $A\beta_{1-40}$ may also decline with disease progression.

- Schupf et al. (2008) studied a sample of 1021 non-demented subjects at least 65 years old at baseline. Plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels were assayed at baseline. One hundred and four (104; 10.2%) subjects developed AD within 4.6 years. Higher plasma $A\beta_{1-42}$ at baseline was associated with a 3-fold increase in the risk of AD. On the other hand, development of AD was associated with a significant decline in plasma $A\beta_{1-42}$ and a decrease in the $A\beta_{1-42}/A\beta_{1-40}$ ratio as dementia progressed.
- Cosentino et al. (2010) studied a sample of 880 subjects, 65 or older and dementia free at the first of two plasma Aβ measurements. High baseline plasma for both $A\beta_{1-42}$ and $A\beta_{1-40}$, and decreasing or stable $A\beta_{1-42}$ were associated with faster decline in multiple cognitive areas.
- Schupf et al. (2010) studied the relationship between plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and the occurrence of dementia among a community-based cohort of 225 Down syndrome adults, dementia-free at baseline. Sixty-one (61, 27.1%) developed AD during follow-up. The mean length of follow-up was 4.1 years. The increase in plasma $A\beta_{1-40}$, decrease in plasma $A\beta_{1-42}$, and decrease in $A\beta_{1-42}/A\beta_{1-40}$ levels were significantly associated with development of dementia. This study was an extension of the follow-up time of an earlier study (Schupf et al., 2007).
- Devanand et al. (2011) studied a small number of patients (n=20) with amnestic mild cognitive impairment (MCI), a harbinger of AD development in the majority of cases, and 19 cognitively normal controls. Plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels were assayed. In addition PET scans determined Pittsburgh compound B (PiB) binding in various brain locations and in the total brain. The plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was decreased in the MCI patients compared to the controls, but $A\beta_{1-42}$ and $A\beta_{1-40}$ did not differ between the two groups. PiB binding levels were significantly higher in the cingulate and parietal brain areas and in the entire brain among the MCI patients compared to the

controls. However, in the prefrontal cortex and parahippocampal gyrus the differences were only marginally significant, but the sample size was relatively small. Low $A\beta_{1-42}/A\beta_{1-42}$ and $A\beta_{1-40}$ were associated with high cingulate, parietal and total brain PiB binding, using regression analyses which included age, gender, and cognitive test scores.

- For completeness, we provide the results of a meta-analysis by Song et al. (2011) of 12 cross-sectional and 7 longitudinal studies of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels related to AD. The results were as follows:
  - ✓ Longitudinal studies: cognitively normal subjects who developed AD had higher baseline plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ (p=0.0001 and 0.006, respectively), but non-significantly increased $A\beta_{1-42}/A\beta_{1-40}$ (p=0.10).
  - ✓ Cross-sectional studies: AD patients had marginally significant (p=0.08) lower plasma $A\beta_{1-42}$. The $A\beta_{1-40}$ levels were not significantly different (p=0.69).

- Noonan *et al.* (2002a) examined 60 electric utility workers in studying the relationship between measured ELF MF exposure during the work day and serum $A\beta_{1-42}$ and $A\beta_{1-40}$ (square root transformed) levels. ELF MF exposure was individually determined by wearing a dosimeter at the waist during work time. Blood samples were obtained between 2:50 pm and 4:50 pm. The primary findings were as follows:
  - **i.** there was an inverse association between physical work and A Aβ levels;
  - **ii.** there was an apparent trend for the $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ levels to be higher for higher magnetic field exposure (significance not provided); and
  - **iii.** the differences (Table 3) in Aβ levels between the highest (≥ 2 milliGauss (mG), n=7) and lowest (< 0.5 mG, n=20) exposure categories were 156 vs 125 pg/ml (p=0.10) for $A\beta_{1-40}$, 262 vs 136 pg/m (p=0.14) for $A\beta_{1-42}$, and 1.46 vs 1.03 for $A\beta_{1-42}/A\beta_{1-40}$ (significance not provided).

  There was a 93% increase in $A\beta_{1-42}$, a 25% increase in $A\beta_{1-40}$, and a 42% increase in the ratio $A\beta_{1-42}/A\beta_{1-40}$ between the lowest and highest ELF MF exposure categories. The 2 mG cutpoint for the highest category is the cutpoint generally used for medium (or at times high) ELF MF exposure in epidemiologic studies. Thus, while the sample size was small, this study provides some evidence that ELF MF exposure may result in higher peripheral production of Aβ for exposures above 2mG.

  Melatonin production was estimated using urinary 6-sulphatoxymelatonin (aMT6s) adjusted for creatinine (Graham *et al.*, 1998). aMT6s is the primary urinary metabolite of melatonin. A complete overnight urine sample was used to estimate overnight melatonin production, normally about 85-90% of total 24-hour production. A post-work urine sample, taken on the same day as the post-work blood sample, was used to estimate work time melatonin blood levels. The overnight creatinine-adjusted aMT6s levels were, on average, about 5 times higher than the post-work creatinine-adjusted aMT6s levels. Noonan *et al*. state that the correlations between overnight creatinine-adjusted aMT6s and amyloid beta levels were not significant. No data were provided. However, post-work creatinine-adjusted aMT6s levels were negatively correlated with both the $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ post-work levels. The Spearman correlation coefficients were -0.22 (p=0.08) and -0.21 (p=0.10), respectively. With adjustment for age and physical work, the correlation with $A\beta_{1-42}$ was marginally stronger (-0.25, p=0.057). The timing of the urine sample with respect to the blood sample appears to be important. Table 4 provides

the Spearman correlations, adjusted for age and physical work, based on the time difference between blood and urine samples, which were all obtained after the blood draw. Some of the workers had their urine sample in the early evening. It is clear that the correlation is strongest when the samples are taken close to one another in time.

In an unadjusted analysis, the post-work creatinine-adjusted aMT6s levels were split into tertiles. Subjects in the highest tertile had the lowest levels of $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ (Table 5). However, subjects in the middle tertile had higher levels than subjects in the lowest tertile.

- In an *in vitro* study, Del Giudice *et al.* (2007) used human neuroglioma cells (H4/APPswe), which stably overexpress a specific human mutant amyloid precursor protein (APP, to examine the effect of ELF MF exposure. ELF MF or sham exposure was 3.1 mT (31,000 mG) for 18 hours. Total Aβ and total $A\beta_{1\text{-}42}$ production was statistically significantly elevated among the ELF MF exposed cells compared to the cells with sham exposure. No gross morphological changes or changes in viability were observed in the ELF MF exposed cells. The 3.1 mT exposure level is 2-3 orders of magnitude higher than the highest occupational mean exposures. The authors state that such high levels were administered because occupational exposures are "much more prolonged than the one described in our experimental setting". There was no indication that any longer duration exposure at lower levels was studied.

A.2. Lowered Melatonin Production: An Alternative/Complementary Pathway

Conclusion: There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

Several *in vitro* and animal studies indicate that melatonin may be protective against AD and thus low or lowered melatonin production may be a risk factor for AD. These studies have generally found that supplemental melatonin has the following effects:

- the neurotoxicity and cytotoxicity of A□ is inhibited, including mitochondria (Pappolla *et al.*, 1997, 1999, 2002; Shen YX *et al.*, 2002a; Zatta *et al.*, 2003; Jang *et al.*, 2005);
- the formation of β-pleated sheet structures and Aβ fibrils is inhibited (Pappolla *et al.*, 1998; Poeggeler *et al.*, 2001; Skribanek *et al.*, 2001; Matsubara *et al.*, 2003; Feng *et al.*, 2004; Cheng and van Breemen, 2005);
- the profibrillogenic activity of apolipoprotein E ε4, an isoform conferring increased risk of AD, is reversed (Poeggeler *et al.*, 2001);
- oxidative stress *in vitro* and in transgenic mouse models of AD is inhibited if given early (Clapp-Lilly *et al.*, 2001a; Matsubara *et al.*, 2003; Feng *et al.*, 2006), but not necessarily if given to old mice (Quinn *et al.*, 2005);
- survival time is increased in mouse models of AD (Matsubara *et al.*, 2003);
- oxidative stress and proinflammatory cytokines induced by $A\square_{1\text{-}40}$ in rat brain are reduced *in vitro* and *in vivo* (Clapp-Lilly *et al*, 2001b; Shen YX *et al.*, 2002b; Rosales-Corral *et al.*, 2003);
- the prevalence of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in the brain is decreased in young and middle aged mice (Lahiri *et al.*, 2004);

- memory and learning is improved in rat models of AD pathology (Shen YX *et al.*, 2001; Weinstock and Shoham, 2004), but not necessarily in Aβ-infused rat models (Tang *et al.*, 2002).

Note that transgenic mouse models of AD mimic senile plaque accumulation, neuronal loss, and memory impairment. See Pappolla *et al.* (2000), Cardinali *et al.* (2005), Srinivasan *et al.* (2006), Cheng *et al.* (2006), and Wang and Wang (2006) for reviews. Thus, chronic low levels of melatonin production may be etiologically related to AD incidence.

A.3. Cytogenetic Hypothesis Relating ELF MF Exposure to Alzheimer's Disease

Conclusion: This is an interesting hypothesis and is deserving of research efforts.

Maes and Verschaeve (2011) review evidence that genomic instability, including aneuploidy, telomere shortening, and gene amplification, is associated with an increased risk of early-onset familial AD and perhaps sporadic AD. The authors then discuss possible genetic effects of ELF MF (or electromagnetic field (EMF)) exposure. Further, directed research into this hypothesis is warranted.

D. Epidemiologic Studies of Alzheimer's Disease/Dementia and ELF MF Exposure

Conclusion: There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are now twelve (12) studies of ELF MF exposure and AD or dementia which . Nine (9) of these studies are considered positive and three (3) are considered negative. The three negative studies have serious deficiencies in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk or protective factor for AD.

D.1. Introduction

First, it is necessary to point out that there are no case-control studies of melatonin as a risk factor for AD. This is primarily because AD results in a precipitous decline in melatonin production due to the destruction of specific neuronal structures and therefore it is inappropriate to use "current" melatonin production of cases as a surrogate estimate of the pre-AD melatonin production. Also there have yet to be any longitudinal studies of melatonin production. This is probably because neither urine nor blood have been collected appropriately to measure nocturnal melatonin production.

If ELF MF exposure is a true risk factor, there are several problematic areas in evaluation and comparison of epidemiologic studies related to occupational ELF MF exposure and Alzheimer's disease, particularly the following.

1. Diagnosis – false positive diagnoses will bias the odds ratio estimator towards 1.0
2. Occupational exposure assessment – inclusion of subjects with low exposure in the "exposed" categories likely biases the odds ratio estimator towards 1.0
    - Definition of ELF MF exposure – published studies have differing definitions

of ELF MF exposure, potentially resulting in "exposure" categories with significant proportions of subjects with low exposure

- Cut-points for non-exposure/exposure categories – some studies use numerical estimates of exposure developed from earlier exposure studies (job exposure matrices) in certain occupations and use average estimates and/or low cut-points to determine "medium" exposure
- Ever versus never exposed – at least one study used ever exposed, with a low threshold for exposure
- Categorized occupational data – categorized data from governmental databases leads to relatively large variation in "exposure" within occupational categories, which results in subjects with low exposure being classified as having been exposed.

Table 6 provides the data on the percentages of ELF MF exposed subjects in the published studies to date. There is a wide range of percentages, due primarily to variation in exposure definition, use of average or mean job-specific estimates, and secondarily to the use of varying job exposure matrices. Table 7 provides the odds ratio estimates of studies discussed in some detail below. The studies which used death certificates or other non-expert databases for the identification of AD cases are not included in Table 7.

The role of seamstresses among workers with high occupational ELF MF exposure in the two *et al.* studies (1995, 1996b) and the Davanipour *et al.* study (2007) is discussed.

D.2. **Death Certificates-Governmental Databases: Alzheimer's Disease Diagnosis**

The use of death certificates or governmental databases to identify AD cases is certainly problematic. False positive diagnoses tend to bias the OR estimator towards 1.0. Most diagnoses of AD have been and still are made by physicians who are not experts in AD, and who seldom have sufficient clinical time to make a proper diagnosis. The determination of dementia and subsequent differential diagnosis of AD by someone other than an expert has a high false positive rate. In addition, many physicians do not think that AD is a "cause of death", which results in an increase in the false negative rate.

Therefore the recent "positive" Feychting *et al.* (2003), Håkansson *et al.* (2003), and Park *et al.* (2005) studies and the "negative" Savitz *et al.* (1998a,b) and Noonan *et al.* (2002b) studies have been excluded from the discussion below of individual studies. The Johansen *et al.* study (2000) has also been excluded because it depended upon the clinical hospital discharge diagnoses of an historical cohort to determine a "diagnosis" of "presenile" AD or "dementia". Evidently, in that study, late-onset (age at least 65) AD was included under "dementia". (It should be noted that Johansen *et al.* found an increased risk of "dementia", but not "presenile" AD, associated with higher ELF MF exposure.)

D.3. ELF **MF Exposure Assessment Rates and Analytic Results**

The Sobel *et al.* (1995, 1996b), the Davanipour *et al.* (2007), and the Harmanci *et al.* (2003) studies have followed nearly the same protocol for ELF MF exposure assessment and classification into low, medium and high ELF MF occupations. In these studies, medium exposure was defined as mean ELF MF occupational exposure above 2 mG, but less than 10 mG, or intermittent exposures above 10 mG, while high exposure was defined as mean ELF M F exposure above 10 mG or

intermittent exposures above 100 mG. The rates of medium or high (M/H) exposure in these studies are considerably lower than the rates in the Feychting *et al.* (1998a), Graves *et al.* ((1999), Qiu *et al.* (2004), and Savitz *et al.* (1998b) studies and somewhat lower than the Feychting *et al.* (2003) study. The remaining three studies (Häkansson *et al.*, 2003; Savitz *et al.*, 1998a; Johansen, 2000) utilized subjects from electrical industries and therefore understandably have high rates of ELF MF exposure. (See Table 6 for these rates.)

Thus, it is likely that a substantial percentage of ELF MF "exposed" subjects in 4 of the 6 comparable studies (Feychting *et al.*, 1998a; Graves *et al.*, 1999; Qiu *et al.*, 2004) (Table 7) had a high rate of somewhat minimal exposure in the "exposed" category, due to classification methodologies, compared to the "exposed" categories in the Davanipour *et al.* (2007), Harmanci *et al.* (2003), and the Sobel *et al.* (1995, 1996b) studies. This would tend to lead to an OR estimate closer to 1.0 in the 4 former studies.

D.3.1. **Sobel *et al.* (1995) Study – <u>Positive Study</u>**

The initial publication of an apparent association between AD and having worked in occupations with likely ELF MF exposure consisted of three case-control studies, two from Helsinki, Finland, and one from Los Angeles, USA (Sobel *et al.*, 1995). Control groups varied: the first case-control study analyzed used VaD patients; the second (and largest study) used non-neurologic hospital patients; and the third (and second largest study) used non-demented well subjects. The study-specific ORs were 2.9, 3.1, and 3.0, while the combined OR was 3.0 (95% CI = [1.6 – 5.4], $p < 0.001$), with no confounder adjustments necessary. The occupational information was apparently primarily related to the last occupation, e.g., judge, high ranking military officer. A total of 386 cases and 575 controls was analyzed in these studies. 9.3% of the cases and 3.4% of the controls were judged to have had an occupation with likely medium or high ELF MF exposure. Among women, 31 (5.3%) were exposed to M/H occupational ELF MF, of whom 29 (95%) were seamstresses, who were classified as having high exposure based on measurements taken during the study. Seamstresses have subsequently been shown to have very high ELF MF exposures (e.g., Hansen *et al.*, 2000; Kelsh *et al.*, 2003; Szabó *et al.*, 2006).

D.3.2. **Sobel *et al.* (1996b) and Davanipour *et al.* (2007) Studies – <u>Positive Studies</u>**

These two studies utilized the databases of the nine (9) State of California funded Alzheimer's Disease Diagnosis and Treatment Centers (ADDTC). Sobel *et al.* (1996b), the second published study of occupational ELF MF and AD, used the Rancho Los Amigos (RLA) ADDTC database. There were 316 cases and 135 controls. Twelve percent (12%) of the cases and 5.3% of the controls had had a medium or high "primary" exposed (ELF MF) occupation. The Davanipour *et al.*, 2007) study used the databases of the other 8 ADDTCs. Seven and one-half percent (7.5%) of the cases and 3.8% of the controls had had a medium or high ELF MF "primary" occupation. Among the women in the RLA ADDTC study, 26 (8.4%) had M/H exposure, of whom 17 (65.4%) were seamstresses. In the Davanipour *et al.* study, among women, 50 (3.8%) had M/H ELF MF exposure, of whom 34 (68%) were seamstresses. This difference is statistically significant ($p < 0.001$). Among the men in the RLA ADDTC study, 14.8% had a medium or high ELF MF exposed occupation, while in the Davanipour *et al.* ADDTC study, 13.5% had a medium or high ELF MF exposed occupation. This difference is not significant. It thus appears that the women in the combined populations from which the ADDTCs in the Davanipour *et al.* study have drawn their patients have a lower rate of ELF MF exposed occupations than the population from

which the RLA ADDTC draws its patients. This is not too surprising because Los Angeles has a large apparel manufacturing industry.

The OR (adjusted for age-at-onset, gender, and education) for medium or high ELF MF exposure in the RLA ADDTC study was 3.9 (95% CI = [1.5 – 10.6], p = 0.006). The ORs for medium or high ELF MF exposure in the Davanipour *et al.* ADDTC study were lower: 2.2 ($p < 0.02$; 95% CI = [1.2 – 3.9]) and 1.9 ($p < 0.04$; 95% CI = [1.04 – 3.6]), using age-at-exam and age-at-onset, respectively, plus gender and history of stroke in the model. These ORs are all statistically significant. In the two studies, the 95% CIs greatly overlap and, under the assumption of normality of the natural logarithms of the odds ratios estimators and a straightforward hypothesis test that the means of two independent normally distributed variables are equal, the null hypothesis that the corresponding ORs are equal cannot be rejected at the 0.05 level.

D.3.3. **Other AD/Dementia and Occupational ELF MF Exposure Studies**

Studies with (at least some) Positive Results

Qiu et al. (2004) *Study* Qiu *et al.* (2004) studied a Swedish cohort of 931 subjects, aged 75+ at baseline, followed for up to 7 years. Job history was usually obtained from the next-of-kin, but only after 4 years of follow-up. ELF MF exposure assessment was estimated using previous occupational exposure studies, specific measurements (e.g., seamstresses and tailors), and expert opinion. During the follow-up period, 265 subjects developed dementia, with 202 receiving an AD diagnosis. Numerical exposure estimates were obtained using both the longest held occupation, last occupation, and any occupation. The estimated average daily ELF MF exposure was used to classify individual exposure.

Exposure for a sample of seamstresses and tailors was measured at the head. They were classified as having low exposure. Exposures of seamstresses who used industrial sewing machines and workers who used home sewing machines likely were under estimated by Qiu *et al.* (2004): 5.5 mG for "industrial seamstresses" and 1.9 for tailors. Qui *et al.* only considered home sewing machines, which at the head had a mean exposure of 10 mG. For "industrial seamstresses, they assumed that 50% of the workday was at a 10 mG exposure and 50% was at background, 1 mG. This gives an average exposure of 5.5 mG. For tailors, they assumed that only 10% of the workday was spent sewing, so the mean exposure was 1.9 mG. There are several problems with this determination of exposure for seamstresses and tailors:

1. exposures to the head are among the lowest body exposures and are not necessarily the sole important exposure;
2. even in Sweden, it is unlikely that home sewing machines were exclusively used. It is more likely that most of the machines were industrial machines, which produce much higher fields constantly, even when sewing is not occurring;
3. seamstresses have exposure most of the workday;
4. ambient exposure levels in industrial settings have been measured at up to 6 mG (Sobel and Davanipour, unpublished Finnish data);
5. tailors would not make a living sewing only 0.8 hours per day.

Hansen *et al.* (2000) found that, at the side of the waist, mean full-shift exposure for industrial machines was approximately 30 mG, while Qiu used a figure of 10 mG. Based on unpublished measurements on AC home sewing machines, Sobel and Davanipour (1996c) found that exposures

to the head were usually the lowest measurements, while the chest, pelvic area, thigh, knee, right arm and hand had much higher exposures (Table 8). In addition, foot pedals can produce high magnetic fields (Table 8). Also, AC/DC converters in the handles (right side) of computerized home sewing machines constantly produce high magnetic fields – about 75 mG at 2 inches away from the handle. The right hand, lower right arm, and knee regularly receive high exposures (Table 8). Thus, the 10% sewing time assumed by Qiu *et al*. (2004) does not mean that significant exposure is not over a longer time period. The biological plausibility of hypotheses discussed above provides an argument that exposure to other body parts may also be deleterious. The numbers or percentages of industrial seamstresses and/or home sewing machine workers were not provided by Qui *et al*. Note: seamstress' exposure assessment is discussed further in Section V.B.

Nevertheless, for the principal occupation, but not for the last occupation or cumulative lifetime exposure, Qiu *et al*. (2004) found statistically significant ORs: OR=2.3 (95% CI = [1.0 – 5.1]) for AD and OR=2.0 (95% CI = [1.1 – 3.7]) for any dementia for men with average exposures greater than 2 mG. For women, no increase in risk was found for the principal occupation, last occupation, and all occupations combined. The average lengths of time in the last and principal occupations were not provided. Thus, comparison with the Feychting *et al.* study (1998a) could not be made.

The proportions of subjects with at least 2 mG exposure were 28.2% for AD cases and 28.8% for controls for the principal occupation (Table 6). For all occupations combined, the proportions were higher. For men, with cases and controls combined, the proportions were 43.1% and 33.0%, respectively, for principal occupation and all occupations combined. For women, the proportions were 24.3% and 32.1%. In the Sobel *et al*. (1995, 1996b) and Davanipour *et al*. (2007) studies, the proportion of female cases and controls with medium or high exposure (considered above 2 mG) was only 5.5%, 80% of whom were seamstresses or had allied professions with significant ELF MF exposure, e.g., cutter. Thus, in these three publications, the exposure category for women contained a higher percentage of subjects with very high exposure. This may explain the lack of findings among women. The occupations which were in the exposure categories 'at least 2 mG' (dichotomized exposure) or 'at least 1.8 mG' (trichotomized) were not provided by Qiu *et al.*(2004).

Harmanci et al. (2003) *Study* Harmanci *et al*. (2003) conducted a cross-sectional, population-based study of Alzheimer's disease by selecting a random sample of 1067 subjects at least age 70, among whom 1019 (96%) agreed to participate in the study. AD was determined in a two-step process: a screening exam using the Turkish version of the Mini-Mental State Exam MMSE, followed by an expert clinical exam among those whose MMSE scored indicated cognitive impairment. Two hundred twenty three (223) were asked to have a clinical exam, and 155 (69.5%) agreed. Among the subjects with a "normal" score on the MMSE, 126 were randomly selected for a clinical examination. Among these 281 subjects, 57 were clinically diagnosed as having possible AD, and 127 were determined to be cognitively normal. These subjects were included in the case-control study. M/H ELF MF exposed occupations were stenographers and typists, carpenters and joiners, metal molders and core makers, tailors, dressmakers, and hatters. Except for stenographers, these occupations were considered to result in medium or high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and current study. A stepwise backwards logistic regression analysis was used. Medium/high ELF MF exposure occupations had an adjusted OR of 4.0, with a 95% CI of [1.02 – 15.78]. It is interesting to note that use of electrical residential heating was also a risk factor (OR = 2.8, 95% CI = [1.1 – 6.9]).

Feychting et al. (1998a) *Study* In the case-control study by Feychting *et al.* (1998a), ELF MF exposure during the last occupation, but not during the longest held occupation, was a risk factor

for dementia not caused by a single stroke. The last occupation was held an average of 24.8 years among cases and 25.9 and 25.1 years among subjects within the two control groups. Consequently exposure during the last occupation was over a significant period of time. Using the two control groups, the ORs for dementia were 3.3 and 3.8 with 95% CIs of [1.3 – 8.6] and [1.4 – 10.2] for occupations with geometric mean ELF MF exposures estimated to be at least 2 mG. Housewives were excluded from the analyses. The ORs for Alzheimer's disease were somewhat lower (2.4 and 2.7). When the analysis was restricted to subjects aged 75 and below at onset or examination, the ORs (5.0 and 4.8) for AD were statistically significant. Also, for subjects of all ages with occupations likely to have resulted in an average ELF MF exposure above 5 mG, the ORs for AD were both high, but significant for one referent group (OR = 8.3), and not for the other (OR = 4.1). The Feychting *et al.* study was small: 44 dementia cases had occupational data, 29 of whom were diagnosed with AD. 43% of the cases were in the ELF MF exposed group, while 23% and 19% of the controls were in this exposure group. Given these high percentages, it is clear that some lower ELF MF exposed occupations were classified in the exposed category than were classified in this study and the earlier Sobel *et al.* studies (1995, 1996b).

Chang et al. (2004) Study Chang et al. (2004) studied exposure to ELF MFs and other possible risk factors for AD among 62 AD patients and 124 controls, all of whom were elderly ex-military personnel, aged 66 to 102. (The published paper is in Chinese and we only have the PubMed English translation of the article's abstract.) Cases and controls were matched for age. Univariate and multivariate logistic regression models were analyzed. "Early" exposure to ELF MFs had an odds ratio of 2.49, with a 95% CI of (0.96-6.45).

Röösli et al. 2007 Study (Röösli et al. 2007) used records from the Swiss Federal Railway on employees who were employed or retired between January 1, 1972 and December 31, 2002. Employees in the following categories were used in analyses: train drivers, shunting yard engineers, train attendants, and station masters. "Average" ELF MF exposure for each year was assessed, based on measurements and "modeling". Five (5) ELF MF exposure indices were used: train drivers vs the other 4 occupations; cumulative work-time exposure (microtesla [μT] years); cumulative time above 10 μT; cumulative exposure up to 10 years prior to death or study closure; exposure within 20 years before death or study closure. Death certificates were used to determine disease status: AD (not coded in ICD-8 and only for subjects whose death was from 1995-2002); senile dementia (including AD); Parkinson's disease (PD); amyotrophic lateral sclerosis (ALS); cardiovascular disease (CVD); and respiratory tumor (RT). The total sample size for analysis was 20,141. Cox proportional hazards models were used to estimate the hazard ratio (HR) with station masters as the referent group. Station masters had, by far, the lowest ELF MF exposure.

Generally, train drivers experienced a very much higher ELF MF exposure than shunting yard engineers, train attendants, or station masters. ELF MF exposure was not associated with death due to (or with) CVD, PD, ALS, or RT. For senile dementia, which included AD, the HR for train drivers was 1.96, with a 95% CI of (0.98-3.92). For AD only, the HR was 3.15 with a 95% CI of (0.90-11.04). It should be noted that the number of deaths due to or with senile dementia or AD were small among the train drivers, shunting yard engineers, train attendants, and station masters, respectively: 30, 3, 17, 11 for senile dementia; 14, 2, 6, 3 for AD. This leads to wide confidence intervals.

Risks associated with increasing cumulative ELF MF exposure were assessed by determining hazard ratios related to exposure tertiles, with the lowest tertile as the referent group. There was an apparent possible increase in risk for subjects in the highest tertile, although the 95% CIs

included 1.0.

Risks were also assessed by determining the HR for the number of years of exposure at or above 10 μT. In this analysis, risk increased by 5.7% for senile dementia and 9.4% for AD. Both figures are statistically significant at the 0.05 level: 95% CIs were above 1.0.

Studies with Only or Mostly Negative Results

Graves et al. (1999) *Study* Graves *et al.* (1999) studied 89 matched case-control pairs. Complete occupational histories were obtained. ELF MF exposure in a given occupation was defined as having at least "probable intermittent exposures (a few minutes)" above 3 mG. A high exposure category was defined as exposure of "1 to several hours" above 3 mG. Two industrial hygienists rated the occupations. Thus, many exposed subjects likely had a low average exposure. 19.1% and 21.4% of the cases were considered to have been 'ever' exposed, while 21.4% and 22.5% of the controls were considered 'ever' exposed. An unknown number of subjects, classified as having experienced ELF MF exposure, would not have been so classified in most or all of the other studies of neurodegenerative diseases or cancer. The estimated adjusted ORs for 'ever' having been exposed were 0.74 and 0.95, depending upon which industrial hygienist's classification was used (Graves *et al.*, 1999).

As noted above, the Feychting *et al.* (1998a) study found elevated odds ratios associated with the last occupation, and in the Sobel *et al.* studies (1995, 1996b) and the Davanipour *et al.* (2007) study, occupational information most likely related to the last occupation. Also, Feychting *et al.* (1998a) did not find an increased risk associated with measures which included earlier occupations, e.g., highest exposed occupation and longest held occupation. Qui *et al.* (2004) found elevated risk associated with the principal occupation for males. Consequently, 'ever' vs 'never' exposed, as used by Graves *et al.* (1999), may not be an appropriate comparison.

Graves *et al.* (1999) also used a cumulative exposure index, the weighted sum of the numbers of years in each occupation with the weights being 0, 1 and 2 for no exposure, only "intermittent exposures" above 3 mG, and exposure for "1 to several hours" above 3 mG, respectively. Using the non-zero cumulative index values, exposure was dichotomized at the median as 'low' or 'high'. Adjusted ORs for 'low' or 'high' cumulative exposure versus no exposure were also close to 1.0. The last or the primary occupation was not separately analyzed.

In summary, the non-significance of the ORs in the Graves *et al.* (1999) study may be due to three reasons: (1) less restrictive definitions of magnetic field exposure resulting in minimally exposed subjects being classified as having been 'ever exposed' or even highly exposed; (2) equal weight given to exposure during any age period, e.g., age 25-45 and age 45-65; (3) a cumulative exposure metric which equates what can be negligible exposure with significant exposure, e.g., negligible exposure for 20 years equals significant exposure for 10 years. In addition, there were no seamstresses among their subjects, who were from an HMO established primarily for union families. Seamstresses are seldom in a union.

Seidler et al. (2007) Seidler et al. (2007) conducted a case-control study by recruiting dementia-diagnosed cases, all 65 or older, from 23 general practices located in Frankfurt-on Main and neighboring cities. Recruitment was primarily based on the Mini-Mental State Examination. The Hachinski Ischemic Score was used in an attempt to differentiate between AD and vascular dementia (VaD). 195 cases (45 men and 150 women) were obtained: 108 were thought to have

"possible" AD, 59 "possible" VaD, 25 had "secondary" dementia, and 3 an "unclassified" dementia. Imaging studies were also used for differential diagnostic purposes, if available. Population controls were randomly selected among those 65+ years of age who scored at least 27 on the MMSE. A second control group was selected from the general practices which contributed dementia cases. These controls needed to be ambulatory and also were required to have a MMSE of 27 or above. The authors state, but do not provide any other information, that "preliminary" analyses using the control groups separately produced "comparable results" with one exception: the ORs for blue collar work were "markedly" higher ($p<0.1$) for ambulatory controls than for population controls. Based on these unpublished analyses, the control groups were combined for "final" analyses. There were 229 controls in these latter analyses: 75 men and 154 women.

Analyses are conducted for dementia, possible AD, and possible VaD cases. However, the diagnostic methods used were really quite insufficient. For example, subjects with depression often have a low MMSE score.

Occupational histories were obtained by interview. Informational items obtained were job phase, job title, industry, and specific job tasks for every job that lasted at least one year. Next-of-kin were used for the dementia subjects, unless there was no next-of-kin and the subject was in the "first signs of dementia". These cases were not excluded in the published results because the results were not "fundamentally" different without them. Only jobs prior to the date of symptom onset or more than 4 years prior to dementia diagnosis if symptom onset timing was unknown were considered. Again, exclusion of these cases did not "substantially" alter the study results. The median time interval between the end of the last job and dementia diagnosis was 17 years for men and 24 years for women, while the for the controls the medians were 10 and 21 years, respectively.

Job titles were coded by experienced members the Frankfurt Institute for Occupational Medicine according to the Classification of the Federal Statistical Office in Germany and the Occupational Classification of the Finnish Censuses. Two-digit occupational codes were used. ELF MF exposure levels for each job were estimated by an "expert" co-author from the German Federal Institute for Occupational Safety and Health, blinded to case-control status. Exposure categories were specified as follows: < 1 mG; 1-2 mG; 2-10 mG, 10-100 mG,; 100-1000 mG, and > 1000 mG. (It is not clear in which category the lower and upper limits of each of the middle 4 categories belong.)

Analyses were based on cumulative exposure and maximum exposure to ELF MF, as determined by the expert co-author. ORs were determined for the 15 primary occupational two-digit categories (ever vs never worked in the category and per 10 years work) and for estimated cumulative exposure and maximum exposure. ORs were adjusted for age, region, gender, dementia in parents, and pack-years of smoking. The referent group consisted of subjects who never worked in the given category and who held white-collar jobs as their main occupation

Statistically significant findings among the ever vs never analyses were as follows:

Dementia Cases

- food & beverage processors; tobacco product makers - OR=4.1, 95% CI = (1.4 , 11.8);

- laborers (unskilled workers) – OR=7.6; 95% CI = (1.7 , 34.2);
- blue-collar work as the main occupation – OR=1.6; 95% CI = (1.0 , 2.5)

AD Cases

- blue-collar work as the main occupation – OR=1.7; 95% CI = (1.0 , 3.1)

VaD Cases

- food & beverage processors; tobacco product makers - OR=7.3, 95% CI = (2.0, 27.3);
- laborers (unskilled workers) – OR=6.3; 95% CI = (1.0 , 39.2).

Analyses based on "per 10 years" of work which were statistically significant or nearly so for possible AD were as follows:

- metal workers (machinery fitters, machine assemblers, mechanics, manufacturers of precision instruments, plumbers, welders, sheet metal and structural metal preparers and erectors – OR=2.2; 95% CI = (1.0 , 5.1),
- electrical and electronics workers – OR=2.7; 95% CI = (0.9 , 8.1),
- spinners, weavers, knitters, dyers, tailors, dressmakers – OR=1.4; 95% CI = (0.9 , 2.2),
- construction workers, including structural engineers, civil engineers) – OR=12.9; 95% CI = (0.9 , 186).

The "ever" versus "never" analyses are really quite inappropriate because the duration of time in the specific and general occupational categories can be quite low. The "per 10 years" analyses are thus more appropriate, but the sample sizes within job categories are quite small, except for "spinners, weavers, knitters, dyers, tailors, and dressmakers". However, it is not clear what the actual ELF MF exposures for spinners, weavers, knitters, and dyers might be.

The categories of (1) metal workers, (2) electrical and electronics workers, (3) spinners, weavers, knitters, dyers, tailors, and dressmakers; and (4), construction workers contain many of the occupations classified as medium or high ELF MF exposed occupations in the Sobel, Davanipour et al. papers and the papers by those who have essentially used the same classification methodology. One of the problems in the Seidel el al. (2007) paper is that the higher classification categories contain may occupations with low exposure.

The authors have available to them the actual specific occupations of each subject. They could therefore classify subject ELF MF exposure using the Sobel-Davanipour et al. methodology to reanalyze their data and determine if their findings for presumptive dementia (cognitive dysfunction) or AD patients replicate (or not) the Sobel, Davanipour et al. findings.

Andel et al. (2010) Study This study uses subjects from the Swedish Twin Registry. All subjects were 65 years or older in 1998. In all, 9,508 subjects had both a dementia/AD diagnostic workup and ELF MF occupational exposure estimates. 27.9% of the subjects were classified as having high exposure – above 2 mG. Among the subjects diagnosed as having dementia, 33.8% were classified as having had high exposure. The figure for subjects diagnosed with dementia was 34.0%. Among

the controls, the corresponding figure was 27.8%. Dementia and AD were diagnosed in a structured, presumably appropriate manner : 216 (2.27%) with dementia; 141 (1.49%) with AD. Age at dementia onset (≤ 75 vs > 75) was determined by informants, presumably family members. Analyses were adjusted for covariates: gender, education, coronary disease, and stroke. Subjects were classified into three (3) exposure groups: < 1.2 mG, 1.2 to < 2.0 mG, and ≥ 2.0 mG. The referent group consisted of subjects with estimated exposure below 1.2 mG. Note that in the manuscript microTesla (μT) units were used: 1 mG = 0.1 μT. For all subjects, the dementia adjusted odds ratios (AORs) were 1.41 (p=0.079) for exposure between 1.2 and <2.0 mG and 1.38 (p=0.108) for exposure ≥ 2.0 mG. The AD AORs were 1.35 (p=0.211) and 1.38 (p=1.53). For age of onset ≤ 75, the AORs were 1.94 (p=0.03) and 2.01 (p=0.022) for all types of dementia and 1.69 (p=0.215) and 1.94 (p=0.090) for AD. For age of onset greater than 75, the AORs were much closer to 1.0 and clearly not significant. Analyses were conducted also for manual and non-manual workers separately. AORs for non-manual workers were clearly non-significant. For manual workers, the AORs for dementia and AD had p-values below 0.05, except for exposure ≥ 2.0 mG for AD when the p-value was 0.056.

It is our opinion that the ELF MF exposure assessment is not accurate in this study and other studies (e.g., breast cancer) which use the same exposure assessment methods and data. Specific occupational information was obtained by interview and then sent to "Statistics Sweden for coding according to categories from the 1980 Swedish Population and Housing Census". For men, occupational exposure assessment was based on measurements of a sample of 1098 Swedish men (Floderus et al., 1996). For women, the results of a study of 49 occupations by Forssén et al. (2004) have been used. This latter paper is also discussed below in our discussion of breast cancer, primarily in Section IV.E. We have two major concerns with the occupational classifications with respect to ELF MF exposure:

1. Generally, government classifications of occupation are wider than occupational determination based on individual subject information. Individual ELF MF exposure classification based on government classifications is therefore not likely to be particularly accurate. This will result in many individuals being misclassified as having exposures above 2 mG. The exposure classification methodology used by Davanipour, Sobel et al. and others has, we believe, much lower misclassification rates for 2.0 mG and above. For example in Davanipour et al. (2007) the rates of classification were 7.5% and 3.8% for AD cases and controls, respectively. As stated above, the comparable classification rate in the Andel et al. (2010) study was 27.9%.
2. The Forssén et al. (2004) measurements for women classified seamstresses as having low ELF MF exposure. This is very much out of line with our experience in Finland and in California and with the experiences of other researchers. Davanipour & Sobel measured ELF MF exposures in two clothing manufacturing companies in Finland. The ambient exposure, except during lunch time, among seamstresses and associated workers (e.g., cutters) in the same areas was over 6 mG. Exposures of individual seamstresses, all of whom used AC current industrial sewing machines, were much higher at every body location. We personally measured scores of seamstresses. The lowest exposure to any body part was 20 mG (e.g., Hansen et al., 2000). The usual work pattern was as follows: (1) the seamstress sits at a U-shaped table; (2) clothes to be sewed are folded on the right hand side; (3) the seamstress selects an article, sews it as specified; and (4) refolds the article, placing it on the left hand side of the desk.

All this time, the sewing machine is producing ELF MFs. This is because the motor is always on and a clutch needs to be engaged in order to move the needle. The seamstresses are doing this work for 6-8 hours per day. Seamstresses who work in drycleaners stores certainly do not sew all day long, so their exposure would be lower.

E. RF Exposure and Alzheimer's Disease

We found no human studies of AD and RF to discuss. The single published epidemiologic study of RF and melatonin is discussed in Section II (Burch *et al.*, 2002).

E.1. Transthyretin Studies

There have, however, been studies related to the effect RF exposure on transthyretin (TTR), also referred to as prealbumin. TTR is found in the brain, cerebrospinal fluid (CSF), and blood. Based on earlier research related to Aβ deposition (discussed below), Söderqvist et al. (2009a,b) investigated the effect(s) of RF on TTR in two studies. Söderqvist et al. (2010) discusses these same studies. In these studies, serum TTR levels are used as indicators for CSF and (presumably) brain TTR levels. However, there is apparently no study demonstrating that this assumption is valid.

1. In the 2009a study, 500 females and 500 males, aged 18-65, were randomly recruited from the municipality of Örebro, Sweden. Consenting subjects initially completed a questionnaire which included employment history, use of specific types of wireless telephones, X-ray, chemical, and radiation exposures (e.g., in medical therapy), and health and lifestyle questions, including physical exercise and disease history. An initial blood sample was collected from each subject as close to the end of a work week as possible. TTR concentrations (g/L) were determined using "standard immunoephelometric techniques". 133 (26.6%) of the male and 184 (36.8%) of the female subjects who were "recruited" fully participated. TTR assay results were log-transformed in all statistical analyses. Short-term wireless telephone use was determined by cumulative use (minutes) on the day the blood sample was delivered. Long-term use had two categories: "cumulative use" in total hours; and years since initial use. <u>These short- and long-term figures were presumably guestimates by the study subjects.</u> High TTR was chosen as the highest quartile (> 0.31 g/L. Low TTR was ≤ 0.31 g/L.

   There was no indication that wireless telephone use for at least 5 years or at least 10 years affected TTR levels as dichotomized. However, using the TTR levels themselves, for cumulative use, among men, there was an indication of increased risk with increasing use of mobile telephones (both analogue and digital). That is, the p-values were between 0.05 and 1.0. For years since first use, among men, the results were stronger. The p-values were below 0.05 for mobile telephones (all phones and analogue only). However, among men, for Universal Mobile Telecommunications System *(*UMTS*)* telephones there was declining risk with higher use (p=0.02).

   For short-term use, there were no findings of significance or, evidently, marginal significance, except in one instance. Among women, the shorter the time between last use of a mobile telephone and blood samples, the lower the TTR value (p-0.03).

There is no indication that the statistically significant or marginally significant finding have any biological importance.

2. Based on these short-term use finding, Söderqvist et al. conducted a "provocation" study, exposing volunteers to an 890 MHz mobile "phone-like" signal. Forty-four volunteers, aged 18-30 were recruited. Exposures occurred during the working day: 8 am – 5 pm. Exposures were over a 2 hour period, with blood samples collected prior to exposure, after a 30 minutes "rest" period, immediately following the provocation, and 60 minutes after the provocation. The provocation exposure had an average $kSAR_{1G}$ of 1.0 watts/kg. Seemingly the study design did not work out very well. The biggest mean change was a decrease between sample 1 and sample 2, when presumably nothing much was happening, except that the subjects were told to rest. The mean changes were very minimal between sample 2 and post-exposure samples 3 and 4, especially compared to the between subject values. There was also a control group who did not have any exposure. Their TTR measurements were not much different from the experimental groups measurements. However, no statistical comparison was presented.

   In short, this study seems to have provided no useful information.

The questions of importance here are (i) whether TTR concentrations in serum are indicative of concentrations in the CSF and brain and (ii) whether TTR inhibits or increases the aggregation and neurotoxicity of Aβ.

i. As mentioned above, we could find no studies of the relationship(s) between serum and CSF or brain levels of TTR.

ii. In *in vitro* studies, Schwarzman et al. (1994, 1996) found that CSF TTR binds to Aβ, possibly preventing or limiting amyloid formation within the brain. Their conclusion was that perhaps TTR helps prevent or delay AD onset. Serot et al. (1997) studied elderly AD patients and controls with ages between 2 and 90. TTR concentrations in CSF increased with age among the controls. TTR concentrations among the AD cases were similar to those controls in middle age and lower than the elderly controls (20.02 mg/l (sd=2.45) vs 17.49 mg/l (sd=2.02), $p<0.001$). The authors suggest that AD development may result in a lowering of TTR secretion. Lovell et al. (2008) studied the "aberrant" protein complex prostaglandin-d-synthase (PSD) and TTR in the CSF of autopsy verified late-onset AD patients, patients with mild cognitive impairment (MCI), and controls. They found that complexed PDS/TTR was significantly increased in the ventricular CSF of the AD and MCI patients compared to normal controls. This possibly explains the results of Serot et al. (1997). Animal and cell studies have found that TTR infusion leads to a reduction in Aβ deposits (Link, 1995), lack of neurodegeneration in the transgenic mouse AD model Tg2576 (Stein and Johnson, 2002), inhibition of Aβ aggregation, toxicity, and induced apoptotic changes in cultured cells (Giunta et al., 2005).

   Wati et al. (2009) then studied TTR and vascular Aβ deposition in two (2) transgenic mouse models of AD: Tg2576/$TTR^{-/-}$ which lacks endogenous TTR, but produces human variant amyloid precursor protein (APP), and Tg2576/$TTR^{+/-}$, which does not lack endogenous TTR. The Tg2576/$TTR^{-/-}$ mice had a significantly reduced Aβ burden compared to the Tg2576/$TTR^{+/-}$ mice, contrary to the researchers expectations. Their result indicates that, in their animal model, TTR appears to be associated with increased

risk of amyloid burden.

On the other hand, using a different mouse model ceAPPswe/PSIΔE9/TTR$^{+/-}$ versus ceAPPswe/PSIΔE9/TTR$^{+/+}$, Choi et al. (2007) found that amyloid deposition in the hippocampus and cortex was elevated in the brains and "accelerated" in the hippocampus and cortex of the ceAPPswe/PSIΔE9/TTR$^{+/-}$ mice compared to the ceAPPswe/PSIΔE9/TTR$^{+/+}$.

Thus, results may be dependent upon differences between experimental species or sub-species. This suggests that (1) replication is warranted and (2) concentration on studies involving humans is appropriate if animal model replications continue to demonstrate differing results.

E.2. RF and Mitochondrial DNA (mtDNA) Oxidative Damage

Coskun et al. (2010) have demonstrated that mutations in the control region of mtDNA accumulate in the brain with age, with AD patients having a significant elevation of these mutations. These mutations in AD patients are associated with a reduced mtDNA copy number. They found that these mutations generally increase with age, both within the brain and in peripheral blood DNA and lymphoblastoid cell DNA. They argue that the mtDNA mutation level is inversely correlated with mtDNA copy number and positively correlated with beta-secretase activity, an indicator of increasing amyloid beta. Consequently, mtDNA damage may be associated with increased risk of AD.

Xu et al. (2010) studied oxidative damage to mitochondrial DNA related to 1800 MHz RF exposure in primary cultured cortical neurons. The neurons were exposed to 1800 MHz modulated by 217 Hz, using an average special absorption rate of 2 watts/kg for 24 hours. Examination of the neurons demonstrated a significant increase in 8-hydrodeoxyguanosine (8-oxodG), an indication of increased DNA damage. In addition, there was a clear reduction in the copy number of mtDNA and in the level of mtRNA after RF exposure. Xu et al. (2010) also conducted replicate assays, but with the addition of melatonin. The effects of RF exposure were reversed, but not completely.

IV. **BREAST CANCER**

Figure 2 provides a schematic outline of the areas of study providing evidence that ELF MF exposure can lead to breast cancer through an effect on melatonin production levels, and, of course, possible but unknown other pathways. Section references are provided in Figure 2.

There is now accumulating evidence that low melatonin production may increase the risk of breast cancer (BC). This evidence comes from *in vitro*, animal, and two longitudinal human studies. The *in vitro* and animal study literature is quite extensive, so only a highlight review is provided. There are numerous published case-control studies of residential and occupational ELF MF exposure as a risk factor for breast cancer. No epidemiologic studies of radiofrequency MF exposures and breast cancer have been published, which do not include ELF MF exposure, and which have reasonable data on RF exposure.

For a review of melatonin from basic research to cancer treatment, see Vjayalaxmi *et al.*, 2002.

- *Conclusion: There is sufficient evidence from in vitro and animal studies, from human biomarker studies, and from occupational and light at night studies to conclude that high ELF MF exposure may certainly be a risk factor for breast cancer. Most of the residential ELF MF exposure studies have been negative. This may be because "high" residential exposures are actually not very high. Individual exposures may be of importance, e.g., home sewing machines, hair dryers, AC/DC converters near the head of the bed, water pipes causing intermittent high exposures near living room or TV room sofas and easy chairs.*

As with Alzheimer's disease, we provide the results of a meta-analysis for breast cancer (Chen et al., 2010) despite our antipathy for such analyses, due primarily to varying study design components, exposure assessments, and subject differences. Chen et al. (2010) chose 15 studies published between 2000 and 2009. They found no associations between ELF MF exposure and (female) BC, including subgroup analyses based on exposure modes, menopausal status, and estrogen receptor status. These results are said to be in agreement with results by Erren (2001). Chen et al. (2010) found no statistically significant association between ELF MF exposure (residential, electric blanket, or occupational) and BC in general or BC based on menopausal status or ER status. There was substantial heterogeneity between studies. On the other hand, Erren (2001) found, using earlier studies not included in Chen et al. (2010), a slightly increased risk (referred to as RR) of BC in general: 1.12, 95% CI = (1.09 , 1.15). This is clearly statistically significant due to the very large sample size. Erren (2001) remarks that the results are quite variable between studies and "in part contradictory". He found that the primary methodologic problems were "probable misclassification of exposure" and "possible misclassification of the disease itself". Thus Chen et al.'s (2010) claims that (1) their results suggest no association between ELF MF exposure and BC and (2) are "in accordance" with Erren's results (2001) should be taken with a grain of salt.

A. *In Vitro* and Animal Studies Relating to Melatonin as a Protective Factor against Breast Cancer

A.1. *In Vitro* Studies Related to Prevention of Oxidative Damage; Comparative *in vivo* Studies with Vitamin C and Vitamin E

Melatonin has been found to neutralize hydroxyl radicals and to reduce oxidative damage in over 800 publications (Reiter et al., 1995; Tan *et al.*, 2002). Melatonin has also been shown to act synergistically with vitamin C, vitamin E and glutathione (Tan *et al.*, 2000) and stimulates the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter *et al.*, 2002).

- Using a cell-free system, Tan et al. and others have demonstrated that melatonin neutralizes hydroxyl radicals more efficiently than does reduced glutathione Tan *et al.*, 1993a; Bromme *et al.*, 2000).
- Melatonin reduces oxidative damage to macromolecules in the presence of free radicals (Reiter *et al.*, 1997, 2001a). One mode of action is as a free radical scavenger (Reiter *et al.*, 2001b).
- Melatonin increases the effectiveness of other antioxidants, e.g., superoxide dismutase, glutathione peroxidase, and catalase (Antolin *et al.*, 1996; Kotler *et al.*, 1998; Pablos *et al.*,

1995; Barlow-Walden *et al.*, 1995; Montilla *et al.*, 1997).

- Melatonin has protective effects against ultraviolet and ionizing radiation (e.g., Vijayalaxmi *et al.*, 1995). Vijayalaxmi et al. studied the effects of melatonin on radiation induced chromosomal damage in human peripheral blood lymphocytes (Vijayalaxmi *et al.*, 1996). Blood from human volunteers was collected before and after administration of a single 300 mg oral dose of melatonin. The post-administration samples of both serum and leukocytes had increased concentration of melatonin compared to the samples prior to melatonin administration. After gamma radiation and mitogen exposure, a sample of cells was cultured for 48-72 hours. Lymphocytes from the sample after melatonin was administered had significantly fewer chromosomal aberrations and micronuclei. Primary DNA damage was reduced. Vijayalaxmi et al. hypothesized that melatonin, in addition to its hydroxyl radical scavenging, may also stimulate or activate DNA repair processes (Vijayalaxmi *et al.*, 1998).

Melatonin has been found to be a more potent protector from oxidative injury than vitamin C or vitamin E (micromoles/kg) in several *in vivo* studies (for a review, see: Tan *et al.*, 2002). Melatonin was also found *in vitro* to scavenge peroxyl radicals more effectively than vitamin E, vitamin C or reduced glutathione (Pieri *et al.*, 1994; Reiter *et al.* 1995), although melatonin is not a very strong scavenger of peroxyl radicals (Reiter *et al.*, 2001b).

A.2. **Animal Studies of Mammary Tumor Prevention with Melatonin**

Several studies have found that melatonin inhibits the incidence of mammary tumors in laboratory animals either prone to such tumors or exposed to a carcinogen (e.g., Tamarkin *et al.*, 1981; Shah *et al.*, 1984; Kothari *et al.*, 1984; Subramanian and Kothari, 1991a,b; Blask *et al.*, 1991). In 1981, Tamarkin et al. found that supplemental melatonin, given on the same day as 7,12-dimethylbenz(alpha)-anthracene (DMBA) and continued for 90 days, lowered the incidence of mammary tumors from 79% in controls to 20% ($p<0.002$) in the melatonin treated Sprague-Dawley rats (Tamarkin *et al.*, 1981). When they treated pinealectomized rats with DMBA, the incidence of mammary tumors increased to 88%, indicating a possible effect on endogenous melatonin on tumor incidence. Similar results, but with somewhat different study designs, using female Holtzman rats given the carcinogen 9,10-dimethylbenzanthracene have been found (Shah *et al.*, 1984; Kothari *et al.*, 1984). Subramanian and Kothari studied the suppressive effect by melatonin in rats treated similarly with DMBA under varying light:dark schedules and time of melatonin administration in both intact and pinealectomized female Holtzman rats (Subramanian and Kothari, 1991a). They found that when administered during the initiation phase, melatonin only suppressed tumor development in intact animals. However, when administered during the promotion phase, melatonin had suppressive effects regardless of the presence or absence of the pineal gland. Subramanian and Kothari (1991b) also studied C3H/Jax mice and spontaneous mammary tumor development. Mammary tumors developed in 23.1% of mice provided with melatonin from 21 to 44 days of age, but in 62.5% of control mice ($p<0.02$). Furthermore, there was a decrease in serum 17-beta-estradiol levels in the melatonin treated mice ($p<0.05$). In a N-methyl-N-nitrosourea (NMU) model of hormone-responsive Sprague-Dawley rat mammary carcinogenesis, Blask *et al.* (1991) found that melatonin, given during the promotion phase, reduced the incidence of tumors and antagonized estradiol's stimulation of NMU-induced tumor incidence and growth. They, however, did not find a decrease in estradiol in the melatonin treated rats.

In two studies, Tan *et al.* (1993b, 1994) found that melatonin protected Sprague-Dawley rats from safrole induced liver DNA adduct formation. The protection was found at both physiological and pharmacological levels of supplementation. The level of protection was dose dependent. Intraperitoneal injection of paraquat causes lipid peroxidation, a decrease in total glutathione, and an increase in oxidized glutathione in Sprague-Dawley rats. Melchiorri et al. found that melatonin inhibits these effects (Melchiorri *et al.*, 1995). In addition, melatonin and retinoic acid appear to act synergistically in the chemoprevention of animal model tumors (Teplitzky *et al.*, 2001) and *in vitro* systems (e.g., Eck-Enriquez *et al.*, 2000).

A.3. **Animal Studies Related to Prevention of Oxidative DNA Damage by Estradiol and Radiation**

Karbownik *et al.* (2001) found that melatonin protects against DNA damage in the liver and kidney of male hamsters caused by estradiol treatment. They also found that in the testes, estradiol did not increase DNA damage, but that melatonin was protective against the natural level of oxidative DNA damage, as indicated by 8-hydrodeoxyguanosine (8-oxodG) levels. Several studies have found that laboratory animals are protected by melatonin from lethal doses of ionizing radiation (e.g., Blickenstaff *et al.*, 1994; Vijayalaxmi *et al.*, 1999; Karbownik *et al.*, 2000). Vijayalaxmi *et al.* (1999) and Karbownik *et al.* (2000) investigated markers of oxidative DNA damage and found that significant decreases in these markers in the melatonin treated animals.

A.4. **Melatonin: Scavenger of $^{\bullet}OH$ and Other ROS**

Melatonin is a powerful, endogenously produced scavenger of reactive oxygen species (ROS), particularly the hydroxyl radical ($^{\bullet}OH$). Other ROS which melatonin scavenges include hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), peroxynitrite anion ($ONOO^-$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Reiter, 1991; Tan *et al.*, 2000, Hardeland *et al.*, 1995; Antolin *et al.*, 1997; Stasica *et al.*, 1998). $^{\bullet}OH$ is produced at high levels by natural aerobic activity. ROS are also produced by various biological activities or result from certain environmental and lifestyle (e.g., smoking) exposures.

Hydrogen peroxide does not appear to react directly with DNA (Halliwell, 1998), but does undergo chemical reactions within the cell nucleus which produce $^{\bullet}OH$, e.g., with Fe^{+2}. On the other hand, $^1O_2$ readily oxidizes the guanine base and causes HOCl, $ONOO^-$, and $NO^{\bullet}$ damage in various patterns (Halliwell, 1998).

However, $^{\bullet}OH$ is the most reactive and cytotoxic of the ROS (Halliwell *et al.*, 1986). $^{\bullet}OH$ appears not to be removed by antioxidative enzymes, but is only detoxified by certain direct radical scavengers (Tan *et al.*, 1999) such as melatonin.

Melatonin is found in every cell of the body and readily crosses the blood-brain barrier. It scavenges ROS at both physiologic and pharmacologic concentrations. In the literature, "physiologic" refers to blood level concentrations of melatonin, while "pharmacologic" indicates 2-3 orders of magnitude higher concentration. Recently, intracellular levels of melatonin, especially within the nucleus, have been shown to be naturally at "pharmacologic" levels for all cellular organelles studied to date (Maestroni, 1999; Reiter *et al.*, 2000).

Tan *et al.* (2002) review the underlying basis for melatonin's scavenging of ROS, which is briefly discussed here. From the known structure-activity relationships, the reactive center of the interaction between oxidants and the melatonin molecule is its indole moiety. This is due to its high resonance stability and quite low activation energy barrier towards free radical reactions. In addition, the methoxy and amide side chains contribute significantly to melatonin's antioxidant activity. The methoxy group in the C5 component of the molecule appears to prevent prooxidative activity. If this methoxy group is replaced by a hydroxyl group, under some *in vitro* conditions, melatonin may exhibit prooxidant capability. The mechanisms of melatonin's scavenging ROS appear to involve the donation of an electron to form a melatoninyl cation radical or a radical addition at site C3 of the melatonin molecule. (There are other possibilities also.) All known intermediates generated by the scavenging of a ROS by melatonin are also free radical scavengers. This is known (by some) as the 'free radical scavenging cascade reaction', which allows one melatonin molecule to scavenge 4 or more ROS. (See Tan *et al.*, 2007, for details).

A.5. Melatonin and Oxidatively Damaged Guanine in DNA

Davanipour et al. (2009) published the results of a study relating overnight melatonin production (as measured by aMT6s/creatinine levels in complete overnight urine samples) to the levels of oxidatively damaged guanine in DNA (as measured by urinary guanine damage/repair guanine products 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanine (8-oxoGua). 8-oxodG is a product of the damage/repair of DNA guanine, while 8-oxoGua is a product of the damage/repair of either DNA or RNA guanine. Fifty-five (55) mother-father-oldest adult daughter families were recruited. All were healthy for their age. The age ranges were as follows: mothers – 43-80; fathers – 46-81; daughters – 18-51. The results were as follows:

- with or without adjustment for BMI or weight, among the mothers there was an inverse relationship between creatinine-adjusted aMT6s and 8-oxodG (p=0.02);
- among the mothers older than the oldest daughter (age 51.6) the significance level of the inverse relationship between creatinine-adjusted aMT6s and 8-oxodG fell to 0.009;
- among the fathers older than the oldest daughter, the inverse relationship between 8-oxoGua and creatinine-adjusted aMT6s was significant at the 0.03 level;
- among the oldest daughters, there was an increase in 8-oxoGua with increasing age.

This study appears to be the only research published to date on the relationship between melatonin production and DNA damage/repair in humans.

B. Longitudinal Human Studies of Low Overnight Melatonin Production as a Risk Factor for Breast Cancer

<u>Conclusion</u>: Five longitudinal studies have now been conducted of low melatonin production as a risk factor for breast cancer. Two of the studies collected urine samples in an optimal manner to estimate the important component of melatonin production – overnight production. However, two (2) used first morning void, which is close to optimal and one (1) had to use 24-hour collection, which hides possible non-circadian rhythm, which can be deleterious. One study, which used first morning void urine, was limited to premenopausal BC. The study which used 24-hour urine samples was negative. Of the remaining 4 studies, three were positive and the one limited to premenopausal BC was problematic, perhaps due to lag times and the likely adverse effect of BC in its very early stage on melatonin production.

Thus, there is increasingly strong longitudinal evidence that low melatonin production is a risk factor for at least post-menopausal breast cancer.

There have been five (5) longitudinal studies, two of which were from the Nurses' Health Study cohort, of low melatonin production as a risk factor for breast cancer. Note that many breast cancers are associated with a decrease in melatonin production (Bartsch *et al.*, 1997). There is often a "rebound" after excision of the tumor, but it is not known if post-excision melatonin production is near the pre-tumor production level (Bartsch *et al.*, 1997). Thus, as with AD, it is not appropriate to use post-tumor melatonin levels in a case-control study of low melatonin as a risk factor for breast cancer.

DNA damage is the pathway through which normal cells become malignant. Thus, the greater the amount of DNA, the greater the probabilities of a malignant transformation and the development of cancer. Davanipour *et al.* (2009) have conducted a study on the association between endogenous melatonin levels and oxidative guanine DNA damage among mothers and their oldest sampled daughters. The mothers' age range was 43-80, while the oldest daughter's age range was 18-51. Nearly all of the mothers, but few of the daughters were postmenopausal. Complete overnight urine samples were obtained. Creatinine-adjusted aMT6s and 6- hydrodeoxyguanosine (8-oxodG) were assayed. 8-oxodG is a measure of the level of oxidative DNA damage. Creatinine-adjustment is not necessary because the 8-oxodG level using complete overnight urine is a measure of the total repair of oxidized DNA guanine during the night. There was a statistically significant (p=0.02) inverse association between the level of nocturnal melatonin production (aMT6s/creatinine) and 8-oxodG for the mothers, but not for the daughters. Statistical adjustment was made for age and weight; however, there was little difference in the results with or without adjustment. The correlation between creatinine-adjusted aMT6s and 8- oxodG was 0.35 (p=0.01).

Positive Studies

Schernhammer and Hankinson (2005) reported on the association between urinary melatonin levels and breast cancer risk in the Nurses' Health Study II. The study had collected first morning void urine samples prior to the diagnosis of any cancer in a sub-sample of the women in the study. Assays of aMT6s and creatinine for 147 women who developed invasive breast cancer, and 291 age-matched controls, plus 43 women who developed in situ breast cancer and 85 matched controls were analyzed. Analyses were based on quartiles of creatinine-adjusted aMT6s developed from the control data, with subjects in the lowest quartile as the referent group. (Thus, the analyses were conducted with a view that higher levels of melatonin production might be protective.) Unadjusted analyses, estradiol level adjusted analyses, and analyses adjusted for age-at-menarche, parity, age-at-first birth, family history of BC and benign breast disease, alcohol use, antidepressant use, and body mass index were conducted. It should be noted that low levels of melatonin are causally associated with earlier age-at-menarche (e.g., Cohen *et al.*, 1978; Sizonenko, 1987). Thus, inclusion of age-at-menarche in the adjustment is perhaps not appropriate. Analyses of cases and controls from the lowest and the highest quartile were statistically significant for each level of adjustment. The odds ratios (OR) were all 0.59. (In terms of risk associated with low melatonin production, the OR was 1/0.59 = 1.69.) Inclusion of the the cases with in situ breast cancer led to OR between 0.68 and 0.70. Significance levels were not provided. However, the 95% CI's for invasive breast cancer did not contain 1.0, while the 95% CIs when in situ breast cancer cases were included just

barely contained 1.0.

In 2008, Schernhammer and Hankinson used the Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) cohort to study low overnight melatonin production as a possible risk factor for postmenopausal breast cancer. The ORDET study was conducted in northern Italy and included 10,786 healthy women aged 35-69 at baseline, 3966 of whom were postmenopausal. Complete 12-hour overnight urine samples were obtained. There were 178 subjects who developed postmenopausal BC prior to the Schernhammer et al. study analysis and met inclusion criteria, e.g., BC as the initial cancer, urine sample availability. Seven hundred ten (710) women were selected as controls, matched on age at enrollment (± 3 years), date of recruitment (± 180 days) and laboratory assay batch. Conditional regression models were used for analyses, adjusting for thirteen (13) known BC risk factors and circulating testosterone, which was a BC risk factor in the ORDET study. Analyses were performed using both aMT6s and creatinine-adjusted aMT6s. Analyses were done by quartiles of aMT6s. 95% CIs and trend p-values were calculated. Trend p-values were 0.05 or below when the analyses excluded in situ BC and below 0.10 when in situ BC was included. When analyses were conducted without current smokers, the trend p-values were below 0.005. Comparing the highest versus lowest quartile of aMT6s, the p-values were at or below 0.05 for invasive BC, including or excluding testosterone. When only non-current smokers were analyzed, the p-values were smaller. (Note: only 95% CIs were actually published.) Results were similar for creatinine-adjusted aMT6s analyses.

In 2009, Schernhammer and Hankinson used to Nurses' Health Study cohort to further investigate the relationship between urinary melatonin levels and postmenopausal BC. Spot morning urine assays for aMT6s were available for 357 postmenopausal women who developed incident BC after recruitment into the cohort and 533 matched controls. The analysis methods were much the same as in the previous paper. Quartiles of aMT6s among the controls were analyzed. In multi-variable adjusted analyses, the subjects in the lowest quartile of aMT6s had an increased risk ($p < 0.05$) of developing BC compared to subjects in the highest quartile. This was true for all BC, for in situ BC only, and for invasive BC only. Subjects in the lowest quartile also had an increased risk compared to subjects in the 3rd (highest) quartile for all BCs and for in situ BC only. Trend p-values were below 0.05 for all three groups: all BCs, invasive BC, in situ BC.

** It should be noted that the first morning void, especially when the subject has had urine voids during sleep time, is not as good as complete overnight urine collection in estimating nocturnal melatonin production. **

<u>Negative Study</u>

Travis *et al.* (2004) conducted a study of melatonin and breast cancer using the Island of Guernsey or Guernsey III longitudinal study. This study recruited women for an eight and one-half year period, ending in 1985. During the follow-up period, 127 women developed breast cancer. Three hundred fifty three (353) controls were selected with matching based on age, recruitment date, menopausal status, day of menstrual cycle (if applicable) when the urine sample was obtained, and number of years post-menopausal (if applicable). Twenty-four (24) hour urine samples were collected. These samples were evidently not divided between overnight and other time-of-day sub-samples. None of the analyses (all cases-

controls, only pre-menopausal cases-controls, or only post-menopausal cases-controls) showed any hint of an increase risk associated with low 24-hour melatonin production.

** It is unfortunate that the 24-hour urine samples were not subdivided by time of day. It is the nocturnal blood level of melatonin that is important. About 85%-90% of pineal melatonin is produced nocturnally. The circadian rhythm appears to be vital for the effects of melatonin in regulation of important biologic functions, including immune response. This particular problem with the study makes the results suspect. (See Hrushesky and Blask, 2004, for further details.) **

Problematic/Peculiar Study

In 2010, Schernhammer et al. used the ORDET cohort to investigate premenopausal BC. There were 180 premenopausal BC cases, with 683 controls selected – nearly 4 per case – using the same matching criteria as was previously used. The urine samples were 12 hour, overnight (7:00 pm – 7:00 am) samples. There was a statistically significant trend towards **increasing risk** with higher baseline aMT6s. This was the opposite of what was likely anticipated. However, when current smokers were excluded, the increasing risk completely disappeared. On the other hand, among non-current smokers, a BC diagnosis within 3 years of urine collection was much more likely for subjects in the highest aMT6s quartile compared to subjects in the lowest quartile. Lag time from urine collection to BC diagnosis was also investigated among non-current smokers. Only after 8 years of lag time was there a statistically significant difference between the lowest and highest quartiles of aMT6s: an increase in risk associated with low production. Thus, this study's results are clearly perplexing. The authors recognize this and suggest that perhaps very early BC is causing an increase in melatonin production.

C. No Case-Control Studies of Low Melatonin Production as a Risk Factor for Breast Cancer

As mentioned previously, breast cancer itself often causes a decrease in melatonin production, e.g., Bartsch *et al.* (1997). It is therefore inappropriate to use current levels of melatonin production of breast cancer cases in a case-control study of whether low levels of melatonin are a risk factor for breast cancer, and none have been published.

D. Light-at-Night and Night Shift Work Studies as a Risk Factor for Breast Cancer – Surrogates for Low Melatonin Production

Conclusion: There is moderately strong evidence that both long-term light-at-night and night shift work increase the risk of breast cancer. Five (5) studies are reviewed, 4 of which are positive. The negative study did find an increased risk for light-at-night, but not shift work. This study classified subjects as having had rather short shift work as exposed. Only very few subjects had at least 8 years of shift work: 8 (1.6%) of cases and 19 (3.7%) of controls.

Several studies have found an increase in risk of breast cancer among women who have rotating night shift work or who otherwise experience light at night. Light at night (LAN) is well-known to cause a decrease in nocturnal melatonin production (e.g., Lewy *et al.*, 1980; Lowden *et al.*, 2004; Schernhammer *et al.*, 2004). Note that occupational studies of ELF MF exposure

(Section E, below) have included jobs with night shift work, e.g., flight attendant and radio/telegraph operators.

Positive Studies

- Lie *et al.* (2006) studied the occurrence of breast cancer among Norwegian nurses. All data were obtained from government registers. Among a cohort 44,835 nurses, who graduated from a 3-year nursing program between 1914 and 1980 and who were alive on January 1, 1953, or born after this date, 537 breast cancer cases which occurred between 1960 and 1982 were identified. (1960 was chosen because that was the first year for which fertility data were available.) Four (4) controls, alive and cancer free, for each case were selected from the nurse cohort, matched by year of birth (± 1 year). Controls were required to have graduated or started their initial job no later than the year the corresponding case was diagnosed with BC. Number of years of night shift work was estimated from work history and work locations. Statistical adjustments in OR estimates included total employment time and parity. The OR for 30+ years of night shift employment versus 0 years, was 2.21 ($p<0.05$), 95% CI = [1.10 – 4.45]. The p-value for trend was 0.01. When the analysis was limited to nurses aged 50+, the OR was 2.01 ($p>0.05$), 95% CI = [0.95 – 4.26]. The number of cases without night shift work was only 50 for all ages, and was 29 for nurses over age 50. The number of cases with at least 30 years of night shift work was 24. (No case below age 50 had 30+ years of night shift work.)

- Schernhammer *et al.* (2001) examined rotating night shift work as a possible risk factor for breast cancer in the Nurses' Health Study. The total number of years in which a subject had worked rotating night shifts of at least 3 nights per month was obtained in 1988. The sample was quite large: 31,761 nurses had not had any years meeting the night shift criterion; 40,993 had had 1-14 years; 4,426 had had 15-29 years; and 1,382 had had 30+ years. During the following 10 year period, 2,441 incident cases of breast cancer were identified. Compared to nurses who had had no qualifying years, the adjusted relative risk (RR) for nurses with 30+ years of rotating night shift work was 1.36, with a 95% CI of [1.04 – 1.78]. All subjects with 30+ of rotating night shift work were post-menopausal Analyses were also conducted within pre- and post-menopausal groups. The RR and 95% CI were the same for 30+ years of exposure, because the number of nurses with no exposure decreased slightly (from 925 down to 801). While not statistically significant, perhaps due to sample size, pre-menopausal nurses who had at least 15 years of shift work had an adjusted RR of 1.34, 95% CI = [0.77 – 2.33], essentially the same RR as post-menopausal women (RR=1.36, 95% CI = [1.04 – 1.78]) who worked night shift for at least 30 years. There were only 14 pre-menopausal nurses with 15+ years of exposure. The trend in RR for increasing years of exposure was statistically significant for post-menopausal nurses and all nurses. Adjustments were made for age, weight change between age 18 and menopause, and many other variables associated with breast cancer. The increase in risk was almost totally due to hormone-receptor positive breast cancers. This was the first prospective night shift and breast cancer study.

- Davis *et al.* (2001b) studied 813 breast cancer patients, aged 20-74, and 793 controls. The controls were obtained through random digit dialing and were frequency matched

by 5-year age intervals. Lifetime occupational history, bedroom lighting, and sleep habits were obtained by interview for the 10 years prior to diagnosis. Not sleeping during nocturnal periods (when melatonin production is usually at its peak) had an OR of 1.14 for each night per week. The 95% CI was [1.01 – 1.28]. Night shift work had an OR of 1.6, 95% CI = [1.0 – 2.5]. There was a significant upward trend (p = 0.02) in the OR with increasing years and more hours per week in night shifts. Statistical adjustments were made for parity, family history of BC, oral contraceptive use (ever), and recent (but discontinued) use of hormone replacement therapy.

- Hansen (2001) studied BC risk among younger Danish women whose work was mostly at night. All women born between 1935 and 1959, and 30-54 years of age, were identified though the Danish Cancer Registry. The number of such women was 7,565. One control per case was randomly selected from the Danish Central Population Registry. Controls were (i) living, (ii) apparently cancer free, and (iii) working before the date of diagnosis of the corresponding case. Work history was obtained from the Danish pension fund database. No work history was found for 530 cases, so the number of case-control pairs for the study was 7,035. Using a national survey (1976) of women and working conditions, 4 occupational categories were identified in which at least 60% of the female employees so some work at night. These were manufacturing of beverages, land transport services, catering, and air transport services. For hospitals, furniture manufacturing, water transport services, and cleaning services, between 40% and 59% of the women work some night shifts. Comparisons were made between occupations in which 60%+ of the women work night shifts and occupations in which less than 40% work night shifts. Only occupations within 5 years of diagnosis were considered. This limit was based on suspected induction time for breast cancer. To be placed in the "exposed" category a women had to have worked at least 6 months in a night shift occupation. Statistical adjustments were made for age, social class, ages at birth of first and last child, and parity. The OR for all "exposed" occupations was statistically significant ($p<0.05$): OR=1.5, 95% CI = [1.3 – 1.7]. For women who worked at least 6 years in "exposed" occupations, the OR was 1.7 ($p<0.05$). The results were essentially driven by the catering and air transport service occupations. (It should be noted that these two occupations may also result in higher ELF MF exposure, compared to manufacture of beverages and land transport services.) The authors state that "(w)hen the 5-year induction time was ignored, the ORT decreased marginally".

Negative Study

- O'Leary *et al.* (2006) studied night shift work, light-at-night and BC in Long Island, NY, as part of the Electromagnetic Fields and Breast Cancer on Long Island Study (EFBCLIS) Group. There were 487 cases and 509 population-based controls, frequency matched to the expected age distribution of the cases in the study. These subjects had to have participated in the earlier Long Island Breast Cancer Study Project (LIBCSP). Each case had to have lived in the same home for at least 15 years prior to the diagnosis of breast cancer, while each control had to have lived in the same residence for at least 15 years prior to recruitment. Cases had to have had their BC diagnosis within the 12 month period beginning August 1, 1996. Controls were concurrently recruited. The LIBCSP had collected, via direct interview, complete job history information, including shift work – all jobs held for at least 6 months beginning at age 16, full time or part-time. The EFBCLIS repeated the job history interview, without the shift work

information, for the period 15 years prior to the date of BC diagnosis (cases) or recruitment (controls). Military assignments were included. Light-at-night information was obtained by interview, and included information about sleep hours, frequency and length of having lights on during sleep time for the 5 year period prior to the reference date.

Exposure to shift work was defined as ever having had a job (≥ 6 months, either part or full time) with at least 1 day per week of shift work, during the 15 years prior to the reference date. Sub-groups were defined as follows: ever had an evening shift job; ever had an overnight shift job; ever had an evening shift, but never an overnight job; ever had an overnight shift; but never an even shift job. Statistical analyses were adjusted for reference date, parity, family history of BC, education, history of benign breast disease.

For any of the various categories of shift work during the 15 years prior to the reference date, there was no elevated risk of BC. However, 'any overnight shift work' had a statistically significant OR below one. The referent group included subjects with a jobs having less than 1 shift work day per week. Such a job could have been held for many years. The OR for at least 8 years of overnight shift work was statistically significantly below 1. For light-at-night within 5 years prior to the reference date, the only statistically significant finding was an OR = 1.65 for waking up and turning on lights at least 2 times per night versus doing so no more than 3 times per month.

The authors conclude that their study "provides mixed evidence for the light-at-night hypothesis". Analyses of shift work within 5 years of the reference date, the "induction" period used by Hansen (2001), were not presented. Overnight shift work was in the work history of only 26 cases and 50 controls; a duration of at least 8 years of overnight shift work was experienced by only 6 cases and 19 controls. Thus, the effective, "exposed" sample size was quite small. Information as to when this shift work occurred relative to the reference date was not provided.

E. Occupational Case-Control Studies of ELF MF Exposure as a Risk Factor for Breast Cancer

Conclusion: There is rather strong evidence from case-control studies that long-term, high occupational exposure to ELF magnetic fields is a risk factor for breast cancer. Six (6) independent studies are reviewed. Four (4) have positive conclusions, while two (2) are negative. The latest study is particularly strong. The two negative studies have serious shortcomings in exposure classification and come from the same research group.

There have been several case-control studies of occupations with more or less high ELF MF exposure and the risk of breast cancer. These studies have been generally positive, in the sense that there appears to be an increased risk. Earlier studies generally lack appropriate exposure information (e.g., Wertheimer and Leeper, 1994).

Positive Studies

- Peplonska *et al.* (2007) have conducted a large, population-based, case-control study of

breast cancer and 73 occupational categories. All incident cases of cytologically or histologically confirmed breast cancer among women aged 20-74 in Warsaw and Lódź, Poland, in 2000-2002 were identified. 2,502 controls were randomly selected using the Polish Electronic System of Population Evidence, which maintains records on all citizens of Poland. Controls were matched to cases by city of residence and age ± 5years. A structured questionnaire was completed by 79% of the cases and 69% of the controls. The questionnaire included items related to demographics, reproductive and menstrual history, hormone use history, physical activity, occupational history for all jobs held at least 6 months, smoking, alcohol use, diet, cancer history in female relatives, medical and screening history, prenatal exposures, and history of weight and height development. Occupational information included job title, start and stop dates, employer, company products and/or services, work activities and duties, physical activity related to work, passive smoking, and exposures to a list of chemicals. The study was funded by the U.S. National Cancer Institute (NCI) and managed by Westat (Rockville, MD).

Statistical adjustment was made for age, age-at-menarche (≤ 12; 13-14; ≥ 15), menopausal status; age-at-menopause, parity ≤ 1; 2; ≥ 3), body mass index (< 25; 25-30; ≥ 30 kg/m^2), first degree female family history of BC, education (< high school; high school; some college or professional training; college degree), previous mammographic screening, and city of residence. Oral contraceptive use, marital status, tobacco and alcohol use, age-at-first full term birth, breastfeeding, recreational and occupational history were not used for adjustment in the final analyses because they had "little impact" on the results.

In the primary analyses, for each specific job category/industry, the referent group consisted of all subjects who did not work in that job/industry for at least 6 months. For each specific "white-collar" occupation, additional analyses using all other white-collar jobs as the referent group were conducted. This was thought to provide at least a partial account for socio-economic factors not accounted for by education. Similar blue-collar job analyses were not conducted. Several job categories containing occupations with elevated ELF MF exposure had statistically significantly elevated ORs.

** These ORs were significantly elevated despite the fact that all other occupations with elevated ELF MF exposure were placed in the referent group. **

ELF MF exposure was determined using a job exposure matrix developed within NCI for a brain cancer study. No, low, medium and high categories were developed by "experienced industrial hygienists". (No reference was provided.) The highest ELF MF exposure category of all jobs for an individual was used in analyses. 99% of the high exposed subjects were so ranked due to employment as machine operators and tenders in the textile apparel and furnishing industry. Information on which occupations were classified as low or medium ELF MF exposure were not provided.

** It should be noted that (1) 'tenders' generally provide maintenance to machinery and (2) operators of machines other than sewing machines, e.g., cutters, both have lower ELF MF exposure than seamstresses. **

The OR for high ELF MF exposure versus no exposure was significant: OR = 1.5,

95% CI = [1.1 – 2.0]. For low exposure, the OR was also significant: OR = 1.2, 95% CI = [1.0 – 1.5]. For medium exposure the OR was also 1.2, but the 95% CI was [0.9 – 1.5]. Additional data analyses were not provided. The OR for high exposure among textile apparel machine operators and tenders is in line with the statistically significantly increased OR for seamstresses in the Forssén *et al.* (2005) study (see below under "negative studies") discussed below. In the Forssén *et al.* study (2004), seamstresses were classified as having medium-low ELF MF exposure.

Specific ORs for occupations classified (surprisingly and for some likely incorrectly) as having high (as opposed to low or at most medium) ELF MF exposure by Forssén *et al.* (2004) (see below) were calculated: cooks (OR=1.0); computer scientists (OR=1.3); computer and peripheral equipment operators (OR=0.7); data entry keyers (OR=0.3); dentists (OR=0.6); dental nurses (OR=1.0); counter clerks and cashiers (OR=1.1); and telephone operators (OR=0.9).

- Labréche *et al.* (2003) studied occupational ELF MF exposure and post-menopausal breast cancer. Cases and controls were identified through pathology department records at 18 hospitals in Montreal, Canada. These hospitals treat most of the breast cancer cases in the area. Age was restricted to 50-75 at the time of initial diagnosis of primary BC. Cases had to be residents of the region and the diagnosis had to have been in 1996 or 1997. Controls had one of 32 other cancer diagnoses and were frequency matched by age and hospital. The following cancers were excluded: liver, intrahepatic bile duct, pancreas, lung, bronchus, trachea, brain, central nervous system, leukemia, lymphoma, and non-melanoma skin cancer, but not gastrointestinal (Schernhammer *et al.*, 2003) or colorectal cancer (Bubenik, 2001).

  Complete occupational history, including task descriptions, and other personal information was obtained by personal interview, either of the subject or a surrogate if the subject was deceased or otherwise unavailable. Specialized occupational questionnaires were used for specific occupations, including sewing machine operators, cooks and nurses. The development of these questionnaires was led by Jack Siemiatycki. See, for example, Siemiatycki *et al.* (1991, 1997). ELF MF exposures were estimated from detailed descriptions of tasks, equipment used, and the work environment by industrial hygienists intimately familiar with Montreal workplaces. The ELF MF exposure categories and primary occupations were as follows: no exposure (< 2 mG; low exposure (2-5 mG, "typical jobs", including VDT operators, electric typewriter operators); medium exposure (5-10 mG; denturists, machinists); and high exposure (≥ 10 mG; sewing machine operators, textile workers). The industrial hygienists "confidence" in each subject's exposure assessment was obtained as definitely no exposure, or low, medium, and high confidence of exposure.

  Exposures to benzene, perchloroethylene, and alphatic aldehyes, chemicals found in the textile industry, were also considered.

  Statistical adjustments were made for age at diagnosis, family history of breast cancer, education, ethnicity, age-at-bilateral oophorectomy, age-at-menarche, age-at-first full-term pregnancy, oral contraception use, duration of HRT, total duration of breast feeding, alcohol use, smoking, and body mass index, as appropriate. Adjustment was also made for proxy versus personal responses because proxies tend to report fewer

jobs. In addition, duration of employment in the textile industry was an adjustment variable. As mentioned previously, adjustment for age-at-menarche is probably not appropriate due to melatonin's causal relationship with age-at-menarche.

In addition to the categorical analyses, the number of hours of medium or high exposure was used as a risk factor. The number of hours from the lower limit of the second quartile to the upper limit of the third quartile of medium/high exposure was 6000 hours. ORs were presented for a difference of 6000 hours.

All analyses, e.g., no exposure vs ever exposed, prior to 10 years before diagnosis, or before age 35, were non-signficant and non-elevated except for the following ones, adjusted for textile industry employment and other factors:

- ✓ No exposure vs medium-to-high exposure – OR = 1.90, 95% CI = [0.99 – 3.85];
- ✓ 6000 hour increase in medium-to-high exposure – OR = 1.21, 95% CI = [0.97 – 1.49];
- ✓ 6000 hour increase in medium-to-high exposure prior to 10 years before diagnosis – OR = 1.31 ($p<0.05$);
- ✓ 6000 hour increase in medium-to-high exposure prior to age 35 – OR = 1.54 ($p<0.05$).

The significant results appear to be primarily due to ELF MF association with progesterone positive and/or estrogen positive breast cancers.

The use of a 10 year lag eliminates exposure periods which may be too near the diagnosis time to be etiologically relevant. The analysis of exposures prior to age 35 identifies the time period when the development of female breast cells appears to cease.

The use of textile industry employment (yes/no) or length of time in the textile industry, as appropriate, as a covariate provides some adjustment for chemical exposures. Thus, the increase in the ORs when adjustment was also made for textile industry employment relates to ELF MF exposure.

Finally, controls also had cancer. While many of the excluded cancers may conceivably have ELF MF as a risk factor, some of the non-excluded ones may also. This is especially true if the melatonin hypothesis is correct. Thus, the OR estimates may be biased towards 1.

- Kliukiene *et al.* (1999, 2003, 2004) and Tynes *et al.* (1996) studied occupational ELF MF exposure and breast cancer among Norwegian women in general and radio and telegraph operators in particular. These were follow-up studies. A population-based cohort of 1.1 million women was developed using the 1960, 1970, and 1980 censuses. All women were working at the time of enrollment and had a potential for occupational ELF MF exposure. The follow-up period was from 1961-1992. Date of birth, and census information about occupation and socioeconomic status was obtained. Incidence of breast cancer was obtained from the Cancer Register of Norway. Out-migration information was obtained.

For the countrywide, all occupations study (1999), ELF MF occupational exposure assessment was not optimal, but was as follows. The first method used expert opinion. An expert panel, using written guidelines, decided whether a given occupation had ELF MF exposure above 1 mG for than 4 hours per week, between 4 and 24 hours per week, or more than 24 hours per week. Occupations were identified by a 3-5 digit industry code and a 3-digit occupation code. For cumulative exposure, the mean of each of the three (3) levels of exposure were used: 2 hours; 14 hours, 32 hours (based on a 40 hour week). It was assumed that each subject was in the same occupation from census to census, unless she died, emigrated or turned age 65.

The second method used the Swedish job exposure matrix used in the Forssén *et al.* (2000) study (below), which was constructed from observations of male workers. Cumulative exposure was categorized as below 9 mG-years, between 9 and 14 mG-years, between 14 and 30 mG-years, and above 30 mG-years. Exposure was also classified by number of work hours of exposure above background (1 mG): below 900 hours; 900-999 hours; 1000-1999 hours; 2000 or more hours.

Poisson regression, with adjustment for age, time period, and socioeconomic status, was used to estimate the relative risk (RR) of breast cancer. 22,543 breast cancer cases were diagnosed during the follow-up period. In the total cohort and the two sub-cohorts for those below or at least 50 years of age at inclusion in the cohort (Kliukiene *et al.*, 2004), the RRs were statistically significantly above 1.0 for each category of number of exposed hours, with below 900 hours as the reference category. For each cumulative exposure category above the reference category (below 9 mG-years, the RR for the total was statistically elevated. For the two sub-cohorts, the RRs were significantly elevated for the 9–14 and 14–30 mG-years categories. For the 30+ mG-years category the RRs were elevated, but lower bounds of the 95% CIs were 0.98 and 0.99.

These studies did not have very good occupational data.

For the radio and telegraph operators studies, the same cohort and occupational determination method was used. The Kliukiene *et al.* (2003) study was identical to the Tynes *et al.* (1996) study, except for a longer follow-up. By the end of May 2002, there were 99 breast cancer cases among the 2619 radio and/or telegraph operators in the cohort. The standardized incidence ratio was 1.30, 95% CI = [1.05 – 1.58].

A nested case-control study was also conducted, using the 99 BC cases and 4 controls per case matched on year of birth ± 5 years for cases born prior to 1920 and ± 1 year for cases born in 1920 or later. It was an update of an earlier study by Tynes *et al.* (1996). The reference category consisted of subjects (all radio and/or telegraph operators) who were not registered in the Norwegian Seamen Registry, i.e., had no history of working on merchant ships. ELF MF exposure was not particularly explicit. It seems to have been assumed that that women who had no history of working on merchant ships had lower MF exposure (ELF and radiofrequency) than those with a history of such work. Spot ELF MF and radiofrequency MF measurements in the radio/telegraph rooms of 2 and 3 ships, respectively, were performed. RF magnetic and electric fields were below the detection level of the instruments at the operator's desks. ELF magnetic fields varied from 0.2 mG to 60 mG at the operator's desks. However, the highest exposures were only to the stretched out leg. "Normal" exposure to the body varied from 1 mG to

2 mG. Thus, exposure was certainly not high.

Tertiles of cumulative exposure at sea were used in the statistical analyses, with adjustment for age-at-first birth and parity. Detailed job histories on each ship were available for each 'exposed' subject. For each ship, the amount of time spent in the radio/telegraph room was estimated by an experienced researcher. A rank of 1-3 was assigned: 1 – 'long voyage' for tankers or dry-cargo ships with longer stays as sea; 2 – 'many calls' for trade ships with several loading and discharge ports; 3 – larger passenger ships. Increasing rank implies increasing percentage of time spent in the radio/telegraph room. Exposure was then calculated by summing the product of the number years of service on ships of each rank by the rank of the ships.

Analyses were conducted for total exposure, and for total exposure with lag times of 10 and 20 years prior to BC diagnosis. Analyses were conducted for (1) all cases and controls, for cases and controls below age 50 in the reference year, and for cases and controls at least age 50 in the reference year, and (2) ER+ and ER- cases.

No OR was statistically significant for any analysis without consideration of ER status. However, there was a statistically significant increasing trend in the ORs over cumulative exposure categories in the analyses for all cases, cases younger than 50, and cases at least age 50. There was also a significant upward trend for a 10 year lag time using all cases. The ORs for the highest exposure category were all elevated, but not significant perhaps because of the sample size.

For analyses by ER status, the only significant finding was for ER- cases, age 50+ in the highest exposure category. There were elevated ORs for all exposure categories for all ER- cases, and for the highest exposure category for ER+ cases and for ER+ cases below age 50.

The authors concluded that "occupational exposure to electromagnetic fields increases the risk of (female) breast cancer" (Kliukiene *et al.*, 2003).

- Loomis *et al.* (1994) investigated BC mortality among female electrical utility workers. This study used U.S. national death certificate information, 1985-1989, to identify cases and controls (without leukemia or brain cancer as a cause or contributing cause of death) and occupations. There were 27,814 women with breast cancer and sufficient occupational information, of whom 68 had an "electrical" occupation. There were 110,750 controls, of whom 199 had an "electrical" occupation. The primary factor limiting the sample size was the availability of occupational information. It should be noted that use of occupational data from death certificates is far from optimal. Statistical adjustments were made for age, ethnicity, and social class. Loomis *et al.* found an elevated risk associated with having an electrical occupation recorded on the death certificate: OR=1.38 ($p<0.05$). The only specific occupation with a statistically significant elevated risk was telephone installers, repairers and line workers: OR=2.17. Electrical engineers and electrical technicians had 'elevated', but not significant risk estimates (OR=1.73 and 1.28). On the other hand, air traffic controllers, telephone operators, data keyers, computer operators, computer programmers did not have 'elevated' risk estimates.

In a letter commenting on the Loomis *et al.* paper, Kantor *et al.* (1995) analyzed essentially the same data set, with the inclusion of data from 1984. They used an industrial hygienist to estimate the probability of occupational ELF MF exposure or video display terminals (0, low, medium or high) among white and black women. The ORs were statistically significant (but not particularly high) for medium or high probability of exposure for both white and black women. When the hygienist actually categorized the level of ELF MF exposure, only medium exposure was associated with a statistically significant OR. High exposure had somewhat lower ORs.

- Forssén *et al.* (2005) published a case-control study of occupational ELF MF exposure and breast cancer. This study may be considered influential, unless reviewed in detail. So considerable detail is provided.

The Forssén *et al.* (2005) study found no association between occupational ELF MF exposure, as determined by Forssén *et al.* (2005) , and breast cancer. The study is singled out because (1) it is essentially well designed, and (2) has a completely inappropriate ELF MF occupational classification scheme based on either non-representative workers in specific occupations or what should be considered quite suspect individual measurements (Forssén *et al.*, 2004). Many occupational groups which are generally considered to contain higher ELF MF exposed occupations have been classified as low or medium-low exposure.

** Forssén *et al.* (2005) did find that seamstresses had statistically significantly elevated risk of breast cancer. However, they classified seamstresses as having medium-low ELF MF exposure. **

Forssén *et al.* (2005) used newly collected exposure data for occupations in which women commonly work (Forssén *et al.*, 2004). The exposure study assessed occupations identified within the Swedish 1980 census. Forty-nine (49) specific occupational titles were identified. Volunteers working in each of these occupations were then ascertained by methods which are not specified. Personal 24-hour ELF MF measurements were obtained on what was presumably supposed to be a typical 24-hour day, using a dosimeter worn at the waist. The volunteers kept a diary so that time periods at work, at home, and elsewhere could be identified. The number of subjects with measurements by occupation ranged from 5 to 24. The total number of subjects measured was 471. There were only 5 observations for Seamstresses, and 5 Radio and Television Assemblers and Repairwomen. The workday measurements were used for classification purposes. In the epidemiologic study of breast cancer, 4 categories of exposure were used: Low (< 1 mG); Medium-Low (1-1.9 mG); Medium-High (2-2.9 mG); and High (≥ 3 mG). The occupations in the categories above 'low' are provided in Table 9. The arithmetic rate of change measure was also calculated. Seamstresses and Radio and Television Assemblers and Repairwomen were both classified as medium-low exposed occupations. The 5 seamstresses measured for exposure had their own small businesses and did not work in apparel manufacturing. They evidently also did not do much sewing. They spent 55% of their workday in fields below 1 mG and only 15% in fields above 3mG. This is only an average of 1 hour and 12 minutes of 'high' exposure during a working day. In the two counties in Sweden in which both the

measurement study and the breast cancer case-control study were performed, there was almost no apparel manufacturing (Forssén *et al.*, 2004; personal communication, M. Feychting, 2007). Still, it is difficult to imagine such low exposures among women who actually work as seamstresses.

The cases and controls were obtained from all women who were employed at any time between 1976 and 1999, based on any of the censuses between 1960 and 1990, in either Stockholm or Gotland counties, Sweden. Subjects entered the study in either 1976 or their 15th birthday, which ever came first, and were followed through 1999 or to the date of their initial breast cancer diagnosis. Cases were identified through the Regional Cancer Registry in Stockholm. The referent year was the year of the case's diagnosis. Controls were selected randomly by age and calendar year, apparently matched to cases. Cases could not also be controls. Both cases and controls had to be living in Stockholm or Gotland counties during the referent year. All information, including occupational history, was obtained from registries. 20,400 cases and 116,227 controls were enrolled in the study. Varying numbers of cases and controls were used in the analyses, depending on the availability of occupational and other data. Statistical adjustment was made for age, referent year, parity, and socioeconomic status.

For statistical analyses, exposure was assessed in various ways: (1) ELF MF exposure for the occupation closest to the time prior to the referent year; (2) ELF MF exposure at the most recent census which was at least10 years prior to the referent date; (3) ELF MF exposure at the most recent census when the subject was at least age 35. Analyses were also carried out by (4) splitting the study period at 1985, by (5) only using subjects who either always had low exposure or ever having had high exposure, and by (6) defining low exposure as a median less than 1 mG and a third quartile of less than 1.7 mG and high exposure as a median greater than 2.5 mG and a first quartile including 1.7 mG. With these definitions, high exposed occupations were cashiers, working proprietors in retail trade, air stewardesses, dental nurses, cooks, post office clerks, and kitchen maids. No time latency period was used in the analyses related to (3).

There were no significant or elevated adjusted ORs for analysis (1) using the 4 categories of exposure, either for all BC cases, ER positive cases, or ER negative cases, for age below or at least 50. The referent group had ELF MF exposure below 1 mG. There were no significant or elevated adjusted ORs for analysis (1) using low versus high (separated) exposure categories defined by (6), above.

Finally, in a series of analyses based on exposure 10+ years before the referent year, before age 35 for post-menopausal women, referent year before or after 1985, maximum point exposure, rate of change, and proportion of time exposure was above 3 mG, only a single adjusted OR was significant. The significant OR=0.87 and was for medium-high ELF MF exposure among post-menopausal women before age 35.

It is thus fair to say that Forssén *et al.* (2005) found no relationship between their assessment of ELF MF exposure and breast cancer. The authors do recognize that "(t)he major concern in the study is exposure misclassification".

Their job exposure classification is at odds with other classifications. Forssén *et al.* (2004, 2005) have classified Dental Nurses, Cashiers in Retail Stores and Restaurants,

Working Proprietors in Retail Trade, Cooks, and Air Stewardesses as high ELF MF exposure occupations. None of these occupations would be classified as having high ELF MF exposure in any other classification scheme. The common cut-point for high exposure is 10 mG. Cashiers, cooks, and air stewardesses may at times have medium or high exposure, depending on (1) the exposure from scanners, (2) the exposure from microwave ovens, mixers, other motorized kitchen equipment, and (3) the exposure time from sitting near electrical panels on takeoff and landing and in the airplane's kitchen areas.

** Forssén *et al.* should conduct a sub-study to determine the actual environment in which the seamstresses in their study worked, the type of machines used (industrial, home; AC or DC operation), and the percent of time spent actually sewing. They also should conduct a study of seamstresses in general in Stockholm and Gotland counties and the in-migration rates. Also, the authors note an occupational category labeled 'textile occupations', which certainly includes seamstresses, but is otherwise undefined in the paper. Textile occupations need to be specified and studied individually, as was done by Hansen *et al.*, 2000. It is important to determine whether the "seamstresses" in the Forssén *et al.* (2005) study have fundamentally different levels of exposure than seamstresses in other studies.**

The only significant occupational finding in this study related to seamstresses. Two analyses were conducted related to seamstresses (Table 10), probably because their exposure assessment was so at odds with every other series of exposure measurements of seamstresses. First, the OR for 'textile occupations', undefined in the paper, versus low ELF MF exposed occupations was 1.37, 95% CI = [1.11 – 1.68]. Second, the OR for 'textile occupations' versus all other occupations, regardless of ELF MF exposure assessment, was 1.33, 95% CI = [1.10 – 1.62]. The authors state that their results "suggest that the increased risk for breast cancer in these occupations might be related to some exposure other than magnetic fields".

'Textile occupations' were not defined, but could certainly have included a multitude of occupations with quite varying chemical exposures, and generally medium or high ELF MF exposures. However, none of the 49 occupational categories, other than seamstress, used in the study appear to relate to textile occupations, if sales and administration are excluded.

The numbers of seamstresses as cases or controls in the study are not provided. However, in the AD studies by Sobel and Davanipour (1995, 1996, 2007), approximately 2% of the controls were seamstresses. Thus, there may have been at least 2000 seamstresses among the controls. Assuming that most, if not all women in "textile occupations" were seamstresses, and based on the OR of "textile occupations" vs ELF MF exposure below 1 mG, the number of seamstresses with BC in the study can be estimated as approximately 475. Rough calculations indicate that if seamstresses are reclassified as having high ELF MF exposure (> 3 mG), the adjusted OR for high occupational ELF MF versus low occupational ELF MF exposure would be about 1.10 and statistically significant. It is worth repeating that the Forssén *et al.* (2004) occupational classification for high ELF MF exposure is (1) not as high as usual and (2) measured workday exposures are unusual for such occupations.

- Forssén *et al.* (2000) conducted an earlier case-control study of occupational and residential ELF MF exposure and breast cancer. The cohort from which the study population was obtained consisted of all Swedish residents who lived within 300 meters of a (high power, 220 or 400 kilovolt) transmission line for at least one year between 1960 and 1985 and were at least age 16 sometime in the period. Subjects in this group living further away from transmission lines essentially had no exposure from such lines. Cases were identified through cancer registries. Controls were randomly selected and matched by age group, residence in the same parish at the time of diagnosis of the case and in the same type of house (single-family/apartment further than 300 meters from the same power line. (The parish/power line criteria were relaxed for 95 cases; a control could not be found for 7 cases.) Residential exposure was calculated from the ELF MF generated by power lines. Occupation information was obtained from census data. An older job- exposure matrix was used to assess occupational ELF MF exposure. Low (< 1.2 mG), medium (1.2 – 1.9 mG), and high (≥ 2.0 mG) exposure categories were selected, based on quartiles. Exposure greater or equal to 2.5 mG was also considered.

  Statistical adjustments were made for the matching variables. Only occupational exposure immediately prior to the diagnosis of BC and only residential exposure at the time of diagnosis was used in the analyses. No information concerning occupations of the subjects was provided. It is unlikely that seamstresses were included in the analyses.

  No significant findings were identified.

  Of 1767 cases and 1766 controls, only 711 and 709, respectively, had residential exposure information, only 744 and 764 had occupational exposure information, and only 197 and 200 had both types of exposure information. For the actual analyses of occupational exposures, with matching variable adjustment, there was complete information for only 440 cases and 439 controls. For analyses using both occupation and residential exposures, and matching variables, there was complete information for only 87 cases and 83 controls.

Partially Positive/Partially Negative Studies

- Coogan et al. (1996, 1998) and McElroy et al. (2007) conducted case-control studies using the same ELF MF exposure classification scheme.
  - The 1996 Coogan et al. study selected breast cancer cases, aged 74 or younger, from the Maine, Wisconsin, Massachusetts, and New Hampshire cancer registries who were diagnosed between April 1988 and December 1991. Controls, aged below 65, were selected from state driver's license lists and were frequency matched to cases by 5-year age intervals. Cases aged below 65 had to have driver's licenses. Controls, aged 65-74, were selected from the Health Care Financing Administration's Medicare beneficiary lists. "Most representative" occupation was obtained via telephone interviews. Occupation duties and industry were obtained if "the occupation was not clear".

    Occupations were coded according to the 1980 Bureau of the Census 3-digit occupational classification. The ELF MF exposure classification scheme identified each of the 3-digit occupation classes as low, medium or high or

background (non-exposed) exposure "potential". It is our opinion that the classification scheme is rather deficient: for example,

1. Welders are classified as having **medium** ELF MF potential exposure;
2. Dressmakers (e.g., seamstress) and tailors are classified as having **low** potential for ELF MF exposure;
3. Shoe repairers are classified as having **low** potential for ELF MF exposure;
4. Electrical/Electronic Engineers are classified as having **high** potential for ELF MF exposure;
5. Statisticians and Scientists are classified as having **medium** potential for ELF MF exposure.

In most classification schemes, including that of Sobel-Davanipour et al., welders, dressmakers (seamstresses) are classified as high ELF MF exposed occupations, shoe repairers, electrical/electronic engineers would be classified as medium exposed occupations, and statisticians and scientists would be classified as low exposed occupations.

Nevertheless, the adjusted OR for breast cancer among subjects having occupations with high potential ELF MF exposure versus background was 1.43, with a 95% CI of (0.99 , 2.09). Among pre-menopausal cases with high exposure potential occupations, the adjusted OR was 1.98, with a 95% CI of (1.04, 3.78).

- Coogan and Aschengrau (1998) essentially replicated the earlier Coogan et al. (1996) study, except for adding non-occupational exposure, e.g., homes close to transmission lines, electric heating, bed-warming device. Cases and controls were obtained from Cape Cod, where elevated rates of breast cancer had been observed. Complete work histories (beginning at age 18) were obtained by interview. Jobs were classified using the methodology in Coogan et al. (1996). There were 259 cases and 738 controls. The crude and adjusted ORs were all below 2.0, except for having a "high" ELF MF job at some point and "other ELF MF exposure". The adjusted OR in this case was 2.3. None of the OR estimates was significant.

- McElroy et al. (2007) replicated the initial Coogan et al. (1996) study with female breast cancer subjects obtained from the Massachusetts, New Hampshire, and Wisconsin cancer registries after the close of recruitment for the Coogan et al. (1996, 1998) studies. Occupational ELF MF exposure using the same methodology as in the Coogan et al. (1996, 1998) studies was estimated for each subject's primary occupation. This was a large study: 6213 cases and 7390 controls. None of the adjusted (or unadjusted) ORs were anywhere near statistical significance. (The largest adjusted OR was 1.21.) However, the trend for increasing adjusted (or unadjusted) ORs for all women and for women who were post-menopausal at diagnosis were statistically significant, with p-values between 0.02 and 0.04.

We emphasize that the ELF MF exposure categories are quite inappropriate.

- Peplonska et al. (2007) conducted a case-control study of 2386 incident BC cases (diagnosed in 2000-2003) and 2502 controls. Lifetime occupational histories and known BC risk factors information were obtained. Occupational information included job title, start and stop dates, work activities and duties, and product(s) made and/or service provided. Occupations were coded to the Standard Industrial Classification Manual (1987) and the Standard Occupational Classification Manual (1980). Occupations were characterized as 'white collar' and 'blue collar'. Analyses are provided by occupation and duration, and by industry and duration. Thus, it is generally not possible to identify subjects with significant ELF MF exposure. For example, the following occupations are combined:
    - ✓ electrical, electronic, agricultural, industrial, mechanical, computer, and other engineers;
    - ✓ engineering and related technologists and technicians;
    - ✓ typists, secretaries, stenographers;
    - ✓ hairdressers and cosmetologists;
    - ✓ machine operators and tenders;
    - ✓ printing machine operators and tenders;
    - ✓ textile apparel and furnishing machine operators and tenders;
    - ✓ textile sewing machine operators and tenders;
    - ✓ welders and solderers.

  Analyses by at least somewhat relevant occupational categories for any duration of work are as follows:
    1. Engineers (electrical, electronic, agricultural, industrial, mechanical, computer, and others): OR=2.0, 95% CI = (1.05 , 3.8);
    2. Health record technologists and technicians: OR=2.4; 95% CI = (1.04 , 5.7);
    3. Machine operators and tenders: OR=1.2 95% CI = (1.03 , 1.5);
    4. Printing machine operators and tenders: OR=3.1; 95% CI = (1.4 , 7.0);
    5. Textile apparel and furnishing machine operators and tenders: OR=1.3; 95% CI = (1.03 , 1.5);
    6. Textile sewing machine operators and tenders (a subset of the previous job category): OR=1.2; 95% CI = (0.9 , 1.5);
    7. Welders and solderers: OR=1.2; 95% CI = (0.6 , 2.8).

  None of these seven occupations showed any trend towards increasing risk with duration of work: ≤ 10 years vs > 10 years.

  The analyses by industry are particularly inappropriate.

  The authors used a job exposure matrix (JEM) developed by the National Cancer Institute for a brain cancer study (unreferenced) to evaluate ELF MF exposure and the risk of BC. They identified a statistically significant trend with ORs equal to 1.2, 1.2, and 1.5 for low, medium, high ELF MF exposure. (The actual data were not provided in the paper or online supplementary materials. The authors state that the "excesses in the highest exposure category" were almost completely due to textile apparel and furnishing machine operators and tenders. These employees evidently formed "99%" of the entire high ELF MF exposure group.

With respect to considering ELF MF as a risk factor for breast cancer, the authors would have been better served to use the actual job title and descriptions to form categories of ELF MF exposure. Nevertheless, the authors state that "occupations with potential exposure to magnetic fields deserve further evaluation".

- Ray et al. (2007) conducted a large and potentially valuable study of breast cancer among female textile workers in Shanghai, China. The authors took advantage of a randomized trial of breast self-examination efficacy to conduct a case-cohort study of occupational exposures and BC risk. 1709 BC cases and an age-stratified reference sub-cohort of 3155 non-cases were studied. Hazard ratios were estimated for duration in various job categories and exposure duration by Cox proportional hazards methodology.

  A job exposure matrix was developed for ELF MF exposure (Wernil et al., 2006). Admittedly based on a small number of subjects, the proportion of specific processes in the following textile industry areas were found to result in ELF MF exposure: spinning (75%, 8 of 12); weaving (88.9%, 8 of 9); cutting and sewing (60%, 3 of 5); and maintenance (30%, 3 of 10). There was no information about the extent (in instantaneous or cumulative mG) of the exposure.

  Among the weavers, cutters/sewers, and maintenance female personnel, only cutters/sewers and maintenance personnel with 10 – 20 years of experience had hazard ratios exceeding 1.0: HR=1.61, 95% CI = (1.16 , 2.25) and HR=1.83, 95% CI = (1.01 , 3.32), respectively. There were no indications of any trend. (Note: individual simple calculations of odds ratios for having worked primarily as a weaver, as a cutter/sewer, or as a maintenance person showed no increase or decrease in risk of BC.

  Evidently, no information as to what the ELF MF exposures were for various jobs, e.g., sewer, was collected.

F. Residential Case-Control Studies of ELF MF Exposure as a Risk Factor for Breast Cancer

Residential ELF MF exposure studies and BC have either used wire configuration coding, proximity to high voltage lines, various protocols of room measurements, or a combination of these methods. These studies have generally not found any increased risk of breast cancer (e.g., Feychting *et al.*, 1998; Davis *et al.*, 2002; London *et al.*, 2003; Schoenfeld *et al.*, 2003). Residential studies have measured actual magnetic fields only in current homes of cases and controls, thus homes which might be etiologically relevant are often or usually without actual measurements. Wire configurations and proximity to high voltage lines were at times used for surrogate measures of exposure related to previous homes. Each of these three methods of assessment of the level of exposure leads to significant classification errors. In addition, residential exposures are, almost always, surely relatively low. Individualized exposure, due for example to home sewing, sitting or sleeping near a panel of circuit breakers, sitting near a water pipe (e.g., in the floor or ceiling), is not identified. For homes near high voltage lines, rooms can have dramatically different ambient levels of ELF MF. For these reasons, these studies are not relevant to the purposes of this review.

G. Radiofrequency Exposure and Breast Cancer

There are no epidemiologic studies of radiofrequency MF exposure and breast cancer which do not include ELF MF exposure and which have reasonable data on RF exposure, e.g., Kliukiene *et al.* (2003).

V. SEAMSTRESSES

Conclusion: Seamstresses are, in fact, one of the most highly ELF MF exposed occupations, with exposure levels generally above 10 mG over a significant proportion of the workday. They have also been consistently found to be at higher risk of Alzheimer's disease and (female) breast cancer. This occupation deserves specific attention in future studies.

A. Sobel-Davanipour et al. Studies

Seamstress was the primary occupation among women with high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies related to AD. No other published AD study has evidently involved populations in which sewing was a somewhat common occupation. In the 5 independent case-control studies presented in the 3 Sobel & Davanipour papers, most of the high ELF MF exposed women (cases and controls) were seamstresses. (Among women in these case- control studies, the Mantel-Haenszel AD odds ratio for seamstresses is 3.13, $p < 0.01$). Information about sewing as a hobby, which at least used to be common, was unavailable. Seamstresses have been shown to have very high ELF MF exposures (e.g., Szabó *et al.*, 2006; Kelsey *et al.*, 2003; Deadman and Infante-Rivard, 2002; Hansen *et al.*, 2000). Forssén *et al.* (2004) measured 5 "seamstresses" who owned independent small businesses and found what they classified as medium-low exposure – a mean of 1.7 mG. These 5 individuals used home sewing machines and evidently did not sew very often. Peplonska *et al.* (2007), using a NCI occupational ELF MF classification scheme found that, at least among women, nearly all high exposures occurred among textile machine operators and tenders. Both Forssén *et al.* (2005) and Peplonska *et al.* (2007) found statistically significantly elevated ORs for breast cancer among seamstresses/textile machine operators and tenders.

Sobel and Davanipour (1996c) measured ELF MF exposure from several home sewing machine models, both AC and DC models, to several parts of the body. The results are provided in Table 10. These results show that (1) high ELF MF exposure occurs to many parts of the body, (2) exposures vary by manufacturer, model, and even by machines of the same model, and (3) exposures depend on whether the machine operates by AC or DC current. For Alzheimer's disease and for breast cancer, it is not known where exposures may be most important. The peripheral Abeta hypothesis, if correct, would indicate that exposure to any location is important for AD. To affect pineal production of melatonin, it is not known whether exposure to the pineal gland is what is most important. For example, a majority of breast cancers causally lower pineal melatonin production. Because the melatonin production rebounds after excision of the tumor, the tumor itself must be secreting something that leads to the decline in melatonin production. Thus, it is conceivable that ELF MF exposure may, at least in some individuals, also lead to the peripheral production of something that also causes a lowering of melatonin production. It is also not known whether ELF MF exposure directly to the breast is etiologically important. Note that the right breast receives higher ELF MF exposure from home sewing machines. No studies of right versus left breast cancer and use of home sewing machines have been published.

B. Examples of Studies with 'Questionable' Seamstress Exposure Assessment:

Swedish and German Studies

Most of the Swedish studies on ELF MF and Alzheimer's disease/dementia or breast cancer (e.g., Forssén et al., 2000, 2004, 2005), Andel et al., 2010, Seidler et al., 2007, Feychting et al., 1998a) have relied on an occupational exposure assessment for seamstresses which significantly under-estimates exposure. For example:

- Seidler et al. (2007) uses governmental census categories which lumps seamstresses together with spinners, weavers, knitters, and dyers, all of whom probably have relatively low exposure. Maximum exposure in this occupational category is given as only1.5 mG, which is below the background levels for seamstresses working in factories.
- Forssén et al. (2004) created a job-exposure matrix for occupational ELF MF exposure among women working in the 49 most common or suspected high ELF MF ISCO job categories in Stockholm County using the Swedish 1980 census (Table 14). (ISCO stands for International Standard Classification of Occupations.) Five (5) to 24 subjects were selected in each of these occupations. Each or many of the ISCO job categories include several different occupations. Thus, workers from subgroups were selected. Sampled workers were instructed to wear their dosimeters for 24 hours and to make diary entries if they need to take off the dosimeter. Seamstresses are described as being rather uncommon in Stockholm County, except possibly for repair of clothing. This may account for the very low ELF MF exposure identified. Seamstresses are listed as having a geometric mean occupational exposure of only 1.7 mG. Only about 15% of their time was about 3 mG exposure. Cooks, kitchen maids, air stewardesses, hairdressers/beauticians all are listed as having greater exposure. Housekeeping service work had comparable exposure levels to seamstresses. As discussed in this report, the research by Davanipour, Sobel, and colleagues demonstrates that actual professional seamstresses have a very different exposure experience.

A re-analysis of the data in these studies with the job exposure classification scheme in the Davanipour & Sobel studies (Table 11) would be useful.

Note: The Kliukiene et al. study (2004) from Norway used a rather unique four division scale depending on how many hours of occupational exposure were above 1 mG per week and is thus not related to this discussion.]

Note: Qiu et al., 2004 exposure assessment problems has been discussed in Section D.3.4, above.

ACKNOWLEDGEMENT

The authors thank Dr. James Burch, University of South Carolina, for his careful review of the original 2007 manuscript He provided quite helpful suggestions and comments.

Figure 1: Hypothesized Biological Pathway from ELF MF Exposure to AD Development (from Sobel & Davanipour, 1996a)

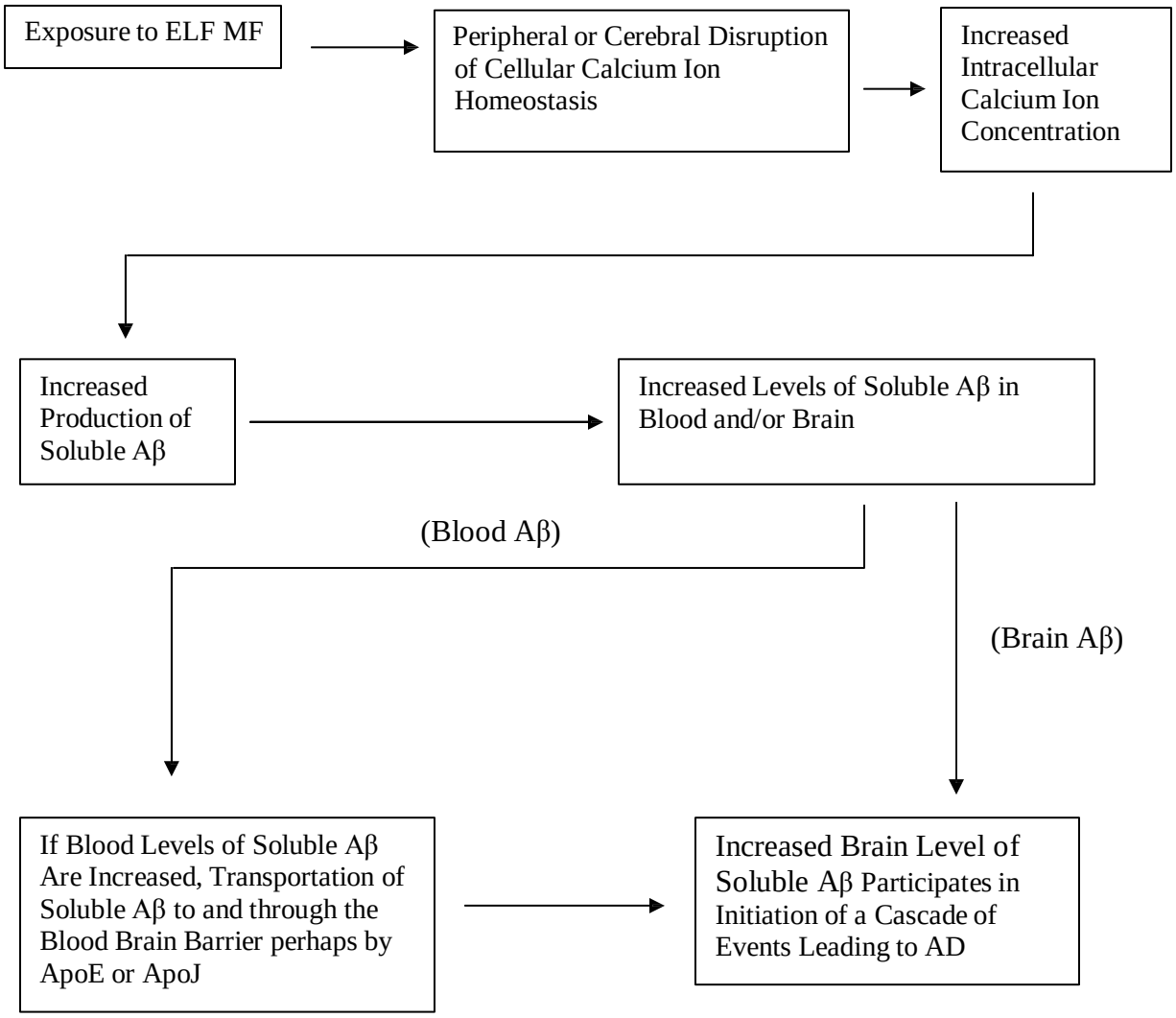
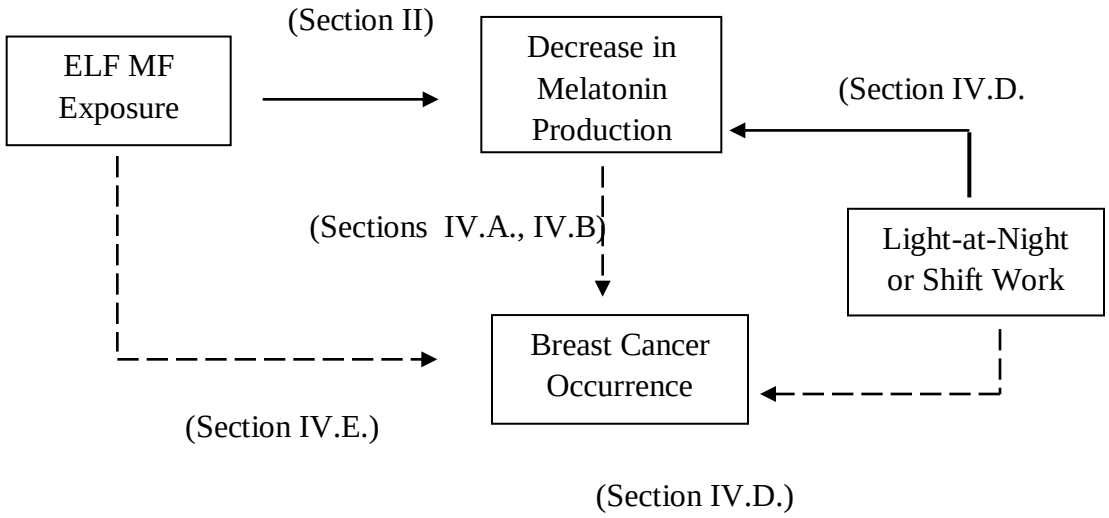


Figure 2: Outline of the Evidence that ELF MF Exposure Causes Breast Cancer through Decreases in Melatonin Production – with Section References



Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night, or shift work to breast cancer occurrence.

Table 1: Baseline Data Results from the 1999 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal at Follow-Up	Developed AD (3.6 Year Average Follow-Up)
Sample Size (n)	105	64
Age	73.4 (5.3)	77.4 (5.9)[a]
Education	9.3 (4.6)	7.5 (3.8)[a]
$A\beta_{1-40}$ (pg/ml)	111.8 (44.1)	134.7 (46.4)[a]
$A\beta_{1-42}$ (pg/ml0	51.5 (42.0)	82.4 (68.8)[a]
$A\beta_{1-42}$/ $A\beta_{1-42}$	0.51 (0.41)	0.67 (0.56)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.0001$; [b] $p < 0.05$.

Table 2: Baseline Data Results from the 2003 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal At Follow-Up	Developed AD (Up to 10 Year Follow-Up)
Sample Size (n)	365	86
Age	75.5 (5.9)	79.3 (6.6)[a]
Education	9.0 (4.6)	6.8 (4.5)[a]
$A\beta_{1-40}$ (pg/ml)	133.3 (61.9)	136.2 (46.7)[c]
$A\beta_{1-42}$ (pg/ml)	58.8 (32.9)	76.5 (59.8)[b]
$A\beta_{1-42}$/ $A\beta_{1-42}$	0.48 (0.3)	0.61 (0.53)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.001$; [b] $p < 0.05$; [c] Not Significant.

Table 3: Post-Work Levels of $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}42}/A\beta_{1\text{-}42}$ by ELF MF exposure among Electrical Workers in the Noonan *et al*. (2002a) Study

ELF MF Exposure	$A\beta_{1\text{-}40}$ (pg/ml)	$A\beta_{1\text{-}42}$ (pg/ml)	$A\beta_{1\text{-}42}/A\beta_{1\text{-}42}$	Sample Size
< 0.5 mG	125	136	1.03	20
0.5 – 0.99 mG	137	163	1.11	25
1.0 – 1.99 mG	128	166	1.19	8
≥ 2.0 mG	156	262	1.46	7

Table 4: Correlation (Corr) between Post-Work Creatinine-Adjusted aMT6s and Amyloid Beta by Number of Minutes between Samples in the Noonan *et al*. (2002a) Study

Number of Minutes	Sample Size	$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}$/ $A\beta_{1-40}$	
		Corr	p-Value	Corr	p-Value	Corr	p-Value
All Subjects	60	-0.25	0.057	-0.19	0.144	-0.23	0.080
≤ 90	46	-0.30	0.047	-0.22	0.154	-0.27	0.080
≤ 60	37	-0.37	0.027	-0.25	0.150	-0.37	0.029
≤ 30	23	-0.43	0.054	-0.28	0.224	-0.42	0.059

Table 5: Amyloid Beta Levels by Tertile of Post-Shift Creatinine-Adjusted aMT6s Levels in the Noonan *et al*. (2002a) Study

aMT6s/Cr Tertiles* (ng/mg)	$A\beta_{1-42}$ Mean**	95% CI	$A\beta_{1-40}$ Mean**	95% CI	$A\beta_{1-42}$/ $A\beta_{1-40}$ Mean**	95% CI
≤ 1.38	177	[112–258]	133	[111–156]	1.30	[0.86–1.74]
1.39–3.3	214	[120–334]	147	[125–170]	1.33	[0.85–1.90]
> 3.3	123	[58–180]	123	[108–139]	0.82	[0.49–1.26]

* n=60 subjects in each tertile

** geometric mean averaged over the work shift

Table 6: Percentages of Subjects with Medium to High ELF MF Occupations Exposure

STUDY	CASES	CONTROLS
Sobel *et al.* (1995a)	9.3 %	3.4 %
Sobel *et al.* (1996b)	12.0 %	5.3 %
Davanipour *et al.* (2007)	7.4 %	3.8 %
Harmanci *et al.* (2003)	10.5 %	3.1 %
Feychting *et al.* (1998a)	43.0 %	23.0 % & 19.0 %[#]
Graves *et al.* (1999)	19.1 % & 21.4 %	21.4 % & 22.5 %[^]
Qiu *et al.* (2004)	28.2 %[*]	28.8 %[*]
	34.2 %[**]	42.7 %[**]
	Cases & Controls Combined	
Feychting *et al.* (1998)	11.1 %	
Håkansson *et al.* (2003)	80.5 % - likely exposed engineering industry workers	
Johansen *et al.* (2000)	56 % - electrical company workers	
Savitz *et al.* (1998a)	electric utility cohort – percentage not supplied	
Savitz *et al.* (1998b)	23.9 %	

Two control groups;
^ Two industrial hygienists
* Based on estimated daily exposure in principal occupation;
** Based on estimated daily exposure in all occupations

Note: The Huss et al. (2009) study was longitudinal and the abstract for the Chang et al. (2004) study did not provide the percentages of cases or controls with high ELF MF exposure.

Table 7: **Odds Ratios for the ELF MF and AD Studies***

Study	Risk Estimate (OR)	95% CI	p-value
Sobel *et al.* (1995) (late-onset; L vs M/H)			
	3.0	1.6 – 5.4	< 0.001
Sobel *et al.* (1996b) (late-onset; L vs M/H)			
	3.9	1.5 – 10.6	0.006
Feychting *et al.* (1998) (mostly late-onset; last occupation; by control group)			
(exposure ≥ 2 mG)	2.4	0.8 – 6.9	--**
	2.7	0.9 – 7.8	--**
(exposure ≥ 5 mG)	4.1	0.7 – 23.5	--**
	8.3	1.1 – 62.7	--**
Graves *et al.* (1999) (late-onset; ever exposed)			
	0.95	0.4 – 2.4	--**
	0.74	0.3 – 2.4	--**
Harmanci *et al.* (2003) (late-onset; exposure as defined in Sobel *et al.* (1995, 1996b)			
	4.0	1.0 – 15.8	--**
Qiu *et al.* (2004) (age ≥ 75; exposure: ≥ 2 mG)			
Men	2.3	1.0 – 5.1	--**
Women	0.8	0.5 – 1.1	--**
Davanipour *et al.* (2007) (exposure as defined in Sobel *et al.* (1995, 1996b)			
M/H vs L	2.2	1.2 – 3.9	< 0.02
H vs L	2.7	0.8 – 9.1	< 0.11
Chang *et al.* (2004) (age: 66-102; exposure: "early exposure to magnetic fields")			
Exp vs No Exp	2.49	0.96 – 6.45	--**

* Studies use various types of controls and definitions of ELF MF exposure. See text.
** p-values were not provided.

Note: the Huss *et al.* (2009) study was longitudinal and is therefore not in this table.

Table 8: Mean ELF MF Exposures (mG) for Home Sewing Machines by Body Location: Continuous 2-Minute Measurements (Sobel & Davanipour, 1996c)

Sewing Machine	Background	Head	Breast		Pelvic Area	Thigh		Knee		Lower Right Arm	Right Hand	Foot Pedal
			Left	Right		Left	Right	Left	Right			
Alternating Current Machines (older machines)												
Bernina 811	0.6	18.6	5.6	12.9	26.9	11.7	90.1	8.9	13.5	251.1	57.0	86.1
Bernina 811	0.9	1.7	2.6	5.4	8.2	4.5	11.6	6.8	36.5	77.1	31.7	102.0
Bernina 817	0.6	8.4	9.6	23.5	41.9	19.1	30.6	9.2	35.4	724.6	135.6	NA
Bernina 817	1.2	12.1	14.2	33.9	51.0	10.3	588.5	8.8	125.7	753.0	132.4	NA
Brother 920D	0.7	2.4	2.1	2.3	1.1	1.3	1.5	1.9	2.3	8.5	16.0	6.2
Necchi Type 525	0.3	5.1	2.0	1.1	2.5	1.1	2.4	2.0	5.1	25.9	22.6	5.9
Sears Kenmore	0.2	1.2	1.9	4.9	5.5	2.2	5.3	2.5	15.8	26.0	17.9	13.8
Singer 625	0.3	4.6	3.6	5.6	5.5	3.9	6.6	6.4	17.2	...	...	...
Singer 5932	0.5	1.2	0.9	2.0	2.7	1.1	2.5	1.0	4.1	8.6	23.0	2.9
Singer 6212C	0.3	7.0	2.8	6.4	2.0	1.4	2.2	1.4	1.9	31.0	26.2	4.4
Viking Husqvarna 6020	0.8	1.5	1.3	1.5	2.7	1.4	2.0	3.1	9.1	5.9	24.9	62.3
White 1410	0.2	2.2	1.6	1.1	1.1	3.2	10.8	4.2	67.5	20.8	18.3	2.8
Direct Current Machines (newer machines)												
Bernina 1000	1.0	1.3	1.6	2.3	2.9	1.9	2.5	2.8	11.2	8.1	41.2	798.0
Bernina 1090S	1.0	1.2	1.6	1.6	1.7	1.2	1.3	1.5	7.7	3.3	22.9	1.0
Elna Diva 900	1.6	5.1	3.9	4.1	4.1	3.0	3.1	3.2	8.4	40.4	57.1	1.8
Singer 3317C	0.7	3.4	1.6	2.9	2.2	2.1	2.2	1.5	11.3	22.1	25.8	5.8
Singer 9015	0.7	2.5	1.9	3.3	4.9	1.7	4.3	2.1	26.2	7.0	28.9	2.3
Viking Husqvarna 500	1.0	3.7	2.7	5.0	3.9	1.8	2.8	2.7	13.8	24.9	39.4	1.1
Percent > 2.0 mG	0%	67%	50%	78%	83%	50%	89%	72%	94%	100%	100%	80%

Note: The Bernina 1000, Bernina 1090S, Elna Diva 900, Singer 3317C, Singer 9015 and Viking Husqvarna 500 were brand new. The Singer 5932, Singer 6212C, and Brother 920D were 3-10 years old. The Bernina 811 and 817 machines, the Sears Kenmore, the Singer 625 the Viking Husqvarna 6020 are probably at least 15 years old. Both the White and the Necchi are fairly old. NA = not applicable, i.e., there was no foot pedal. "..." = no measurements were taken, e.g., because of machine malfunction.

Table 9: Classification of Occupations in Forssén *et al.* (2005)

Classification	Occupation	24-Hour Geometric Mean Average (mG)
High (≥ 3 mG)	Dental Nurse	3.0
	Air Stewardesses	3.0
	Cooks	3.1
	Working Proprietors Retail Trade	3.4 in
	Cashiers in Retail Stores and Restaurants	4.5
Medium-High (2 – 2.9 mG)	Computer Operators	2.0
	Motor Vehicle Drivers	2.0
	Shop Managers	2.1
	Shop Assistants	2.1
	Hairdressers/Beauticians	2.1
	Bank Clerks	2.2
	Kitchen Supervisors	2.4
	Post Office Clerks	2.5
	Waitresses in Restaurants and School Kitchens	2.5
	Kitchen Maids	2.8
Medium-Low (1 – 1.9 mG)	Registered Nurses	1.0
	System Analysts/Programmers	1.2
	Telephone Operators	1.5
	Radio & Television Assemblers and Repairwomen	
	Seamstresses	1.6

Table 10: Odds Ratio Estimates for Textile Occupations in the Forssén *et al.* (2005) Study

Comparison	OR	95% Confidence Interval
Textile Occupations vs Occupations with 24-Hour Exposure Below 1 mG	1.37	[1.11 , 1.68]
Textile Occupations vs All Other Occupations (Regardless of ELF MF Exposure)	1.33	[1.10 , 1.62]

Table 11: Sobel-Davanipour Occupations Classified as Being Likely to Have Resulted in Medium or High ELF MF Exposure

Medium Exposure	High Exposure
Beautician	Cutter
Carpenter	Power Plant Operator
Clothes Inspector: Manufacturing Company	Repair Sewing Machines
Electric Lineman	Seamstress/Tailor
Electrician	Welder
Electronics Technician	
Electronic Assembler	
Equipment Repair	
Fabric Cutter	
Foam Cutter	
Forklift Operator	
Furniture Maker	
Machine Operator	
Machinery Repair	
Machinist (	
Newspaper Pressman	
Presser: Clothing Manufacturing Company	
Seamstress/Tailor – Part-Time	
Sheet Metal Machine Operator	
Shoemaker/Shoe Repairer	
Typist	
Upholstery; Re-Upholstery	
Welder - Parttime	
Wood Cutter; Machinery Repair - Forestry	
Wood Sander – Furniture	

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