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EFFECTS OF 60 Hz ELECTRIC AND MAGNETIC FIELDS ON PRODUCTIVITY, REPRODUCTIVE HORMONES, PLASMA MINERALS AND MINERALS AND NEUROTRANSMITTER METABOLITES IN CEREBROSPINAL FLUID IN DAIRY CATTLE

by

Javier F. Burchard

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Animal Science
Macdonald Campus of McGill University
Montréal, Québec

SUGGESTED SHORT TITLE: Electric and magnetic fields and cattle

Dedicated to:

Maria Olga Levine
Raquel Señoret
Alejandra Burchard-Levine
Antonia Burchard-Levine
Vicente Burchard-Levine,
For their patience.

EFFECTS OF 60 Hz ELECTRIC AND MAGNETIC FIELDS ON PRODUCTIVITY, REPRODUCTIVE HORMONES, PLASMA MINERALS AND MINERALS AND NEUROTRANSMITTER METABOLITES IN CEREBROSPINAL FLUID IN DAIRY CATTLE

ABSTRACT

Ph.D.

Javier F. Burchard **Animal Science**

This study was designed to determine the potential biological effects of electric and magnetic fields (EMF), generated by 735 kV alternating current (AC) high tension lines upon the hormonal profile, some health-related parameters, stress response and productivity in dairy cattle. An EMF exposure chamber to house eight animals at one time was constructed. Forty-nine cows were divided according to their production stage; 8 pregnant non-lactating cows, 16 pregnant lactating cows, 16 non-pregnant lactating cows and 9 non-lactating non-pregnant heifers. They were exposed to an EMF of 10 kilovolts per meter (kV/m) and 30 micro-Tesla (μT) in two different fashions: a) for three consecutive periods of 28 days in two sequences either with the EMF on/off/on or off/on/off, in a switch back design; b) for three consecutive periods with the sequence OFF (5 days), ON (30 days) and OFF (5-12 days). The intensity

of the EMF chosen for the experiments resembled a situation in which the cattle are standing continuously under a 735 kV AC high tension line when the line has a maximum load of current. In reality, these conditions are found only for a few days during the winter in the Province of Québec.

Milk production and composition, feed consumption, blood hormonal profiles and cerebrospinal fluid (CSF) components were assessed during the different periods of exposure. Most of the variables assessed were not affected by EMF. However, there was a positive association between EMF and feed consumption, milk fat content, blood plasma progesterone during pregnancy and estrous cycle length. Also, there were changes in the mineral and neurotransmitter metabolite concentrations in the CSF that showed a relationship to the EMF.

In conclusion, it could be stated that EMF caused a biological response in dairy cattle. It is speculated that these changes do not represent a health hazard for exposed cattle, although they warrant further research.

Les Effets des Champs Électriques et Magnétiques de 60 Hz sur la Productivité, les Hormones de Reproduction, les minéraux dans le plasma sanguin et les minéraux et neurotransmetteurs du Liquide Cérébro-spinal chez la Vache Laitière.

RÉSUMÉ

Ph.D.

Javier F. Burchard **Sciences Animales**

Cette étude visait à déterminer, chez la vache laitière, les effets biologiques potentiels des champs électriques et magnétiques (EMF) générés par des lignes de haute tension conduisant un courant alternatif (AC) de 735 kV. Les paramètres étudiés étaient le profil hormonal, la réponse face au stress, certains paramètres reliés à la santé et la productivité des vaches. Une chambre déxposition à EMF a été construite pour héberger huit têtes à la fois. Quarante-neuf vaches ont été réparties selon leur stade de production; 8 vaches gestantes non en lactation, 16 vaches gestantes en lactation, 16 vaches non-gestantes en lactation et 9 génisses non-gestantes et non en lactation. Elles étaient exposées à un EMF de 10 kilovolts par mètre (kV/m) et 30 microtesla (μT) de deux façons différentes: a) pendant trois périodes consécutives de 28 jours basées sur deux séquences différentes avec les EMF en marche / à lárrêt / en marche ou à lárrêt / en marche / à lárrêt de façon alternative; b) pendant trois périodes consécutives avec une séquance À LÁRRÊT (5 jours), EN MARCHE (30 jours) et À LÁRRÊT (5-12 jours). Líntensité des

EMF choisie pour cette étude représentait une situation à laquelle les vaches font face lorsqu'elles se tiennent continuellement sous une ligne de haute tension de 735 kV AC lorsque la ligne a une charge maximale de courant. En réalité, ces conditions se manifestent seulement pendant quelques jours d'hiver dans la province de Québec.

La composition et la production laitière, la prise alimentaire, les profils hormonaux et les composantes du liquide cérébro-spinal (CSF) ont été évalués durant les différentes périodes d'exposition. La majeure partie des facteurs évalués n'étaient pas affectés par les EMF. Cependant, une association positive a été observée entre les EMF et la prise alimentaire, le taux de gras dans le lait, la progestérone dans le plasma du sang durant la gestation et la durée du cycle œstral. De plus, il y a eu des changements au niveau des concentrations de minéraux et de métabolites neurotransmetteurs dans le CSF qui ont démontré être en rapport avec les EMF.

En conclusion, il pourrait être énoncé que les EMF ont causé une réponse biologique chez la vache laitière. Il est spéculé que ces changements ne représentent pas un danger au niveau de la santé des vaches exposées, mais ils requièrent de plus amples recherches.

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GUIDELINES CONCERNING THESIS PREPARATION

Faculty of Graduate Studies and Research, McGill University.

This thesis consists of five papers in compliance with section B.2 of the "GUIDELINES CONCERNING THESIS PREPARATION" (Revised January, 1993) form the Faculty of Graduate Studies and Research, McGill University. These guidelines require that the following statement be quoted in the thesis:

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3. Additional material (procedural and design data, as well as descriptions of equiped used) must be provided where appropiate and in sufficient detail (e.g. appendices) to allow to clear and precise judgement to be made of the importance and originality of the reserach reported in this thesis.

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The complete text of the above must be cited in full in the introductory sections of any thesis to which it applies (this will inform the external reader of Faculty regulations)."

ACKNOWLEDGEMENTS

I wish to thank the following persons for their valuable contribution to this study:

- **Dr. E. Block,** for his supervision, unlimited support and sense of humor.

- **Mr. J. Carreño,** for his constant support during this study.

- **Dr. R. Cue,** for his advice in statistical analyses.

- **Dr. B. Downey,** for his advice in reproductive physiology.

- **Dr. L. Phillip,** for being part of my Graduate Committee

- **Dr. H. Monardes,** for his support and guidance.

- **Dr. C. Gianoulakis Ph.D.,** from the Douglas Hospital Research Centre for her assistance with the Beta-endorphin radioimmunoassay.

- **Dr. S. Young,** from the Department of Psychiatry of McGill University for his assistance with the neurotransmitter analyses.

- **Mrs. F. Renaud, Dr. D. Goulet and Dr. C. Cardinal,** Scientific research advisors from the Environment branch of Hydro-Québec.

- **Mr. Louis Richard,** from the Institut de Recherche d'Hydro-Québec (IREQ), as an assisstant in developing the software and hardware for the EMF control system.

- **Dr. D. H. Nguyen** from IREQ, for developing the EMF chamber and support.

- **Dr. M. Misakian** (National Institute of Standards and Technology, USA), **Dr. L. Rivest** (Université Laval), **Dr. J. Lee jr** (Bonneville Power Administration, USA), **Dr. P. Lamothe** (Université Montréal) and **Dr. S. Maruvada** (Institut de Recherche d'Hydro Québec); for their participation in the Scientific Committee.

CONTRIBUTIONS OF CO-AUTHORS

Chapters III, IV, V, VI and VII are authored by J. F. Burchard and E. Block and coauthored by D.H. Nguyen and L.Richard (Chapters III, IV, V, VI and VII) and S. Young (Chapter VI). Dr. E. Block was my supervisor, Drs. D.H. Nguyen and L. Richard designed the electric and magnetic field exposure chamber and provided technical assistance throughout this study. Dr. S. Young assited me with the neurotransmitter metabolite analyses.

I. GENERAL INTRODUCTION

Several studies conducted during the last three decades have concluded that electric and magnetic fields elicit a response in different biological systems. Nevertheless, the scientific evidence produced until now is still controversial and, therefore, calls for a word of caution. This implies that there is still a considerable amount of reseach to be done in order to produce feasible recommendations for the public and electric utilities.

Practically the entire Hydro-Québec energy system is operated on alternating current and milk production accounts for the largest share of Québec's agriculture. This prompted, in 1984, the Government of Québec to request that Hydro-Québec conduct a study on the health of livestock subjected to electric and magnetic fields from high-voltage transmission lines. This request was linked to the issuing of an authorization certificate for construction of a 735-kV line between the Nicolet and Kingsley substations.

To respond to the Québec government's request and attempt a worthwhile scientific contribution to knowledge in this field, Hydro-Québec included livestock studies in its research program on the possible biological effects of electric and magnetic fields. This research program is part of a structured plan of action adopted by Hydro-Québec in 1986. Therefore, Hydro-Québec requested the Animal Science Department of McGill University, located on Macdonald Campus, to develop various

research protocols for studying the possible influence of the fields generated by alternating current from high-voltage lines on milk production.

In November 1989, the different research proposals submitted were presented to the Dairy Cattle subcommittee of the Conseil des Productions Animales du Québec (CPAQ) and to the person responsible for the dossier at the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ).

Among the research proposals submitted, a controlled-environment study, reproducing common farming conditions, was finally chosen on the experts' recommendation. This was the only one of the proposed studies that could have provided cause-and-effect relationships, compared to field and retrospective studies. The design of this study provided control over all electrical and some environmental parameters.

The study sought to determine whether exposure to electric and magnetic fields, similar to those that exist at ground level under 735 kV alternating current (AC) transmission lines, has any effect on health and milk production of dairy cows. More specifically, the study intended to determine whether exposure to the fields causes significant changes in estrous cycles, hormonal profiles during gestation, pineal gland function, milk production and composition and cerebrospinal fluid composition.

II. LITERATURE REVIEW

1.0 Introduction

Electric and magnetic fields (**EMF**) have been accompanying biological organisms since the beginning of their evolution. The source of these naturally occurring EMF are the earth acting as a permanent magnet, lightning discharges, atmospheric changes as well as solar radiation. However, during the last decades there has been an important exponential growth of man-made EMF derived from the use of electrical energy in industrialized countries. Since EMF are capable of inducing electric currents and fields in the tissues of exposed subjects (Tenforde, 1989; Kaune and Gillis, 1981; Kaune and Forsythe, 1988), the hypothesis that EMF may represent a hazard to human and animal health has motivated a considerable number of scientists to direct their research efforts toward a better understanding of the biological effects of EMF.

This literature review does not pretend to be exhaustive about the above mentioned subject, but will tend to focus upon those aspects relevant to the potential EMF effects on exposed farm animals.

2.0 Electrical concepts

Electric power transmission lines and all electrical devices produce electric and

magnetic fields with a certain intensity dependant on the voltage and the current.

2.1 Electric field

All materials are constructed with atoms and these atoms have essentially three elements, neutrons, protons (positive charged) and electrons (negatively charged). Most of the materials tend to have a balanced charge since they have the same number of protons as electrons. However, in some instances when these materials are disturbed, such as the case of a plastic rod rubbed with some fur, they get charged. This means that the amount of protons and electrons are not equal and the material obtains either a negative or positive charge. If this material permits an electric charge to move from one region of the material to another it is called a conductor. If there are two bodies with a positive charge at an adequate distance they will exert mutual repulsion. The force that makes possible this repulsion or attraction, depending on the charges of the bodies, is a vectorial force. When one of the charged bodies is removed, its charge produces a vectorial force or electric field that exists at all points in the region around the charged body. If an electric field exists within a conductor, a force is exerted on every charge in the conductor. The motion of the free charges brought about by this force is called a current. Conversely, if there is no current in a conductor and hence no motion of the free charges, the electric field in the conductor must be zero. In summary, electric field is the force per unit charge on a test charge at any point, provided the test charge is small enough so it does not disturb the charges that cause the field. The electric field caused by a collection of

charges is always a force field. If any charged particle is moving within this electric field, the latter will exert its force over the particle and this will result in work which is represented by the potential energy of the collection of charges of the electric field. From this, potential can be defined as the potential energy per unit of charge and the unit is a volt. Electric fields found near transmission lines are normally measured in units of kilovolts per meter (**kV/m**). For example, 1 kV/m means that there is a difference of 1 kV (1000 volts) between two points in the air 1 m apart. The maximum electric field near the ground usually occurs just outside the outer conductors. The highest commercial AC transmission line voltages in the United States and Canada are 765 and 735 kV, respectively, and they produce maximum electric fields of 10-12 kV/m. The electric field force is an inverse function of the distance. For example, directly underneath a 500 kV transmission line there is an electric field of 7 kV/m, whereas at 100 meters from the transmission line the field decreases to 0.1 kV/m (Lee **et al.**, 1989, Sears **et al.**, 1987).

2.2 Magnetic field

One charge at rest creates an electric field in the space surrounding it. This electric field exerts a force on a charge placed in the field. Following the same pattern, a moving charge or a current sets up a magnetic field in the space surrounding it and this magnetic field exerts a force on a moving charge or current. This magnetic field is a vector field and the magnitude of its force is proportional

to the charge and the speed of the charge. This is different from an electric-field force, which is the same whether the charge is moving or not. The unit of the magnetic field B is (Newton)*(second)*(Coulomb)$^{-1}$*(meter)$^{-1}$ or since 1 ampere is 1 coulomb per second, N*A^{-1}*m^{-1}. This unit is called 1 tesla (T). The unit gauss (G) is also used (1 T=10^{4} G). Similar to the electric field, magnetic field intensities decrease with distance from the origin. For example a 500 kV transmission line carrying peak current exerts a magnetic field of 140 mG (14 μT) at ground level directly underneath the power line. At 100 meters from the transmission line tower, the magnetic field decreases to 3 mG (0.3μT) (Sears **et al.**, 1987, Lee **et al.**, 1989).

3.0 Mechanism of interaction between EMF and a biological response

There is inconclusive evidence from both epidemiological and laboratory studies that electric and magnetic fields similar to those produced by electrical power transmission lines may contribute to certain diseases including cancer. Electric and magnetic fields can induce a cellular response without producing a macroscopic thermal change (the temperature in biological samples exposed to EMF increases less than 0.001 °C; Basset, 1989). The prevailing thought is that effects of EMF are caused in another non-thermal way, because it has been shown that fluctuations in the potential across membranes in the cell due to thermal motion are several orders of magnitude greater than variations in potential caused by external 50/60 Hz EMF (Adair, 1991). Also it has been determined that fluctuations in electric fields induced

by an oscillating external 50/60 Hz 300V/m EMF are about 20 mV/m across the cell membrane, which is well below the endogenous fields produced by endogenous currents associated with normal membrane processes (Weaver and Astumian, 1990; Adair, 1991). A comparison of current densities and characteristics associated with field-related phenomena such as electrosensitivity in species of fish (Pickard, 1988), night-time melatonin depression in rodents (Wilson and Anderson, 1990), limb regeneration in amphibians (Borgens **et al.**, (1977) and magnetophosphenes in humans (Lovsund **et al.**, 1980) reveal little that can be of use in determining a 'response metric', yet guide-lines for the general public are in fact based on this quantity, for immediate effects at least. Indeed, currents induced by the electric component of environmental 50 Hz fields are of similar magnitude to those induced by the magnetic component, yet epidemiological studies have identified surrogates of the latter as the significant exposure metric in relation to the incidence of cancer (Wood, 1993).

The available evidence suggests that EMF interacts with biological systems by altering the normal membrane permeability to differnt ions especially Ca (Blackman, 1989) and/or by inhibiting the secretion of melatonin in a similar way as light does, based on the hypothesis that light is also an electromagnetic wave and the retina of some animals would be reactive to it (Wilson, 1989). Experiments on the effects of EMF on cells of the immune system, T-lymphocytes in particular, suggest that the external field interacts with the cell at the level of intracellular signal transduction

pathways. These are directly connected with changes in the calcium-signaling processes of the cell. Based on these findings, a theoretical model for receptor-controlled cytosolic calcium oscillations and for external influences on the signal transduction pathway has been proposed. It is suggested that the external field acts on the kinetics of the signal transduction between the activated receptors at the cell membrane and the G-proteins activating phospholipase C beginning a biochemical cascade that produces inositol triphosphate releasing Ca from internal stores and signaling influx of extracellular Ca (Eichwald and Kaiser, 1995). This hypothesis was tested in neurosecretory cells with carbachol stimulated Ca response and exposed to 1, 60 and 200 Hz 1G EMF. The exposure to EMF did not alter maximun Ca during carbachol stimulation (Thomas **et al.**, 1995). The total current of Ca ions through patch-clamped cell membranes was measured while exposing clonal insulin-producing beta-cells (RINm5F) to a combination of DC and AC magnetic fields at so-called cyclotron resonance conditions. The transport of Ca ions through the protein channels of the plasma membrane did not show any resonant behavior in the frequency range studied (Hojevik **et al.**, 1995). Recently the hypothesis that EMF would affect radical pair chemistry has been gaining some support with the EMF scientists. Radical pairs are produced when the electron sharing bonds are broken and they are common in many biochemical reactions. The half-life of a radical pair is relatively short but, under certain conditions, a magnetic field may prolong its lifespan. The limits which apply to chemical effects induced by low frequency and constant fields indicate that magnetic fields weaker than the earth's fields (around 50

μT) cannot affect biological entities significantly through effects on radical pairs recombination. However, in unusual circumstances one cannot categorically exclude the possibility that, by mechanisms not understood now, the radical pairs are held together for as long as 100 nanoseconds allowing fields as small as 5 μT to generate significant biochemical effects (Adair, 1995).

4.0 Effects of EMF on Calcium dynamics

Kavaliers and Ossenkopp (1987) have suggested that EMF affect the functioning of calcium channels that could result in an alteration of the effectiveness of the opiate system to cope with pain (Testkey **et al.**, 1988). There is evidence that that EMF have an effect on the mobilization of calcium (Ca) from brain cells. This evidence has been produced **in vitro** using freshly isolated avian and feline brains. However, some authors have found that EMF decrease the efflux of Ca from nervous tissues (Bawing and Adey, 1976) and others have demonstrated an increase in the efflux of Ca from brain preparations (Blackman, 1989). It has been have been suggested that these effects are caused by a direct interaction of the EMF with the cell membrane (Blackman, 1989; Goodman **et al.**, 1989).

Experiments conducted in several independent laboratories studying the effect of time-varying magnetic fields with intensities varying from 0.02 mT to 10 mT have found an effect on intracellular Ca concentrations (Walleczek, 1992). Concanavalin

A activated rat thymic lymphocytes exposed to a 0.021 mT, 14.5 Hz magnetic field for 1 hour showed a 60% increase in Ca uptake (Walleczek and Liburdy, 1990). The mobilization of Ca is among the earliest responses of lymphocytes after binding of an antigen or ligand to a receptor on the cell surface. The ligand-receptor reaction induces mobilization of Ca that results in an initial rise of Ca from intracellular stores followed by a Ca influx from the extracellular medium (Weiss, 1987). It is known that blocking this response may result in detrimental effects to the cell activity (Howell and Martz, 1988). Human peripheral lymphocytes exposed to a 2.5 mT, 60Hz magnetic field showed an increase of 30-60% in [^{3}H]thymidine uptake when they were mitogen-activated. No effect was detected in resting cells (Cossarizza **et al** , 1989). Brain microsomes from rats showed 20% less net ATP-dependant Ca uptake when exposed to a 60 Hz 1 G magnetic field for 3, 5 and 10 min. This may represent inhibition of the Ca ATPase or enhanced leakage from the microsome (Stagg **et al.**, 1994). Exposure of a human leukemic T-cell line JURKAT to a 50 Hz (50 mV/cm) electric field resulted in a 11% increase in Mn influx to the cells although, this electric field did not induce changes in Ca flux (Walleczek **et al.**, 1992). Low-frequency magnetic fields can increase the cytosolic calcium concentration in lymphocytes in the same manner as a physiological stimulus such as antibodies directed towards the CD3 complex. In a study, magnetic fields with various frequencies and flux densities were used, while cytosolic calcium concentration changes were recorded using microfluorometry with fura-2 as a probe. The applied sinusoidal magnetic field induced oscillatory changes of cytosolic calcium

concentration in the leukemic cell line JURKAT in a manner similar to that seen with stimulation by antibodies. The response at 0.15 mT was over a frequency range from 5 to 100 Hz, with a fairly broad peak having its maximum at 50 Hz. The result of testing increasing flux densities at 50 Hz was a threshold response with no effect below 0.04 mT and a plateau at 0.15 mT. On the basis of the characteristic Ca pattern resulting from an applied magnetic field, it was suggested that the magnetic field influences molecular events in regular signal transduction pathways of T cells (Lindstrom **et al.**, 1995). There are a number of claims in the literature that specific combinations of low-level DC and AC magnetic fields can cause biologically significant effects. The combinations of fields required to elicit these responses fulfill the theoretical conditions for classical cyclotron resonance of the selected ion. Because of the biological importance of Ca, any effects on it is of particular interest. For instance the claimed increase in Ca uptake by electromagnetically exposed lymphocytes. Measurement of the intracellular Ca concentration, by means of a sensitive fluorescent probe, during a 60 min exposure of mouse lymphocytes to EMF at two frequencies (16 Hz and 50 Hz) were tested, with a range of DC field amplitudes used to shift the frequency up to 25% either side of the calculated optimum. Treatment of the lymphocytes with concanavalin A was used as a positive control and caused a significant increase in intracellular Ca concentration. No change in intracellular Ca concentration could be detected when lymphocytes were exposed to the magnetic fields (Coulton and Barker, 1993).

Rat embryonic neuronal cells exposed to 500 mG (50 μT) 60 Hz EMF did not show any change in Na/K/Cl cotransport or Na/K active transport (Winters **et al.**, 1992). Adult male rats exposed to 50 Hz electric fields (0, 4 and 40 kV/m) for 5 h daily and 66 days showed decreased Ca concentrations in the hippocampus and hypothalamus (Yao **et al.**, 1992). Other ions, such as Cu and Zn have multiple functions as cofactors of enzyme activity and their variations have been suggested as part of the effects of EMF on health (Brugère **et al.**, 1995). Male rats were exposed for different amounts of time (20 min, 1,2,4,6,12,24,and48 h) to 50 Hz 10-100 μT EMF to investigate the effect of EMF on Cu and Zn in blood. Overall, effects were observed and the authors could not conclude that EMF influenced Cu and Zn blood concentration in rats (Brugère **et al.**, 1995).

5.0 Effects of EMF on the central nervous system, pineal gland secretion and its possible relationship to cancer.

A number of epidemiological studies have suggested that exposure to 50/60-Hz EMF from power lines and electrical equipment may be associated with a modestly increased incidence of various types of cancer (Wertheimer and Leper, 1979; Savits **et al.**, 1988). The available animal data on 50/60-Hz EMF exposures seem to indicate that intermediate EMF exposure exerts co-promoting effects in different tumor models, particularly cocarcinogenesis models of breast cancer,

whereas chronic (up to life-time) exposure may exert promoting effects on spontaneous development of certain tumors. The tumor promoting or co-promoting effects of 50/60-Hz EMF exposure found in several animal studies could be related to actions of EMF on gene expression, immune surveillance, and Ca homeostasis as demonstrated by **in vitro** experiments in cell cultures (Blackman, 1989). However, the most plausible evidence for an **in vivo** effect of EMF exposure which could be related to tumor promotion is reduction of circulating levels of melatonin (Wilson **et al.**, 1986, 1990; Lerchl **et al.**, 1991; Martinez-Soriano **et al.**, 1992; Rogers **et al.**, 1992; Haggren **et al.**, 1992; Yaga **et al.**, 1993; Kato **et al.**, 1994; Yellon **et al.**, 1994), a hormone which is inhibitory to the growth of a wide range of cancers, particularly breast cancer. Animal studies have shown that 50-Hz magnetic fields (MF) exposure at flux densities as low as 0.3-1 μT significantly reduces nocturnal melatonin levels in plasma (Lerchl **et al.**, 1990). Whereas decrease of melatonin levels alone could explain tumor promoting or co-promoting effects of MF exposure, recent data indicate that MF exposure also impairs the effects of melatonin at the cellular level. Thus, the oncostatic effect of melatonin on cell proliferation of a human breast cancer cell line was antagonized by 60-Hz EMF exposure at a flux density of about 1 μ T. All these data indicate that interactions between 50/60-HZ MF exposure and melatonin may be the key mechanism of any carcinogenic effect (Loscher and Mevissen, 1994). Magnetic resonance imaging (MRI) at 1.5 T was evaluated for its ability to modulate the level of melatonin in eight male volunteers. Subjects were exposed to three conditions, respectively, between 1:00 and 2:00 AM on different

nights: (a) a series of routine MRI pulse sequences for brain imaging in dark conditions, (b) dark control conditions, and (c) bright-light control conditions. Plasma was analyzed for melatonin and cortisol levels. These conditions were associated with significant differences in melatonin concentrations. Subjects exposed to darkness showed a typical increase in melatonin concentration. Subjects exposed to bright light showed a characteristic suppression of melatonin concentration. Those exposed to the MRI fields showed an increase in melatonin level similar to that seen in the dark control condition. Light and MRI had no significant effects on cortisol levels. Thus, MRI at field strengths known to modulate melatonin levels in rats did not suppress melatonin production in human subjects (Schiffman **et al.**, 1992, 1994)

The relationship between neurotransmitters and the body responses to stress are well established. Therefore, it is reasonable to assume that variations of their concentrations and/or their metabolite concentrations in cerebrospinal fluid (**CSF**) may lead to neurophysiological changes. Exposure of pig-tailed macaques to EMF ranging from 3kV/m and 0.1 G (10 μT) to 30 kV/m and 0.9 G (90 μT) for three 21-day periods resulted in a decrease of up to 20-30% in the CSF concentration of homovanillic acid (**HVA**) and 5-hydroxyindoleacetic acid (**5HIAA**), metabolites of dopamine and serotonin, respectively. These neurotransmitters are associated with higher functions of the central nervous system (Seegal **et al.**, 1988). Exposure of these macaques to above mentioned EMF (highest exposure level was about three times the maximum intensity under high-voltage power lines) did not result in a

significant change in blood chemistry, blood cell counts, and cerebellum and cerebrum macromorphology. Close observation with video tapes failed to detect any animal reaction to field activation (Wolpan **et al.**, 1989). Studies on the effect of 60 Hz EMF on evoked potentials (specific neural activity in the central nervous system (CNS) which, due to the conductivity of the body, can be detected in the surface of the skin as changes in voltages over time) did not show any difference in the various potentials assessed in pig-tailed macaques. An exception was the amplitude of the cortical somatosensory evoked potentials that decreased significantly during 10 kV/m, 0.3 G and 30 kV/m, 0.9 G EMF exposure. Post-exposure amplitudes were normal. The authors speculated that EMF could have an inhibitory effect over the endogenous opiate system (Dowman **et al.**, 1989). The antagonistic effects of EMF upon the analgesic and behavioral effects of opiates have been documented previously (Kavaliers and Ossenkopp, 1984; Ossenkopp and Kavaliers, 1987; Ossenkopp and Kavaliers, 1988-1989). Treatment of mice with EMF resulted in the potentiation of the opiate antagonistic effect of methylnatrexone (an opiate receptor blocker). It is argued that this potentiation is not the result of an alteration of receptor or cellular function, but an alteration of the permeability of the blood-brain barrier (Qouck **et al.**, 1986). This agrees with the temporary alteration of the blood-brain barrier permeability demonstrated in rats exposed to MRI (Shivers **et al.**, 1987). Mice treated with MRI magnetic fields (0.6 T/second) had a reduced fentanyl-induced analgesia. This also indicates an interference of the EMF with the opiod system to abolish pain (Teskey **et al.**, 1988). Exposure of rats to vertical 60 Hz, 39 kV/m

electric fields, 20 h/d for 4 wk resulted in a shift in the periodicity of the daily rhythm of norepinephrine, dopamine and serotonin's metabolite 5HIAA in the hypothalamus but not in the striatum or hippocampus. One of the biological clocks (suprachiasmatic nucleus) is located in the hypothalamus and a phase shift in the periodicity of the neurotransmitter levels may alter a variety of physiological systems (Vasquez **et al**, 1988). Also, this hypothalamic nucleus, via a multisynaptic pathway through the superior cervical ganglia, is the principal source of noradrenergic input to the pineal gland (Vasquez **et al.**, 1988). Rats exposed to 0.005 mT 1kV/m and 0.1 mT 5kV/m EMF for 8 mo had a decreased 5-HIAA content in the brain. This effect was reversed two months after the EMF were turned off (Zecca **et al.**, 1995).

Dopamine levels in light adapted retinae from albino rats were reduced by EMF. A similar effect was observed in the con-dominant retina of the pigmented ground squirrel. On the other hand, dopamine levels increased in the rod-dominant retina after daytime EMF exposure of golden hamsters. In spite of these differences among various rodent species, the authors indicated that the retina may play an important role in the perception of magnetic fields by mammals (Olcese and Hurlbut, 1989). Male and female rats were exposed to 1 G (100 μT) direct current (DC) EMF generated with Helmholtz coils for 1 mo or 4 mo and the effects of these exposures on regional brain neurotransmitter metabolism and circulating amino acid concentrations were determined. After 1 mo of EMF exposure the concentration of serotonin was elevated in the hypothalamus of male rats. Levels of the dopamine

metabolite, 3-methoxytyramine, were increased in the corpus striatum of male and female rats that were exposed to EMF for 1 mo. Hypothalamic concentration of norepinephrine was elevated in both groups of male rats, as compared to respective female groups, but was not affected by EMF. Similarly, levels of tyrosine were increased in hypothalamus, corpus striatum and nucleus accumbens of male rats, but were not affected by EMF exposure. Following 4 mo of EMF exposure, no significant effect of EMF was observed (Chance **et al.**, 1995).

Rats exposed to 0.7 T EMF 20 min/d for 2 wk developed morphological changes in the pineal gland cells. Mammary tumors were induced by intragastric administration of 20 mg (5 mg/wk) 7,12-dimethylbenz(a)anthracene (DMBA) in female Sprague-Dawley rats. Groups of 36 rats were either sham-exposed or exposed for 91 days to 50-Hz 0.3-1 μT EMF, which is a relevant range for elevated domestic EMF exposure as arising from neighboring power lines. Nocturnal melatonin levels were significantly reduced by exposure to this weak alternating EMF. However, histopathological evaluation of mammary lesions did not disclose any significant difference between EMF and sham-exposed animals. Incidence of mammary tumors was 61% in controls versus 67% in EMF-exposed rats. The predominant tumor type was the invasive adenocarcinoma, which was found in 21 rats of both groups. Examination of tumor size did not indicate significant differences in tumor burden between groups. Furthermore, the incidence of preneoplastic lesions was not altered by EMF exposure. Thus, the data of this study indicate that alternating EMF do not

exert significant tumor promoting or copromoting effects at environmentally relevant flux densities in the rat mammary cancer system (Loscher **et al.**, 1994). Stress resulting from the experiment handling enhanced the peptidergic activity in both light and dark control pinealocytes. Rats exposed to EMF had a reduced peptidergic activity in light pinealocytes (Milin **et al.**, 1988). Two groups of Wistar male rats were exposed to 50-Hz magnetic fields of either 1, 10 or 100 μT. The first group was exposed for 12 h and the second for 30 d (18 h/d). During this time the animals were kept under a standard 12: 12 light : dark cycle at 25° C and a relative humidity of 45 to 50%. Control (sham-exposed) animals were kept in a similar environment but without exposure to a magnetic field. The animals were sacrificed under red dim light. Serum melatonin concentration and pineal N-acetyltransferase (**NAT**) and hydroxyindole-O-methyltransferase (**HIOMT**) activities were studied. Long-term exposure to a magnetic field (10 and 100 μT) significantly depressed the nocturnal peak of serum melatonin concentration and pineal NAT activity whereas no effect was observed on HIOMT activity. Short term exposure depressed both pineal NAT activity and nocturnal serum melatonin concentration but only with the highest intensity used (100 μT). These suggest that sinusoidal magnetic fields alter the production of melatonin through an inhibition of pineal NAT activity. Both duration and intensity of exposure play an important role in this effect. Therefore, sinusoidal EMF depresses NAT activity as static magnetic field does whereas HIOMT activity remained unaltered whatever the type of experiment and the intensity used. The effect observed is related to both the duration of exposure and the intensity of

magnetic fields and the sensitivity threshold to magnetic fields varies with the duration of exposure which strongly suggests a cumulative effect of sinusoidal magnetic fields on pineal function (Selmaoui and Touitou, 1995). The pineal gland HIOMT, which catalyzes the conversion of N-acetylserotonin to melatonin, has been implicated to be involved in the regulation of the internal biological clock (Axelrod **et al.**, 1965, Axelrod and Fraschini, 1982). The normal nocturnal increase of melatonin (MLT), which has been described as the translator of environmental conditions (Cremer-Bartels **et al.**, 1984), has been shown to be depressed when rats were exposed to 60-Hz, 1.8, 39 and 65 kV/m EMF (Wilson **et al.**, 1981). When rats were not exposed to EMF, normal circadian rhythm in pineal serotonin and MLT concentrations returned to normal concentrations within 3 d (Wilson **et al.**, 1986). It is discussed that EMF may produce a functional pinealectomy in chronically exposed rats (Wilson, 1988.), which could be comparable to that induced by constant light (Shah **et al.**, 1984). Changes in the earth's magnetic field resulted in a decrease in the concentration of pineal MLT during the night (Welker **et al.**, 1983). Artificial low-intensity EMF can alter the electrical activity of certain cells in the pineal gland in pigeons, guinea pigs, and rats, and also cause a decrease in MLT in guinea pigs and rats (Semm **et al.**, 1980; Semm, 1983). It has been suggested that part of the nighttime inhibitory effect of EMF on morphine-induced analgesia in mice may arise through the pineal gland. However, it was also indicated that other non-pineal mechanisms must be involved since inhibition of morphine-induced analgesia can be achieved with EMF during daytime, when metabolic activity and MLT output are

minimal in the pineal (Teskey **et al.**,1988; Ossenkopp and Kavalier, 1988-1989). Electric and magnetic fields may affect pineal function by interfering with neuronal input to the pineal gland from the CNS. Such alterations of the circadian rhythm in the pineal synthesis of MLT may result in behaviour problems (Wilson, 1988). One possible mechanism for the effects of EMF on the pineal gland is that this gland requires tonic sympathetic input from nerves such as the internal carotid nerve in order to maintain MLT secretion rhythmicity. This necessary input may be disrupted by currents resulting from EMF (Bowers **et al.**, 1984). Another proposed mechanism for the EMF-caused pineal desynchronization is consistent with the view that the mammalian retina (Krauze **et al.**, 1985), the pineal gland, or both, may be directly responsive to EMF. Exposure to magnetic fields produces the isomerization of 11-cis to all-trans retinal in retinas of rats. This represents the initial step in the photostimulation of the visual system; therefore, it can be suggested that, as with light, the magnetic fields may stimulate the retina and hence inhibit the pineal activity (Reiter **et al.** 1992). Non-invasive inhibition of nighttime pineal activity, either by light pulses or the amino-acid decarboxylase inhibitor, benserazide, has been shown to abolish the nocturnal analgesia noted in mice receiving morphine (Kavaliers **et al.**, 1983; Kavaliers and Ossenkopp, 1984). This is consistent with the nocturnal inhibition of morphine-induced analgesia caused by EMF (Kavaliers **et al.**, 1984), and the hypothesis that the pineal gland has magnetic sensor capabilities (Demaine and Semm, 1985).

Epidemiological studies have generated evidence that appears to link the exposure to EMF to certain kinds of cancer, especially leukaemia and lymphomas (Wertheimer and Leeper, 1979; Savitz **et al.**, 1988). Wilson and Anderson (1989) suggested a relationship between EMF and the etiology of cancer. They demonstrated that when rats were exposed to 60 Hz, 39 kV/m EMF for 3 wk their nocturnal levels in the pineal NAT activity and MLT concentrations decreased (Wilson **et al.**, 1986). Rats exposed to a repeatedly inverted horizontal component of the geomagnetic field for 30 to 60 min during a dark period in which the magnetic intensity varied between 20 to 30 μT showed a depressed NAT activity and MLT levels in their pineal gland 45 min after the initiation of exposure (Reiter **et al.**, 1992). The EMF produced by the ion cyclotron resonance depresses pineal MLT synthesis **in vitro** (Lerchl **et al** 1991). Sixty Hz EMF produced by electric blankets altered the urinary excretion 6-hydroxymelatonin sulphate, a stable urinary metabolite of MLT, suggesting an effect of EMF in humans (Wilson **et al.** 1990). Men exposed to 60 Hz 200 mG (20 μT) EMF did not show any effect on nocturnal MLT (Graham **et al.**, 1995). On the other hand, a negative association between total nocturnal 6-hydroxymelatonin sulphate and mean magnetic field exposure was found in electric utility workers carrying magnetic field meters and exposed to 12-18 mG-h (Reif **et al.**, 1995). Since MLT is a hormone that is antagonistic to the secretion of gonadotropins (Axelrod et al., 1982), its nocturnal decrease could result in an increased amount of circulating hormones required for the growth of certain hormone-dependant breast, ovarian and prostate cancers (Wilson and Anderson, 1989). On the other hand MLT has been

demonstrated as an oncostatic agent (Lissoni et al, 1991) or a cancer antipromoting hormone that could antagonize the tumour promoting actions of estradiol (Blask et al., 1991). Maestroni et al. (1989) have demonstrated that MLT possesses immuno-enhancing and anti-stress properties. On the other hand, when female lambs were exposed to the EMF produced by a 500-kV transmission line (4-7 kV/m, 1-50 mG), they did not show any significant variation in the mean nighttime MLT concentration at 18 or 32 wk of age (Thompson et al., 1991; Lee et al., 1992; Lee et al., 1995). Exposure of rats for 1 h during the dark cycle to a magnetic field of 40 μT failed to show any decrease in the amount of MLT present in the pineal gland (Sasser et al. 1992).

6.0 Biological effects of EMF on cattle

Two herds with a total of 55 cows grazing near 400 kV transmission lines did not systematically avoid the area underneath the power lines (Algers et al., 1981). Thirty six herds that were grazing during the summer in an area underneath a 400 kV line did not show any difference in fertility, according to artificial insemination records, that could be related to EMF (Algers et al., 1981). Two herds exposed to 400 kV lines in Sweden reported fertility problems. In the first herd, exposure time in the period 1974-1976 was about 25 d per year and in the 1977-1978 period about 55 d per year. The second herd was exposed about 26 d per year. Information obtained from both herds indicated then when exposure to 400 kV lines started, a detrimental effect on fertility was noticed (Algers et al., 1981). Busby et al., (1974)

carried out a milk production survey in four dairy farms. In two of the farms, it was claimed that milk production was higher before the installation of a 765 kV power transmission line near the farm. In the other two farms, no effect of high voltage was indicated. A study of 55 dairy farms over a period of 4 to 6 yr, before and after the construction of 765 kV power lines, revealed no effect in fertility, calf mortality and birth abnormalities that could be associated with the high tension lines (Williams and Beiler, 1979). Another retrospective study of cow fertility based on artificial insemination records of 106 farms in Sweden that were exposed to 400 kV lines for 15 or more days revealed no effect on reproductive performance due to high tension lines (Algers and Hennichs, 1985). Fifty eight heifers kept beneath a 400 kV, 50 Hz transmission line were exposed for 120 d to an EMF calculated to be 4 kV/m, 2 μT. No differences in regularity of the estrous cycle, mid-cycle plasma progesterone level, intensity of estrus, and conception index in response to EMF exposure were detected (Algers and Hultren, 1987). Exposure of 774 beef cattle to an average of 5.6 kV/m under a 500 kV direct current transmission line over a period of 3 yr did not affect feed consumption, health, mortality, breeding, number of calves weaned, calving, sexual development in bull calves and carcass weight (Raleigh, 1988).

7.0 Conclusions

The studies reported until now in the bovine species are retrospective studies based on surveys to farmers (Algers **et al.**, 1981, Busby **et al.**, 1974, Williams and Beiler, 1979), retrospective studies based on existing data banks (Algers and

Hennichs, 1985) or prospective studies (Algers and Hultren, 1987). The best attempts, thus far, to evaluate the biological effects of EMF on production and reproduction variables in a semi-controlled environment in cattle have been conducted in Sweden (Algers and Hultgren, 1987) and in the USA (Raleigh, 1988). Even though the variables measured in these experiments were not affected by EMF, the uniformity of the fields and the sensitivity of the variables assessed could be questioned as a source of considerable variation. On the other hand, the CNS has been suggested, because of it's richness in electrical activity, to be the system that could be affected the most by the EMF (Adey, 1981). None of the bovine studies addressed or measured any variable that could accurately assess the validity of this hypothesis.

One approach to study the CNS metabolism and activity **in vivo** is to rely heavily upon the analyses of CSF composition (Vogt, 1975). The CSF is a clear watery fluid produced by the CNS that is in intimate contact with the brain and the spinal cord. While concentrations of chemical substances in blood plasma (electrolytes, hormones, nutrients, etc) fluctuate over a wide range their concentrations in the CSF remain relative stable (Feldman, 1989).

In general, it can be stated that the methodology used in the different experiments to assess the effects of EMF upon biological entities is extremely diverse insofar as the EMF intensities and exposure times are concerned. Results obtained in several areas by different authors, in general, have a low level of repeatability,

which makes it difficult to draw orienting information. However, there is enough controversial evidence that warrants further research in this area. From the works consulted it appears that, if one attempts to assess the physiological implications of exposure to EMF, logical aspects to measure are those related to the stress response, reproductive hormonal profiles, the pineal gland secretion, and mineral variations in CSF and blood **in vivo.** It is hypothesized here that: **A)** Exposure to EMF alters the CNS activity resulting in changes in the concentration of neurotransmitter metabolites in the CSF, milk production and feed intake; and **B)**: the EMF are perceived by the animals either in the same fashion as light, through the retina, or act directly on the pineal gland altering its secretion. This change in secretion affects the pituitary secretion of gonadotropins. In order to test these hypotheses it was proposed to evaluate the effects of simultaneous and continuous exposure to alternating current EMF of dairy cattle with EMF similar to those prevailing directly under a 735 kV line at ground level (10 kV/m and 30 μT). Specifically, the effects of EMF on feed consumption, milk production and composition, progesterone, MLT and cortisol profiles and neurotransmitter metabolite, ß-endorphin and mineral concentrations in CSF were evaluated.

III. BIOLOGICAL EFFECTS OF 60 Hz ELECTRIC AND MAGNETIC FIELDS (EMF) ON PRODUCTIVITY OF DAIRY CATTLE.

Submitted and accepted by the Journal of Dairy Science (J. Dairy Sci. 79:submitted)

Running head: Electric and magnetic fields and dairy cows

Biological effects of Electric and Magnetic Fields on Productivity of Dairy Cows.

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ABSTRACT

Sixteen multiparous Holstein cows (weighing 600 ± 50 kg, in 184.8 ± 52 d of lactation, and at 101.9 ± 43 d of gestation) were confined to wooden metabolic cages and exposed to a vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 μT (micro-Tesla). The trial was conducted as a switchback statistical design. Cows were divided into two replicates of 8 cows each. One replicate was exposed for three periods of 28 d each. During the first period, the electric and magnetic fields were off; during the second period they were on; and during the final period, they were off. The second replicate was exposed for three periods also, but the activity of the fields was reversed (first period, on; second period, off; last period, on). Blood samples were obtained twice a week for the determination of cortisol and progesterone and once a week for determination of pH and blood gases. Milk samples were collected once a week to determine milk components (fat, protein, SNF and SCC). Milk yield and feed consumption were measured daily. Most of the variables studied (bicarbonate, pH, O_2 and CO_2 partial pressures, cortisol concentration in blood, uncorrected milk yield, and milk components, except milk fat) did not show any changes that could be attributable to exposure to electric and magnetic fields. Associations among the electric and magnetic fields and increased DMI, 4% FCM yield, milk fat content, and plasma progesterone were found.

(**Key words:** electric field, magnetic field, extremely low frequency, alternating current)

Abbreviation key: EMF = electric and magnetic fields, **MLT** = Melatonin.

INTRODUCTION

During the last few decades, there has been growth of manmade electric and magnetic fields (EMF) derived from the use of electrical energy. Because EMF are capable of inducing electric current and fields in the tissues of exposed subjects (17, 18, 34), the hypothesis that EMF might represent a hazard to human and animal health has motivated a considerable number of scientists to direct their research efforts toward a better understanding of the biological effects of EMF. Additionally, the electrical network of Hydro-Québec (QC, Canada) traverses rural areas where dairy farming and milk production represent the most important economic activity in agriculture in Québec. Because of this exposure Hydro-Québec was motivated to study the effect of extremely low frequency EMF on dairy cows. To evaluate these effects, a chamber was constructed that generated EMF similar to those prevailing directly under alternating current 735 kV power lines and that also resembled normal dairy farming conditions in confined housing. Some previous studies (4, 9, 37) were based on surveys of farmers. Others (1, 2, 23, 37) were retrospective studies based on surveys and existing data banks. The best attempts, thus far, to evaluate the biological effects of EMF on yield and reproduction variables of cows in a semi-controlled environment have been conducted in Sweden (3) and in the US (27). In those experiments, both direct and alternating current lines were used, and the variables (fertility, behavior, yield and health) measured for farm animals did not show evidence of an effect attributable to EMF. However, the uniformity of the EMF and the sensitivity of the variables assessed have varied considerably. Two herds with a total of 55 cows grazing near an 400-kV AC line did not systematically avoid the

area underneath the power lines (1). Using AI records 36 herds that were grazing during the summer in an area underneath a 400-kV AC line did not show any difference in fertility, that could be related to EMF (1). Also, two herds exposed to 400-kV AC lines in Sweden experienced fertility problems (1). In the first herd, exposure time between 1974-1976 was about 25 d/yr, and exposure time between 1977 and 1978 was about 55 d/yr. The second herd was exposed about 26 d/yr. Information obtained from both herds indicated that when exposure to 400-kV AC lines commenced, a detrimental effect on fertility was noticed. Other authors (9) carried out a milk yield survey of four dairy farms. On two of the farms, milk yield was higher prior to the installation of a 765-kV AC power transmission line near the farm. On the other two farms, no effect of the high voltage line was indicated. A study of 55 dairy farms during a period of 4 to 6 yr before and after the construction of a 765-kV AC power line revealed no effect of the lines on fertility, calf mortality, or birth abnormalities (38). Another retrospective study of the fertility of cows based on artificial insemination records of 106 farms in Sweden that were exposed to 400-kV AC lines for $\geq$ 15 d, revealed no effect on reproductive performance because of the high tension lines (2). Fifty-eight heifers housed beneath a 400-kV AC, 50-Hz transmission line were exposed for 120 d to an EMF calculated to be 4 kV/m and 2 μT (micro-Tesla). No differences in regularity of the estrous cycle, midcycle plasma progesterone concentration, intensity of estrus, or conception index in response to EMF exposure was detected (3). Furthermore, exposure of 774 beef cattle to a mean EMF of 5.6 kV/m under a 500-kV DC transmission line over a period of 3 yr did not

affect feed consumption, health, mortality, breeding, number of calves weaned, calving, sexual development in bull calves, or carcass weight (27). Exposure of cows to a mean EMF of 4 kV/m and 2 μT for a mean of 120 d did not influence the expression of estrus, midcycle blood progesterone, ovary weight, size of corpus luteum, or conception rate (15).

The problems with those studies were that the intensity and time exposed to EMF varied considerably. In fact, in some of the trials, the exposed cattle may have been receiving no EMF exposure for days or portions of days because the current in the power lines varied as did the distance of the cattle from the lines, both of which affect the intensity of the EMF. The trial described here was designed to study the effect of maximal and continous exposure to 60-Hz AC EMF generated by 735-kV power lines on blood gases, milk yield and composition, feed intake, cortisol, and progesterone in pregnant dairy cows.

MATERIALS AND METHODS

Sixteen multiparous pregnant lactating Holstein cows (weighing 600 ± 50 kg, in 184.8 ± 52 d of lactation and at 101.9 ± 43 d of gestation) were confined to wooden metabolic cages in an EMF exposure chamber. During the experiment, they were exposed to 12 h of light, 12 h of darkness and a mean temperature and humidity of 16.25 ± 1.86C and 44.3 ± 7%, respectively. The chamber was 15 m long, 10 m wide, and 3 m high; contained eight wooden box stalls, each holding one cow, and was designed to allow simultaneous exposure of the eight cows during long uninterrupted periods. Wooden (rather than metal) box stalls were used to minimize disturbance of the electric field. The chamber was ventilated and heated artificially.

Water and feed were available **ad libitum**. Cows were fed twice daily total mixed diets formulated according to NRC requirements (24). Total feed consumption was estimated by weighing feed offered and orts daily. The cows were milked twice daily. During milking all cows were removed from the EMF chamber for 30 min to be milked in stalls. Exposure to EMF was in a switchback design (10).

The cows were divided into two replicates of 8 cows each. One replicate was exposed for three periods of 28 d each. During the first period, the EMF were off, during the second, they were on; and during the third period the EMF were off. For the second replicate, the EMF sequence was reversed (first period, on; second period, off; third period, on).

The magnetic field in the chamber was generated by 14 rectangular coils of 10 m long and 4 m high. A current of 1.03 A generated a uniform horizontal magnetic field of 30 μT. The electric field was generated by two plates that were 9 m long and 6.5 m wide and were suspended 0.4 m from the ceiling by synthetic isolators. The total electric capacity of the plate was 4.3 nF (nanofarad). Within the chamber, a vertical electric field of 10 kV/m was produced. The intensity of the EMF chosen for this experiment resembled a situation in which the cows were standing continuously under a 735-kV AC power line when the line had a maximun load of current. In reality, these conditions are found only for a few days during the winter in the province of Québec.

Feed samples were collected weekly, composited, and analyzed on a monthly basis. Venous blood samples were obtained from the coccygeal vein in heparinized vacutainer tubes (Becton Dickinson, Rutherford, New Jersey) twice per week for the determination of cortisol and progesterone and once a week for the determination of pH and blood gases. Blood samples collected were immediately analyzed for pH, bicarbonate, O_2, and CO_2 using a blood gas analyzer to measure gas partial pressures following the Van Slyke procedure (IL1306; Instrument Lab., Milan, Italy). An aliquot was centrifuged, and the plasma that was obtained was frozen at -20°C pending radioimmunoassay. Plasma cortisol and progesterone concentrations were estimated using a solidphase radioimmunoassay (Coat-a- Count; Diagnostic Products Corp., Los Angeles, CA) without extracting the plasma. The cortisol standard curve ranged from

0 to 50 μg/dl with a maximum binding of 90% and a sensitivity of 0.2 μg/dl; the sample size was 0.5 μl and intra and inter assay coeficients of variation were 2.55 and 10.8% respectively. The progesterone standard curve ranged from 0 to 40 ng/ml with a maximum binding of 39% and a sensitivity of 0.03 ng/ml; the sample size was 100 μl and intra and inter assay variations were 2.12 and 9.33 %, respectively. Milk sub-samples were obtained on Mondays (pm) and Tuesdays (am) and proportionately composited into one weekly sample. Milk samples were collected in vials containing bromopol to preserve milk components and were sent to the Dairy Herd Analysis Service laboratory, Sainte Anne de Bellevue, QC, to determine milk components (fat, protein, SNF) using infrared spectroscopy (Bentley 2000, Chasca, Minnessota) and somatic cell counts using flow cytometry (Somacount 300, Chasca, Minnesota).

Statistical Analysis

The data were analyzed using analysis of variance of a switchback design with the following regression model:

$$Y_{ijkl} = \mu + C_{ij} + MC_{ij} + P_k + T_l + E_{ijkl}$$

Where:

Y_{ijkl} = dependent variable,

μ = overall population mean,

C_{ij} = effect of the cow j in the sequence group i (j=1,...,16 and i=1 or 2),

MC_{ij} = linear time trend of cow j in the sequence group i,

P_k = effect of the period k (k= 1, 2 or 3),

T_l = effect of the treatment l (l= 1 EMF off or 2 EMF on) and

E_{ijkl} = random error (normally and independently distributed with zero mean).

Treatments were compared using orthogonal contrast between the differences

of the means using a *t* test. Significant differences between means were declared at $P < 0.05$.

RESULTS

Feed Consumption, Milk Yield and Composition

With exception of milk fat, exposure to EMF did not change milk composition (Table 1). Milk yield uncorrected for fat content, did not vary when the EMF periods were compared. However, for 4% FCM, an increase ($P < 0.001$) of 9.1% was detected during EMF exposure. Dry matter intake (DMI) increased ($P = 0.002$) 5.5% during EMF exposure. The SCC was not affected by treatment (Table 1).

Plasma Cortisol and Progesterone

Results for cortisol and progesterone are presented in Table 2. Cortisol concentration in plasma did not differ ($P > 0.05$) between cows exposed and cows not exposed to EMF. Progesterone concentration in plasma was increased ($P = 0.002$) during exposure.

Bicarbonate Concentration, pH, and Gas Partial Pressures in Blood

No differences in bicarbonate concentration, pH, or CO_2 and O_2 partial pressures in venous blood were detected when cows that had been exposed to EMF and cows that had not been exposed to EMF were compared (Table 3).

DISCUSSION

Mean daily milk yield was not affected by exposure to the EMF. However, milk fat content increased from 4.06 to 4.43% ($P = 0.008$) during the exposure period. This increase in milk fat content during exposure was coincident with an increase in DMI when the EMF were on (Table 1). These changes are important biologically, but were within the normal range of productivity (16). In this study, the increased DMI could hypothetically be related to a decrease in the nocturnal MLT secretion by the pineal gland that has been shown to occur with exposure to EMF. Exposure to EMF depressed pineal MLT synthesis **in vitro** in rats (22). The 60-Hz EMF produced by electric blankets altered the urinary excretion of 6-hydroxymelatonin sulfate, a stable urinary metabolite of MLT, suggesting an effect of EMF in humans (41). In the present experiment, a hypothetical decrease in the nocturnal peak of MLT caused by exposure to EMF might have increased prolactin in plasma, leading to an increase in voluntary DMI, analogous to the effect of longer day length. An increase in day length has been associated with a decrease in nocturnal MLT in prepubertal heifers (36), an increase in voluntary DMI (11), body weight and prolactin in sheep (13) and red deer (33). Melatonin decreases prolactin concentrations and decreases DMI (11). Similarly, immunization against MLT tended to result in higher plasma prolactin concentrations in red deer (5), and prolactin injections resulted in increased DMI and live BW gain in red deer (28). Photoperiods of 16 h of light and 8 h of darkness increased the rate of body growth in cattle by 10 to 16% (26), and cows exposed to 16 h of light and 8 h of darkness yielded 3 kg more

milk than cows under natural photoperiod; however, the percentage of milk fat was unaffected (25). Rats exposed to a repeatedly inverted horizontal component of the geomagnetic field for 30 to 60 min during a dark period, in which the magnetic intensity varied between 20 to 30 μT, showed depressed N-actetyl transferase activity and MLT concentrations in the pineal gland 45 min after the initiation of exposure (42). Rats exposed to 60-Hz, 39-kV/m EMF for 3 wk had decreased nocturnal N-acetyltransferase activity and decreased MLT concentrations in the pineal gland (39,40). The inhibition of the N-acetyltransferase activity might have resulted because of the accumulation of serotonin in the pineal gland (21) and, eventually, higher concentrations of serotonin in the cerebrospinal fluid.

The role of serotonin in feeding behavior has been examined in rats by intracranial injections of this neurotransmitter, which inhibits feed intake (14, 32). In contrast to the studies of rats, intracranial injections of serotonin dissolved in synthetic CSF in monkeys (30), sheep, and cattle (7) increased feed intake. The decrease in body temperature observed following intracranial serotonin injections in cattle (12) and sheep (8) could have led to the increased feeding response. Conversely, when female lambs were exposed to the EMF produced by a 500-kV AC transmission line (4 to 7 kV/m, 0.1 to 5 μT), the lambs did not show any significant variation in the mean nighttime concentration of MLT at 18 or 32 wk of age (19, 20, 35). Exposure of rats for 1 h during a dark cycle to a magnetic field of 40 μT failed to decrease the amount of MLT present in the pineal gland (29). Conversely, MLT

is an antigonadotropic hormone (6, 31, 39), and its decreasing secretion might have resulted in an increased gonadotropin secretion that, in turn, could have stimulated the secretion of steroidal hormones, increasing the progesterone concentration in pregnant cows. On the other hand, as showed in chapter IV of this thesis, EMF exposure did not produce a significant change in MLT nocturnal secretion. Nevertheless, the ambiguity of the nocturnal secretion of MLT, when analyzed by replicate, has to be considered when giving a plausible explanation to the increased DMI observed in this experiment.The EMF did not have any significant effect on cortisol plasma concentrations, which agrees with previously reported work in female lambs (32).

From the results obtained in this experiment, most of the variables assessed (bicarbonate, pH, O_2 and CO_2 partial pressures, cortisol concentration in blood, uncorrected milk yield, and milk components, except milk fat) did not show any variation that could be attributable to EMF exposure. Associations between EMF and DMI, 4% FCM yield, milk fat content, and plasma progesterone were found.

CONCLUSIONS

In conclusion, exposure of dairy cows to EMF caused an increase in DMI milk fat percentage and blood progesterone. No indications of observable health hazards to dairy cattle where found by the exposure to 10kV/m and 30 μT EMF .

ACKNOWLEDGMENTS

The authors thank J. Carreño, B. Allard, R. Cue, B. Downey, M. Misakian, L.P. Rivest, J. Lee, Jr., P. Lamothe, S. Maruvada, F. Renaud, D. Goulet, and C. Cardinal for their contributions. This research was sponsored by the Environmental Branch of Hydro-Québec, Montréal.

REFERENCES

1 Algers, B., I. Ekesbo, and K. Hennichs. 1981. The effects of ultra high-voltage transmission lines on the fertility of dairy cows. A preliminary study. Rep. N°. 5 Swed. Univ. Agric. Sci. Dep. Anim. Hygiene with Farrier School, Skara, Sweden.

2 Algers, B., and K. Hennichs. 1985. The effects of exposure to 400 kV transmission lines on the fertility of cows. A retrospective cohort study. Prev. Vet. Medi. 3:352.

3 Algers, B., and J. Hultgren. 1987. Effects of long-term exposure to 400-kV, 50 Hz transmission line on estrous and fertility in cows. Prev. Vet. Medi. 5:21.

4 Amstutz, H., and E.D.B. Miller. 1980. A study of farm animals near 765 kV transmission lines. Bovine Pract. 15:51.

5 Ataja, A. M., P. R. Wilson, T. N. Barry, J. Hodgson, R. M. Hoskinson, W. J.

Parker, and R. W. Purchas. 1992. Early venison production from red deer (Cervus elaphus) as affected by grazing perenial or annual ryegrass pastures, pasture surface height and immunization against melatonin. J. Agric. Sci. 118:352.

6 Axelrod, J., F. Fraschini, and G. P. Velo, ed. 1982. The Pineal Gland and its Neuro-endocrine Role. Plenum Press, New York, NY.

7 Baile, C. A., J. S. Beyea, L. F. Krabill, and M. A. Della Fera. 1979. Intracranial injections of 5-HT and db cAMP and feeding ion sheep and cattle. J. Anim. Sci. 49:1076.

8 Bligh, J., W. H. Cole, and M. Maskrey. 1971. Influence of ambient temperature on the thermoregulatory responses of 5-hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerbral ventricles of sheep, goats and rabbits. J. Physiol. 212:377.

9 Busby, K., D. Driscoll, and W. E. Washbon. 1974. A field survey of farmer experience with 765 kV transmission lines. Agric. Res. Commiss., State of New York, Albany, NY.

10 Cochran, W. G., and G. M. Cox. 1957. Page 141 in Experimental Designs. John Williams and Sons, NY.

11 Dominique, B.M.F.,P. R. Wilson., D. W. Dellow, and T. N. Barry. 1992. Effects of subcutaneous melatonin implants during long daylength on voluntary feed intake, rumen capacity and heart rate of red deer (Cervus elaphus) fed on a forage diet. British J. Nutr. 68:77.

12 Findlay, J. D., and G. E. Thompson. 1968. The effects of intraventricular injections of noradrenaline 5-hydroxytryptamine, acetylcholine and tranylcypromine on the ox at different environmental temperatures. J. Physiol. 194:809.

13 Forbes, D. M., P. M. Driver, W. B. Brown, G. G. Scannes, and I. C. Hart. 1979. The effect of daylength on the growth of lambs. 2. Blood concentrations of growth hormone prolactin, insulin and thyroxine, and the effect of feeding. Anim. Prod. 29:43.

14 Goldman, H. W., D. Lehr, and E. Friedman. 1971. Antagobistic effects of alpha and beta-adrenergically codded hypothalamic neurons on consummatory behaviour in the rat. Nature 231:453.

15 Hultgren, J. 1989. Cows and electricity study, biological interaction and effects on cow behaviour, health and reproduction of stray voltage, electric cow-trainers and high voltage transmission lines. Rep. N$_o$ 27. Swed. Univ. Agric. Sci. Dep. Anim.

Hygiene with Farrier School, Skara, Sweden.

16 Jairath, L. K., J. F. Hayes, and R. I. Cue. 1995. Correlations between first lactation and lifetime performance traits of canadian holsteins. J. Dairy Sci. 78:438.

17 Kaune, W. T., and W. C. Forsythe. 1988. Current densities induced in swine and rat models power-frequency electric fields. Bioelectromagnetics 9:1.

18 Kaune, W. T., and M. F. Gillis. 1981. General properties of the interaction between animals and ELF electric fields. Bioelectromagnetics 2:1.

19 Lee, J. M. Jr, F. Stormshak, J. M. Thompson, D. L. Hess, and D. L. Foster. 1995. Melatonin and puberty in female lambs exposed to EMF: A replicate study. Bioelectromagnetics 16:119.

20 Lee, J. M. Jr, F. Stormshak, J. M. Thompson, P. Thinesen, L. J. Painter, E. G. Olencheck, D. L. Hess, R. Forbes, and D. L. Foster. 1993. Melatonin secretion and puberty in female lambs exposed to environmental electric and magnetic fields. Biol Reprod. 49:857.

21 Lerchl, A., K. O. Nonaka, K. A. Stokkan, and R. J. Reiter. 1990. Marked rapid

alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. Biochem. Biophys. Res. Com. 169:102.

22 Lerchl, A., R. J. Reiter, K. A. Howes, K. O. Nonaka, and K. A. Stokkan. 1991. Evidence that extremely frequency calcium ion cyclotron resonance depresses pineal melatonin synthesis **in vitro.** Neurosci. Lett. 124:213.

23 Martin, F. B., A. Bender, G. Steuernagel, R. A. Robinson, R. Revsbech, D. K. Sorensen, N. Williamson, and A. Williams. 1986. Epidemiological study of Holstein dairy cow performance and reproduction near a high-voltage direct-current powerline. J. Toxicol. Environ. Health. 19:303.

24 NRC. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Nat. Acad. Sci., Washington DC.

25 Peters, R. R., L. T. Chapin, K. B. Leining, and H. A. Tucker. 1978. Supplemental lighting stimulates growth and lactation in cattle. Science 199:911.

26 Petitclerc, D., L. T. Chapin, R. S. Emery, and H. A. Tucker. 1983. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 57:892.

27 Raleigh, R. J. 1988. Rep. DOE/BP-21216-3. Joint HVDC agricultural study. Oregon State University, Boneville Power Administration, Portland, Oregon. DOE, Portland OR.

28 Ryg, M., and E. Jacobsen. 1982. Effects of thyroid hormones and prolactin on food intake and weight changes in young male reindeer (Rangifer tarandus). Can. J. Zool. 60:1562.

29 Sasser, L. B., J. E. Morris, D. L. Miller, and L. E. Anderson. 1992. Effect of acute exposure to magnetic fields on pineal function in the rat. Page 28 in The 1st World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San Francisco Press, San Francisco, CA.

30 Sharpe, L. G., and R. D. Myers. 1969. Feeding and drinking following stimulation of the diencephalon of the monkey with amines and other substances. Exp. Brain Res. 8:295.

31 Silman, R. 1991. Melatonin and the human gonadotropin-releasing hormone pulse generator. J. Endocrinol. 128:7.

32 Singer, G., I. Sanghvi, and S. Gershon. 1971. Exploration of certain behaviourial patterns induced by psychoactive agents in the rat. Commun. Behav. Biol. 6:307.

33 Suttie, J. M., and R.N.B. Kay. 1985. Influence of plane of winter nutrition on plasma concentrations of prolactin and testosterone and their association with voluntary feed intake in red deer stags (Cervus elaphus). Anim. Reprod. Sci. 8:247.

34 Tenforde, T. S. 1989. Electroreception and magnetoreception in simple and complex organisms. Bioelectromagnetics 10:215.

35 Thompson, J. M., F. Stormshak, L. J. Painter, and P. K. Thinesen, J. Corvallis, M. Lee, D. L. Hess. 1991. Endocrine responses of ewe lambs exposed to a 500 kV a-c transmission line. J. Anim. Sci. 69(Suppl 1):229.(Abstr.)

36 Tucker, H. A. 1992. Prolonged suppression of serum concentrations of melatonin in prepubertal heifers. J. Pineal Res. 12:181.

37 Ware, B. J. 1974. Effect of 765-kV Transmission Lines on Animal Grazing Habits. Rep. American Electric Power Service Corp. Columbus, OH.

38 Williams, J. H., and J. E. Beiler. 1979. An investigation of dairy farm operations in association with 765-kV transmission in Ohio. Rep. Ohio Power Siting Commission and Cleveland Electric Illuminating Co. Cleveland, OH.

39 Wilson, B. R., and L. E. Anderson. 1989. ELF Electromagnetic field effect on the pineal gland. Page 157 in Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. B. W., Wilson, R. G. Stevens, and L. E. Anderson ed. Battelle Press, Columbus OH.

40 Wilson, B. W., E. K. Chess, and L. E. Anderson. 1986. 60Hz Electric-field effects on pineal melatonin rhythms: time course for onset and recovery. Bioelectromagnetics 7:239.

41 Wilson, B. W., C. W. Wright, J. E. Morris, R. L. Buschbom, D. P. Brown, D. L. Miller, F. R. Sommers, and L. E. Anderson. 1990. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J. Pineal Res. 9:259.

42 Yaga. K., R. J. Reiter, L. C. Manchesten, H. Nieves, J. H. Sun, and L. D. Chen. 1993. Pineal sensitivity of pulsed magnetic field changes during the photoperiod. Brain Res. Bull. 30:153.

Table 1. Feed intake, milk yield and milk composition of samples obtained from lactating pregnant dairy cows exposed (On) or not exposed (Off) to EMF. Results are leastsquares means (± SE) and the probability of significance ($P > t$).

	Off	On	$P > t$
Feed intake kg of total mixed diet per cow	31.6 ± 0.3	33.5 ± 0.3	0.001
DMI, kg/d per cow	18.0 ± 0.2	19.0 ± 0.2	0.002
Milk yield, kg/d per cow	19.1 ± 0.2	19.4 ± 0.2	0.156
Milk yield, kg/d per cow 4% FCM	18.7 ± 0.3	20.4 ± 0.3	0.001
Fat, %	4.06 ± 0.08	4.43 ± 0.08	0.008
Protein, %	3.35 ± 0.02	3.34 ± 0.02	0.938
Lactose, %	4.45 ± 0.02	4.49 ± 0.02	0.240
SNF, %	8.32 ± 0.02	8.37 ± 0.02	0.142
SCC, x10^3/ml	264 ± 66	213 ± 66	0.605

Table 2. Cortisol and progesterone concentrations in blood samples obtained from lactating pregnant dairy cows exposed (On) or not exposed (Off) to EMF. Results are leastsquares means (± SE) and the probability of significance ($P > t$).

	Off	On	$P > t$
Cortisol μg/dl	1.11 ± 0.1	1.11 ± 0.1	0.995
Progesterone ng/ml	5.6 ± 0.1	6.2 ± 0.1	0.002

Table 3. pH, partial pressures of gases and bicarbonate concentrations in venous blood samples obtained from lactating pregnant dairy cows exposed (On) and not exposed (Off) to EMF. Results are leastsquares means (± SE) and the probability of significance ($P > t$).

	Off	On	$P > t$
pH	7.41 ± 0.008	7.40 ± 0.008	0.461
pCO_2, mm Hg	41.5 ± 0.4	41.4 ± 0.4	0.932
pO_2, mm Hg	55.6 ± 2.5	52.1 ± 2.5	0.345
Bicarbonate, mq/L	26.2 ± 0.1	26.4 ± 0.1	0.267

CONNECTING STATEMENT

Electric and magnetic fields create electromagnetic waves and it has been suggested that they act in the same manner as light suppressing or decreasing the pineal gland secretion of melatonin. In chapter III it was demonstrated that 4% fat corrected milk, feed consumption and plasma progesterone increased when cows where exposed to electric and magnetic fields.

Since melatonin is an anti-gonadotrophic hormone, the effect of electric and magnetic fields on nocturnal melatonin plasma concentrations in pregnant cows was studied in chapter IV.

IV. EFFECT OF 60 Hz ELECTRIC AND MAGNETIC FIELDS ON NOCTURNAL MELATONIN CONCENTRATIONS IN DAIRY COWS

Submitted to the Journal of Dairy Science

Running head:ELECTRIC AND MAGNETIC FIELDS AND MELATONIN IN COWS

Effects of Electric and Magnetic Fields on Nocturnal Melatonin Concentrations in Dairy Cows

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ABSTRACT

Sixteen multiparous, pregnant, lactating Holstein cows (weighing 600 $\pm$ 50 kg, in 184.8$\pm$52 d of lactation, and at 101.9$\pm$43 d of gestation) were confined to wooden metabolism cages and exposed to a vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 μT (micro-Tesla). The trial was conducted as a switchback statistical design. Cows were divided into two replicates of eight cows each. One replicate was exposed for three periods of 28 d each. During the first period, the electric and magnetic fields were off; during the second period, they were on; and during the final period, they were off. The second replicate was exposed for three periods also, but the activity of the fields was reversed (first period, on; second period, off; last period, on). On d 25 of each exposure period, blood samples were obtained every half hour for a period of 14 h starting at 1700 h to determine melatonin concentration. Nocturnal melatonin did not show any variation that could be attributable to exposure to electric and magnetic fields.

(Key words: melatonin, electric field, magnetic field, cows)

Abbreviation key: EMF = electric and magnetic fields, **MLT** = melatonin.

INTRODUCTION

Because electric and magnetic fields (EMF) are capable of inducing electric currents and fields in the tissues of exposed subjects (13, 14, 31), the hypothesis that EMF may represent a hazard to animal health has motivated research efforts to better understand the biological effects of EMF. Studies reported until now in the bovine species were based on farm surveys (4, 7, 33), were retrospective analyses (1, 2, 20, 34), or were prospective studies (3). Cows exposed to EMF had an increase in DMI, milk fat content and plasma progesterone (6).

Rats exposed to 60 Hz, 39 kV/m EMF for 3 wk had decreased pineal N-acetyl transferase activity and decreased melatonin (MLT) concentrations (36, 37). Rats exposed to an inverted, horizontal geomagnetic field of 20 to 30 μT (micro-Tesla) for 30 to 60 min during a dark period showed depressed N-acetyl transferase activity and decreased MLT concentrations in their pineal gland 45 min after the initiation of exposure (17, 18, 39). Exposure to magnetic fields produces the isomerization of 11-cis to all-trans retinal in retina of rats. This isomerization represents the initial step in the photostimulation of the visual system; therefore, as with light, magnetic fields may stimulate the retina and inhibit the N-acetyl tranferase activity in the pineal gland(25). Conversely, when ewe lambs were exposed to the EMF produced by a 500-kV transmission line (4 to 7 kV/m, 0.1 to 5 μT), they did not show any significant variation in the mean nighttime MLT concentration at 18 or 32 wk of age (15, 16,

32). Rats exposed to alternating EMF with intensities similar to domestic EMF exposure arising from neighboring power lines showed significantly reduced nocturnal MLT concentrations (37).

All the prospective studies in the bovine species have been conducted using existing commercial high voltage alternating current lines (3). The amount of current in these lines varies considerably across time; therefore, the uniformity of the EMF is very unpredictable. This motivated the construction of a bovine exposure chamber to generate uniform and controlled EMF (23). Because MLT has been proposed as a mediator of the EMF biological effects in severals species (36, 38), this study was designed to evaluate whether EMF exposure of cows had an effect on the nocturnal concentration of MLT in blood when cows are exposed to uniform and controlled EMF.

MATERIALS AND METHODS

Sixteen multiparous pregnant lactating Holstein cows (weighing 600 $\pm$ 50 kg, in 184.8$\pm$52 d of lactation, and at 101.9$\pm$43 d of gestation) were confined to wooden metabolism crates in an EMF exposure chamber during the experiment. The chamber was exposed to 12 h of light followed by 12 h of darkness; mean temperature and humidity of 16.25 $\pm$ 1.86° C and 44.3 $\pm$ 7 %, respectively. The chamber was 15 m long, 10 m wide, and 3 m high; contained eight wooden metabolism crates, each

holding one cow, and was designed to allow simultaneous exposure of the eight cows during long, uninterrupted periods. Wooden, rather than metal, metabolism crates are used to minimize disturbance of the electric field (25). The chamber was ventilated and heated artificially. Water consumption and feed intake was **ad libitum**.

Cows were fed twice daily total mixed diets formulated according to NRC requirements (22). Total feed consumption was estimated by weighing the feed offered and orts daily. The cows were milked twice daily, for which all cows were removed from the EMF chamber for a period of 30 min.

Exposure to EMF was in a switchback design (8). The cows were divided into two replicates of eight cows each. One replicate was exposed for three periods of 28 d each. During the first period, the electric and magnetic fields were off; during the second period, they were on; and during the final period, they were off. The second replicate was exposed for three periods also, but the activity of the fields was reversed (first period, on; second period, off; last period, on). After 25 days of exposure within each period, blood samples were collected into heparinized vacutainer tubes (Becton Dickinson, Rutheford, NJ) for a period of 14 h every half hour commencing at 1700 h and finishing at 0630 h on d 27 of exposure.

The magnetic field in the chamber was generated by 14 rectangular coils (10 m long and 4 m high). A current of 1.03 A (ampere) generated a uniform horizontal

magnetic field of 30 μT. The electric field was generated by two plates (9 m long and 6.5 m wide) that were suspended 0.4 m from the ceiling by synthetic isolators. The total electric capacity of the plate was 4.3 nanofarad. These plates produced a vertical electric field of 10 kV/m, measurments of the electric and magnetic fields were recorded with a data acquisition system every 5 min (25). The intensity of the EMF chosen for this experiment resembles a situation in which the cows would stand continuously under an 735-kV alternating current power line when the line has a maximun load of current. In reality, these conditions are found only for a few days during the winter in Québec, Canada.

Catheters were inserted in the jugular vein of cows 2 d before blood sample collection. Briefly, the cows were restrained in a chute and tranquilized with 4 mg of xylazine (Rompun^r Bayvet, Etobicoke, ON, Canada) intravenously and, using a 14-gauge needle, an angiocatheter (Becton Dickinson, Rutherford, NJ) was inserted into the left jugular vein and filled with heparinized saline (1 IU/ml). The catheter was fixed to the skin with nonresorbable sutures. A 5-m long catheter was fitted to the angiocatheter. This catheter was fed into the laboratory adjacent to the EMF chamber, which allowed blood sample collections from the laboratory without altering the cows or the EMF. After each 14-h collection period, the catheters were removed. The light was turned off and on at 1800 and at 0600 h, respectively. The samples taken at 1800 and at 0600 h the next day were obtained while the lights were off and on, respectively.

Blood samples were centrifuged at 1000 x g for 15 min and the plasma was stored at -20°C pending analyses. Plasma MLT concentrations were estimated using direct radioimmunoassay without extracting the plasma (10). The standard curve ranged from 0 to 500 pg/ml, maximum binding was 54 %, approximate sensitivity was 3 pg/ml; sample size was 0.5 ml. Intra and inter assay coeficients of variation was 4.1 and 18.5 %, respectively.

Statistical Analysis

The data were analyzed using analysis of variance of a switchback design and the following regression model (8):

MLT concentrations:

$$Y_{ijklmn} = \mu + S_i + C(S)_{ij} + P_k + T_l + MC(S)_{ij} + C*T(S)_{ijl} + N_m + (T*N)_{lm} + E_{ijklmn}$$

Y_{ijklmn} = dependent variable,

μ = overall population mean,

S_i = effect of sequence group i (i = 1 or 2),

$C(S)_{ij}$ = effect of cow j in sequence group i (j = 1, . . . ,16),

P_k = effect of period k (k = 1, 2, or 3),

T_l = effect of treatment l [l = 1 (EMF off) or 2 (EMF on)],

$MC(S)_{ij}$ = linear time trend of cow j in sequence group i,

$C*T(S)_{ij}$ = effect of the interaction between the l treatment and j cow in sequence group i,

N_m = effect of time of sampling m (m = 1, . . .,28),

$(T*N)_{lm}$ = effect of the interaction between treatment l and time of sampling m, and

E_{ijklm} = random error, normally and independently distributed with zero mean and common variance.

Comparisons between treatments were carried out using contrasts between the differences of the means using a *t* test. Significant differences between means were declared at $P < 0.05$.

RESULTS

Results for MLT nocturnal peak are expressed as mean plasma MLT concentrations (pg/ml of plasma) and as areas under the curve of the nocturnal secretion. Both results are presented as least squares means ($\pm$ SE). Mean MLT concentrations ($P = 0.76$), and MLT areas under the curve ($P = 0.77$) did not differ between cows that were exposed or not exposed to EMF (Table 1). The results obtained by replicate indicated, in replicate 2, a tendency of MLT to decrease when the EMF were on (Table 2). Plasma MLT concentrations throughout the 14-h

sampling across the three periods, separated by replicate, are presented in Figures 2 and 3.

DISCUSSION

Overall, EMF did not have any significant effect on the nocturnal secretion of MLT. Nevertheless, some ambiguity was detected in the MLT results. When the MLT values are separated by replicate, MLT concentrations of cows in replicate 2 had a tendency to decrease when cows were exposed to EMF (EMF on = 90.3 pg/ml, EMF off = 105.8 pg/ml, and EMF on = 65.3 pg/ml; $P = 0.06$). This tendency was not observed for cows in replicate 1 (EMF off = 23.6 pg/ml, EMF on = 76.22 pg/ml, and EMF off = 51.72 pg/ml; $P = 0.30$) (Table 2). Melatonin, in some species, has antigonadotropic properties (5, 30). The decrease in plasma MLT concentrations observed during the EMF exposure of the second replicate was coincidental with the increased progesterone concentration reported previously in cows exposed to EMF (6). This increase in progesterone was also numerically greater in replicate 2 in chapter III (See appendix 2, Table 19).

Reports exist that show that EMF cause a decrease in MLT concentrations in the blood and pineal gland (11, 12, 27, 35, 37, 39 40) and other reports (15, 16, 28, 29, 32,) have shown no effect. Apparently, EMF can interfere with the activity of the rate-limiting enzyme, serotonin N-acetyltransferase (Figure 3), which catalyzes the conversion of serotonin to MLT (17, 18, 21, 26, 39). This interference could result in

an accumulation of serotonin in the pineal gland; however, serotonin was not measured in this experiment. There are no studies related to the effect of EMF on MLT in dairy cows.

If the tendency for a decrease in nocturnal serum MLT is an effect of exposure to EMF on the synthesis of MLT, this could lead to an increase in the plasma concentration of prolactin, increasing feed consumption (9) and possibly milk production (6, 24). In the rat, the decreases in MLT and serotonin N-acetyltransferase activity caused by EMF were observed in mid and late dark phases but not in the early dark or light phases. Therefore, the possibility of having an association between the circadian or annual circadian rhythms or both, and the temperatures should be considered when evaluating the onset of EMF effects (39, 40) in cows.

CONCLUSIONS

In this experiment, EMF of 10 kV/m and 30 μT did not cause any change in the nocturnal concentration of MLT in plasma of dairy cows. However, since the fact that the second replicate clearly showed a tendency towards lower concentrations of nocturnal MLT during the exposure period, further research should be considered.

ACKNOWLEDGMENTS

The authors thank J. Carreño, B. Allard, R. I. Cue, B. Downey (McGill University.); M. Misakian (National Institute of Standadrd and Technology), L-P. Rivest (Laval University.), J. Lee Jr (Department of Energy), P. Lamothe (University of Montreal), S. Maruvada, F. Renaud, D. Goulet and C. Cardinal (Hydro Québec), for their contributions. This research was sponsored by the Environmental Branch of Hydro-Québec.

REFERENCES

1 Algers, B., I. Ekesbo, and K. Hennichs. 1981. The effects of ultra high-voltage transmission lines on the fertility of dairy cows. A preliminary study. Rep. No. 5 Swed. Univ. Agric. Sci., Dep. Anim. Hygiene with Farrier school, Skara, Sweden.

2 Algers, B., and K. Hennichs. 1985. The effects of exposure to 400 kV transmission lines on the fertility of cows. A retrospective cohort study. Prev. Vet. Med. 3:352.

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

4 Amstutz, H. E., and D. B. Miller. 1980. A study of farm animals near 765 kV transmission lines. Bovine Pract. 15:51.

5 Axelrod, J., F. Fraschini, and G. P. Velo, ed. 1982. The Pineal Gland and its Neuro-endocrine Role. Plenum Press, New York, NY.

6 Burchard, J. F., D. H. Nguyen, L. Richard, and E. Block. 1996. Biological effects of 60 Hz electric and magnetic fields (EMF) on productivity of dairy

cattle. J. Dairy Sci. 79:1549.

7 Busby, K., D. Driscoll, and W. E. Washbon. 1974. A field survey of farmer experience with 765 kV transmission lines. Agric. Res. Commiss., State of New York, Albany.

8 Cochran, W. G., and G. M. Cox. 1957. Switchback or reversal designs. Page 141 in Experimental designs. John Williams and Sons, New York, NY.

9 Dominique, B.M.F., P. R. Wilson, D. W. Dellow, and T. N. Barry. 1992. Effects of subcutaneous melatonin implants during long daylength on voluntary feed intake, rumen capacity and heart rate of red deer (Cervus elaphus) fed on a forage diet. Br. J. Nutr. 68:77.

10 Fraser, S., P. Cowen, M. Franklin, C. Franey, and J. Arendt. 1983. Direct radioimmunoassay for melatonin in plasma. Clin. Chem. 29:396.

11 Haggren, W., S. M. Yellon, J. L. Phillips, and W. R. Adey. 1992. Effect of magnetic field exposure in adult djungarian hamsters on melatonin rhythms and molecular markers. Page 105 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL. San Francisco Press, San Francisco, CA.

12 Kato, M., K. Honma, T. Shigemitsu, and Y. Shiga. 1994. Circularly polarized 50-Hz magnetic field exposure reduces pineal gland and blood MLT concentrations of Long-Evans rats. Neurosci. Lett. 166:59.

13 Kaune, W. T., and W. C. Forsythe. 1988. Current densities induced in swine and rat models power-frequency electric fields. Bioelectromagnetics 9:1.

14 Kaune, W. T., and M. F. Gillis. 1981. General properties of the interaction between animals and ELF electric fields. Bioelectromagnetics 2:1.

15 Lee, J. M., Jr., F. Stormshak, J. M. Thompson, D. L. Hess, and D. L. Foster. 1995. Melatonin and puberty in female lambs exposed to EMF: a replicate study. Bioelectromagnetics 16:119.

16 Lee, J. M. Jr, F. Stormshak, J. M. Thompson, P. Thinesen, L. J. Painter, E. G. Olencheck, D. L. Hess, R. Forbes, and D. L. Foster. 1993. Melatonin secretion and puberty in female lambs exposed to environmental electric and magnetic fields. Biol. Reprod. 49:857.

17 Lerchl, A., K. O. Nonaka, K. A. Stokkan, and R. J. Reiter. 1990. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. Biochem. Biophys. Res. Com.

169:102.

18 Lerchl, A., R. J. Reiter, K. A. Howes, K. O. Nonaka, and K. A. Stokkan. 1991. Evidence that extremely low frequency calcium ion cyclotron resonance depresses pineal melatonin synthesis in vitro. Neurosci. Lett. 124:213.

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

20 Martin, F. B., A. Bender, G. Steuernagel, R. A. Robinson, R. Revsbech, D. K. Sorensen, N. Williamson, and A. Williams. 1986. Epidemiological study of Holstein dairy cow performance and reproduction near a high-voltage direct-current powerline. J. Toxicol. Environ. Health 19:303.

21 Martinez Soriano, F., M. Gimenez Gonzalez, E. Armanazas, and T. Ruiz. 1992. A pineal "synaptic ribbons" and serum melatonin levels in the rat following the pulse action of 52-Gs (50-Hz) magnetic fields: an evolutive analysis over 21 days. Acta Anatomica 143:289.

22 National Research Council. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.

23 Nguyen, D. H., L. Richard, J. F. Burchard, E. Block. 1995. An electric and magnetic field exposure chamber for studies of biological effects on dairy cows. Page 15 in The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. Palm Springs, CA. W/L Associates, Ltd. Frederick, Maryland.

24 Peters, R. R., L. T. Chapin, K. B. Leining, and H. A. Tucker. 1978. Supplemental lighting stimulates growth and lactation in cattle. Science (Washington, DC) 199:911.

25 Reiter, R. J., K. Yaga, L. C. Manchester, J. S. Sun, L. D. Chen, M. Abdolsalami. 1992. Parametric and mechanistic studies on the perturbation of the circadian melatonin rhythm by magnetic field exposure. Page 5 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL. San Francisco Press, San Francisco, CA.

26 Richardson, B. A., K. Yaga, R. J. Reiter, and D. J. Morton. 1992. Pulsed static magnetic field effects on in vitro pineal indoleamine metabolism. Biochim. Biophys. Acta 1137:59.

27 Rogers, W. R., R. J. Reiter, and J. L. Orr. 1992. 60-Hz electric and magnetic fields and primate melatonin. Page 29 in The First World Congress for

Electricity and Magnetism in Biology and Medicine. Orlando, FL. San Francisco Press, San Francisco, CA.

28 Sasser, L. B., J. E. Morris, D. L. Miller, and L. E. Anderson 1992. Effect of acute exposure to magnetic fields on pineal function in the rat. Page 28 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL. San Francisco Press, San Francisco, CA.

29 Schiffman, J. S., H. M. Lasch, D. M. Rollag, A. E. Flanders, G. C. Brainard, and D. L. Burk. 1992. Effect of magnetic resonance imaging (MRI) on plasma melatonin in normal humans. Page 74 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San Francisco Press, San Francisco, CA.

30 Silman, R. 1991. Melatonin and the human gonadotropin-releasing hormone pulse generator. J. Endocrinol. 128:7.

31 Tenforde, T. S. 1989. Electroreception and magnetoreception in simple and complex organisms. Bioelectromagnetics 10:215.

32 Thompson, J. M., F. Stormshak, L. J. Painter, P. K. Thinesen, J. Corvallis, M. Lee, and D. L. Hess 1991. Endocrine responses of ewe lambs exposed to

a 500 kV a-c transmission line. J. Anim. Sci. 69(Suppl 1):229.(Abstr)

33 Ware, B. J. 1974. Effect of 765-kV transmission lines on animal grazing habits. American Electric Power Service Corp., New York, NY.

34 Williams, J. H., and J. E. Beiler. 1979. An investigation of dairy farm operations in association with 765-kV transmission in Ohio. Rep. Ohio Power Siting Commission and Cleveland Electric Illuminating Co., Cleveland, OH.

35 Wilson, B. W., C. W. Wright, J. E. Morris, R. L. Buschbom, D. P. Brown, D. L. Miller, F. R. Sommers, and L. E. Anderson. 1990. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J. Pineal Res. 9:259.

36 Wilson, B. R., and L. E. Anderson. 1989. ELF Electromagnetic field effect on the pineal gland. Page 157 in Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. Wilson, B. W., R. G. Stevens, and L. E. Anderson, ed. Battelle Press, Columbus, OH.

37 Wilson, B. W., E. K. Chess, and L. E. Anderson. 1986. 60Hz Electric-field effects on pineal melatonin rhythms: time course for onset and recovery.

Bioelectromagnetics 7:239.

38 Wood, A. W. 1993. Possible health effects of 50/60Hz electric and magnetic fields: review of proposed mechanisms. Australian Physical & Engineering Sciences in Medicine. 16(1):1-21

39 Yaga K., R. J. Reiter, L. C. Manchesten, H. Nieves, J. H. Sun, and L. D. Chen. 1993. Pineal sensitivity of pulsed magnetic field changes during the photoperiod. Brain Res. Bull. 30:153.

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

TABLE 1. Mean nocturnal melatonin concentrations in blood plasma samples obtained from lactating, pregnant dairy cows exposed (on) or not exposed (off) to electric and magnetic fields. Results are for both replicates and are least squares means (±SE) and the probability of significance ($P > t$).

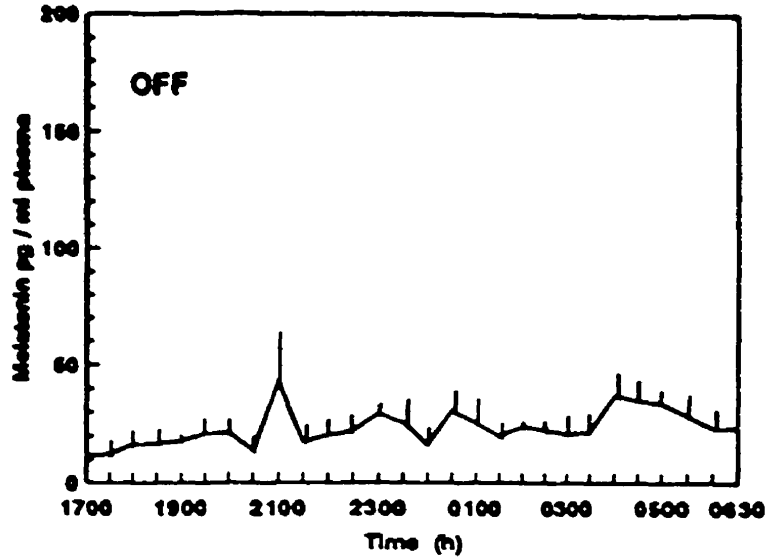
Dependant variable	OFF	ON	$P > t$
Melatonin, pg / ml of plasma	70.82 ± 14	77.02 ± 14	0.760
Area under the melatonin curve, pg / ml of plasma x sampling time	1799 ± 352	1962 ± 352	0.754

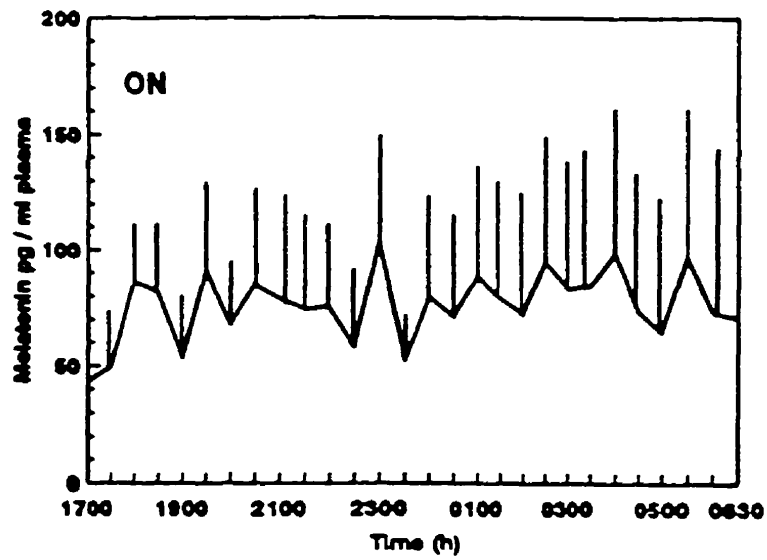
TABLE 2. Mean nocturnal melatonin concentrations (pg / ml plasma) in blood samples obtained from lactating, pregnant dairy cows exposed (on) or not exposed (off) to electric and magnetic fields. Results are divided by replicate and are least squares means (± SE) and the probability of significance ($P > t$).

Hormone	Replicate*	Period 1	Period 2	Period 3	$P > t$
Melatonin	1	23.60 ± 1.6	76.22 ± 5.4	51.72 ± 3.0	0.309
	2	90.30 ± 3.8	105.80 ± 5.6	65.30 ± 3.8	0.065

* For replicate 1, during the first period, electric and magnetic fields (EMF) were off; during the second period, EMF were on; and during the last period, they were off. For replicate 2, during the first period EMF were on; during the second period they were off; and during the final period they were on.

Figure 1. Nocturnal melatonin concentrations in blood plasma samples obtained from the first replicate of lactating pregnant dairy cows not exposed (Off) or exposed (On) to electric and magnetic fields. One blood sample was collected every half hour during 14 h on day 25 of each exposure period and then averaged according to treatment.


OFF
Melatonin pg / ml plasma
0
50
100
150
200
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)


ON
Melatonin pg / ml plasma
0
50
100
150
200
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)

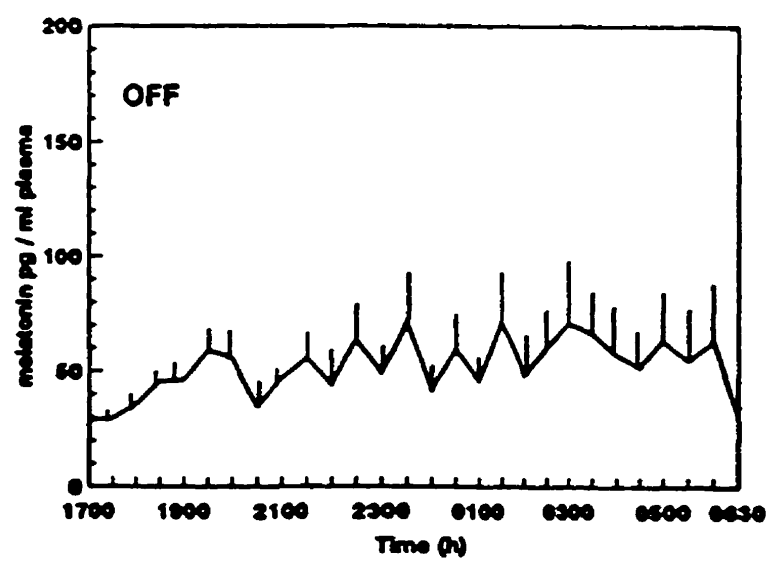
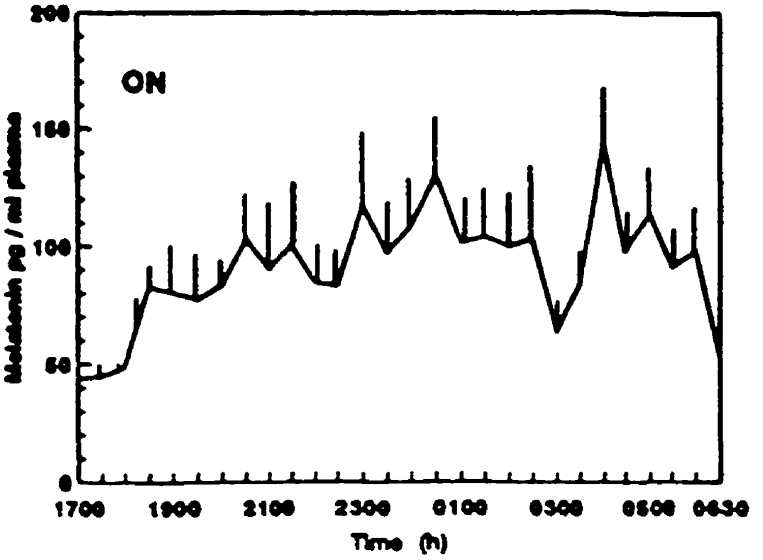
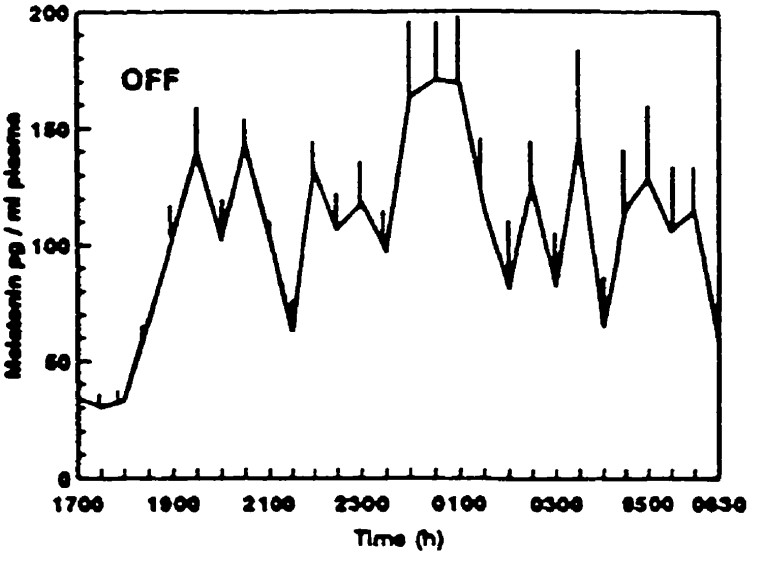

OFF
melatonin pg / ml plasma
0
50
100
150
200
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)

Figure 2. Nocturnal melatonin concentrations in blood plasma samples obtained from the second replicate of lactating pregnant dairy cows not exposed (Off) or exposed (On) to electric and magnetic fields. One blood sample was collected every half hour during 14 h on day 25 of each exposure period and then averaged according to treatment.


ON
Melatonin pg / ml plasma
200
150
100
50
0
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)


OFF
Melatonin pg / ml plasma
200
150
100
50
0
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)

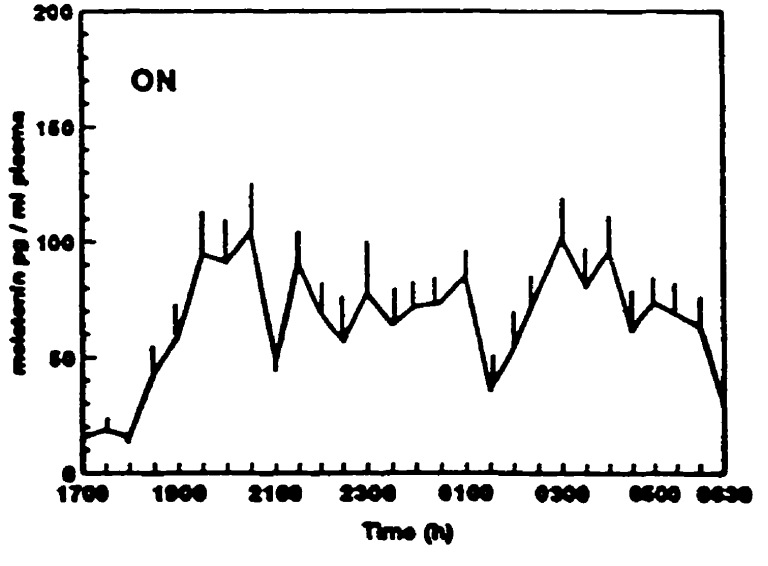
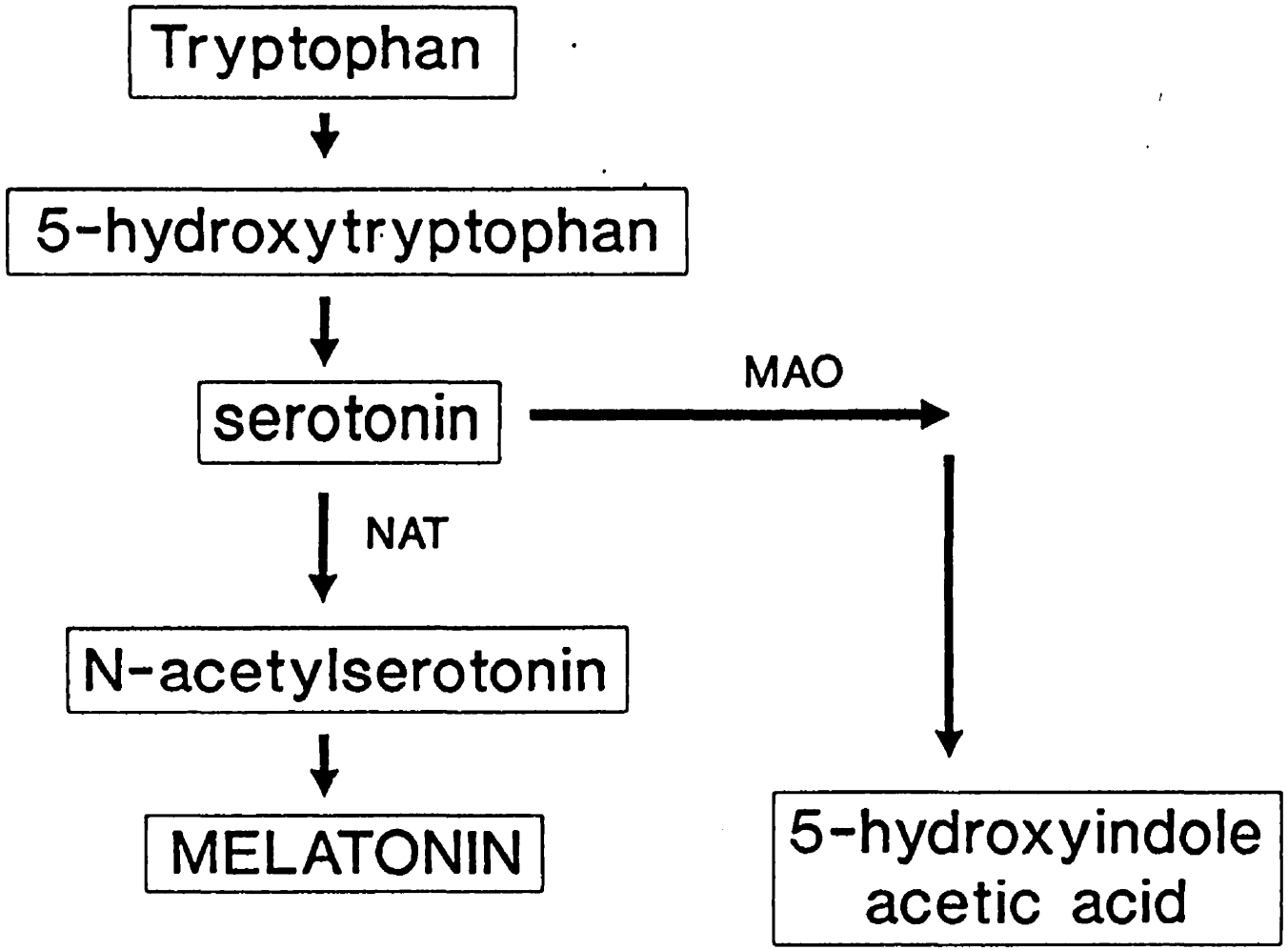

ON
melatonin pg / ml plasma
200
150
100
50
0
1700
1900
2100
2300
0100
0300
0500
0630
Time (h)

Figure 3. Biochemical pathway for the synthesis of melatonin. NAT = Serotonin N-acetyl transferase; MAO = monoamine-oxidase.


Tryptophan
5-hydroxytryptophan
serotonin
MAO
NAT
N-acetylserotonin
MELATONIN
5-hydroxyindole
acetic acid

CONNECTING STATEMENT

The literature review provides evidence that electric and magnetic field exposure causes variations in the concentration of minerals in nervous tissues. Also, it is suggested that the mechanism of action of electric and magnetic fields would involve alterations in the cellular flux of calcium. In Chapter V, the effect of electric and magnetic field exposure upon the concentrations of several minerals is tested in vivo.

V. MACRO- AND TRACE ELEMENT CONCENTRATIONS IN BLOOD PLASMA AND CEREBROSPINAL FLUID OF DAIRY COWS EXPOSED TO ELECTRIC AND MAGNETIC FIELDS.

Submitted to the Journal of Dairy Science

Running head: ELECTRIC AND MAGNETIC FIELDS AND MINERALS IN COWS

Macro- and Trace Element Concentrations in Blood Plasma and Cerebrospinal Fluid of Dairy Cows exposed to Electric and Magnetic Fields.

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ABSTRACT

Eight multiparous, nonlactating pregnant Holstein cows (at 198 ± 35 d of gestation and weighing 608 ± 24 kg) and seven nonlactating nonpregnant ovariectomized heifers (weighing 369.5 ± 29.4 kg) were confined to wooden metabolism crates in an electric and magnetic field chamber. The chamber was exposed to 12 h of light followed by 12 h of darkness. Subarachnoidal catheters were inserted 5 d before the activation of the electric and magnetic fields. For 30 d cows and heifers were continuously exposed in separate trials to electric and magenetic fields [60 Hz, 10 kV/m and 30 μT (micro-Tesla)] except for time spent feeding and cleaning. Mean exposure times for the groups were 21.44 ± 1.4 and 22.5 ± 2.1 h/d.

Blood plasma and cerebrospinal fluid samples were collected daily for three days before the exposure period, the last 3 d of the exposure period, and for 3 d starting 5 d after the exposure period. Concentrations of Ca, Mg, Cu, Zn, Fe, Mn, Na, P and K in blood plasma and cerebrospinal fluid were determined. Overall, trace minerals were much more affected by the exposure to electric and magnetic fields than macrominerals.

(**Key words**: minerals, pregnant cows, electric field, magnetic field)

Abreviation key: EMF = electric and magnetic fields, **CSF** = cerebrospinal fluid.

INTRODUCTION

During the last decade, there has been a considerable increase in man-made electric and magnetic fields (EMF) in industrialized countries due to a higher demand of electricity for different applications. This grater demand implies an increase in the current load of existing high tension lines. This increase has prompted the evaluation of effects of EMF on biological systems. Because the central nervous system (CNS) has a considerable amount of electrical activity, it seems fundamental to study the effects of EMF upon the CNS.

Kavaliers and Ossenkopp (1987) have suggested that EMF affect the functioning of Ca. This disruption could result in an alteration of the effectiveness of the opiate system to cope with pain (Testkey **et al.**, 1988). In vitro evidence exists that EMF have an effect on the mobilization of Ca from brain cells using freshly isolated avian and feline brains. However, some researchers (Bawin and Adey, 1976) have found that EMF decrease the efflux of Ca from nervous tissues; others (Blackman, 1989) have demonstrated an increase in the efflux of Ca from brain preparations . Blackman, (1989) and Goodman et al (1989) have suggested that these effects are caused by a direct interaction of the EMF and the cell membrane. The study of metabolism and activity of the CNS **in vivo** has to rely heavily on the analyses of the cerebrospinal fluid (CSF) composition (Vogt, 1975). The CSF is a clear watery fluid produced by the CNS that is in intimate contact with the brain and the spinal cord.

Although concentrations of chemical substances in blood plasma e.g. electrolytes, hormones, nutrients, etc, fluctuate over a wide range, concentrations in the CSF remain relatively stable (Feldman, 1989). Consequently, the objective of the following study was to assess the effect of EMF on macro- and trace mineral concentrations in blood plasma and CSF.

MATERIALS AND METHODS

Animals

In this study two groups of cows were used to test the effects of EMF on the CNS in two separate trials. Eight multiparous nonlactating Holstein cows (group A) at 198 $\pm$ 35 d of gestation and weighing 608 $\pm$ 24 kg and seven nonlactating nonpregnant ovariectomized Holstein heifers (group B), weighing 369.5 $\pm$ 29.4 kg, were confined to wooden metabolism crates (1.79 m wide, 1.62 m high and 2.43 m long) during the experiment. The EMF chamber was exposed to 12 h of light followed by 12 h of darkness. The mean room temperature and humidity for groups A and B were 15.6 $\pm$ 1.4°C and 33.6 $\pm$ 6% and 26.3 $\pm$ 2.5°C and 59.0 $\pm$ 6.4%, respectively. Two additional nonlactating ovariectomized heifers (group C) housed in the main dairy barn, adjacent to the EMF chamber, were used as controls for Group B only to evaluate the effect of the insertion of subarachnoidal catheters on the concentration of minerals in the CSF. Water and feed were available for ad libitum intake. The cows were offered a total mixed diet twice daily according to NRC requirements (NRC, 1988).

Exposure Chamber

The EMF exposure chamber was designed to resemble the EMF prevailing directly underneath an alternating current, high tension line of 735 kV. The chamber was 15 m long, 10 m wide, and 3 m high; it contained eight wooden metabolism crates, each capable of housing one cow, and was designed to allow simultaneous exposure of up to eight cows during long, uninterrupted periods. Wooden, rather than metal, metabolism crates were used to minimize disturbance of the electric field. The chamber was ventilated and heated artificially.

The magnetic fields were generated by 14 rectangular coils (10 m long and 4 m high). A current of 1.03 A (ampere) generates a uniform horizontal magnetic field of 30 μT (micro-Tesla). The electric field was generated by two plates (9 m long and 6.5 m wide) that were suspended 0.4 m from the ceiling by synthetic isolators. The total electric capacity of the plate was 4.3 nanofarad. These plates produced a vertical electric field of 10 kV/m (Figure 1)(Nguyen **et al.**, 1995).

Insertion of the Subarachnoidal Catheters

The subarachnoidal catheters were inserted 5 d before activation of the EMF. Briefly, the cows were restrained in a chute and tranquilized with 4 mg of xylazine (Rompun^r, Bayvet; Etobicoke, ON, Canada) intravenously. The surgical area was shaved, cleaned with alcohol, and disinfected with 2% iodine. Anesthesia of local skin and subcutaneous tissues was achieved by injecting 20 ml of 2% lidocaine (Astra

Pharma, Mississauga, ON, Canada). A 17 gauge x 9-cm Touhy needle (Becton & Dickinson, Rutheford, NJ) was inserted in the midline between the sixth lumbar and first sacral vertebrae, approximately 3 cm behind the lumbar vertebra with a 50° caudocranial angle. Once CSF was obtained, 35 cm of a 19 gauge x 91 cm-epidural catheter (Becton & Dickinson, Sandy, UT) were fed into the Touhy needle and then inserted into the subarachnoid space of the spinal cord. Once the catheter was in place and verified to be patent, the Touhy needle was withdrawn, and the catheter was fixed to the skin with a nonresorbable stitch. The plastic guide of the catheter was reinserted and left in place to provide further protection against bending and kinking. A piece of surgical tape was attached to the skin with contact glue in order to provide a surface to further secure the catheter.

Exposure and Sampling Schedule

For 30 d, cows and heifers in groups A and B were continuously exposed, separately, to EMF except for time spent feeding and cleaning. Mean exposure times were 21.4 ± 1.4 and 22.5 ± 2.1 h/d, respectively. Blood from the caudal vein and CSF samples were collected daily for three days before exposure, three days before the end of the exposure and five days after the exposure period. Heifers in group C were housed in the main dairy barn, adjacent to the EMF chamber, and were never exposed to EMF.

Sample Analyses

Blood samples were centrifuged at 2000 x g for 15 min. Plasma and CSF samples were stored at 5° C pending analyses to estimate their mineral content. Concentrations of Ca, Mg, Cu, Zn, Fe, Mn, Na, and K in blood plasma and in CSF were determined using atomic absorption spectrophotometry (Perkin Elmer 2830; Perkin Elmer Corp., Norwalk, CT). Phosphorus was determined spectrophotometrically by the ammonium molybdate method (AOAC, 1980).

Statistical Analyses

The data were analyzed with repeated measures ANOVA using the SAS general linear models procedure (SAS Institute Inc., Cary North Carolina , U.S.A). Comparisons between treatments were done using ANOVA of the contrast variables. Significant differences between treatments were declared at $P < 0.05$.

RESULTS

Blood Plasma Samples

Results for nonlactating, pregnant cows (group A) are presented in Table 1 as actual means ($\pm$ SE). The concentrations of Mg decreased ($P = 0.023$) 8.4%, and Fe tended to decrease ($P = 0.059$) 32.9%, during exposure. Concentrations of these two minerals were similar before and after exposure. Concentrations of Cu tended to decrease ($P = 0.082$) 9.3% during the exposure period, and after exposure,

concentrations did not return to values similar to those before the EMF were activated. Manganese and Na did not change during exposure, while Mn concentration tended to decrease ($P = 0.093$) 25.0%, and Na decreased ($P < 0.001$) 6.2% after exposure. Phosphorus and K did not change, either during or after exposure. Calcium had a tendency to decrease ($P = 0.11$) 2.3% during exposure, and Zn tended to decrease ($P = 0.12$) 18.4% after exposure.

Results for non-pregnant, ovariectomized heifers (group B) are presented in Table 2. Two of the exposed heifers developed meningitis, (probably due to catheter manipulation that resulted in an infection) and had to be euthanized because permanent nervous damage was diagnosed.

Calcium and Mg tended to increase ($P = 0.09$) 4.8% and ($P = 0.11$) 12%, during exposure, and then, after exposure, returned to concentrations that were similar to those before exposure. Concentrations of Cu decreased ($P = 0.02$) 7.2% during exposure, and after exposure, quantities were similar to those before the EMF were activated. Iron, K, Mn, Na, P, and Zn did not show any change during exposure or after exposure.

CSF Samples

Results for group A are presented in Table 1. Comparisons between values before and during exposure and before and after exposure indicated that the macro-

and trace mineral concentrations in the CSF were affected to a greater extent by EMF than were those in plasma. The concentrations of Ca and P during exposure increased ($P < 0.05$) 11.1 and 523%, respectively, and after exposure, these levels did not return to concentrations that were similar to those measured before the exposure. Magnesium decreased ($P < 0.01$) 9.4% during exposure, and after exposure, these concentrations did not return to levels similar to those taken before the EMF were activated.

Copper and Zn did not change during exposure although the concentration of Zn increased ($P < 0.05$) 183% after exposure. Iron and Mn decreased ($P < 0.05$) 12-fold during exposure, Fe returned ($P = 0.16$) to 58.3% of the concentration that had been measured before exposure, and Mn maintained lower ($P < 0.05$) concentrations (46.2%) after exposure than before. Sodium decreased ($P < 0.05$) 2.8% during exposure and 7.4% after exposure. Potassium did not change.

Results for group B are presented in Table 2. A CSF sample for one heifer could not be collected after exposure because of a nonpatent catheter; therefore, this heifer was not considered for statistical analysis. In CSF samples, the concentrations of Ca tended ($P = 0.059$) to increase 9.1%, and P increased ($P = 0.004$) 5.7% during exposure, and after exposure, these concentrations returned to levels that were similar to those measured before exposure. Zinc and Fe did not change either during or after exposure. Manganese decreased ($P = 0.04$) 14% during exposure, and after

exposure, the quantity of Mn returned to a level similar to that measured before the EMF were activted. Sodium, K, Mg, and Cu did not show any change either during or after exposure.

Results for group C are presented in Table 3. No changes in any of the minerals were observed during the time that the heifers from group A or B were housed in the EMF chamber.

DISCUSSION

Minerals in Blood Plasma

In mature cows (group A), the decrease in concentrations of Mg and Fe in plasma that occurred during exposure and the fact that 5 d after exposure the values returned to concentrations that were similar to those taken before the EMF were activated suggests an effect of EMF on the concentration of these minerals in plasma. The concentration of Cu tended to decrease during exposure; however, mean concentrations did not return to a level similar to that measured before exposure. Although EMF might have had an effect on Cu, it should be noted that the values for Cu decreased in the period after exposure, suggesting a possible effect of gestation length. Alternatively, it might be that 5 d after the deactivation of the EMF were not long enough to allow for the recovery of plasma Cu concentrations to its former levels. Concentrations of Mn and Na were not affected by EMF exposure, and their values tended to decrease and decreased significatlly, respectively, after exposure. This result could be attributable to gestation length and might not have any relationship with EMF exposure unless there was a residual effect of the EMF. Conversely, in the experiment with nonmature cows (group B), this possible effect on Mn and Na was not detected. However, Ca and Mg concentrations showed an inverse response to that observed with group A. In group A, Ca tended to decrease ($P = 0.11$), and in group B, Ca tended to increase ($P = 0.09$), during exposure. Magnesium decreased in group A ($P = 0.02$) and tended to increase in group B ($P = 0.11$). In both groups, after exposure, Ca and Mg returned to concentrations

similar to those measured before exposure. In group A, Cu tended to decrease (P = 0.08), in group B decreased ($P < 0.01$), during exposure; in group A Cu concentrations in plasma decreased ($P = 0.02$) further after exposure.

The data from group A and Group B were combined and statistically analyzed with the addition to the linear regression model the efect of group and the interaction between group and treatment. For the combined group, Cu in plasma decreased ($P < 0.01$) during exposure and then remained at the same concentration after exposure (Table 4). Because Cu did not return to a concentration similar to that measured before exposure, this additional statistical analysis did not support the hypothesis that EMF has an effect on the Cu concentration in blood plasma unless the EMF effect on Cu in plasma is permanent or the time of recovery after the exposure period precludes Cu concentrations from returning to concentrations that are similar to those measured before exposure. In the case of Mg in plasma, there was a clear association between EMF and a decrease in Mg in group A; however, in group B, there was a tendency for an increase in Mg. The combined statistical analysis of groups A and B suggested that there was no evidence to firmly support the hypothesis that EMF affect Mg concentrations in blood plasma (Table 4).

Minerals in CSF

The fluid environment of the CNS is constant and different from plasma, due to endothelial cells of the capillaries surrounding the CNS that constitute a barrier that

selects the substances that enter the CSF and, hence, substances that contact the CNS. Once these substances enter the CSF, they diffuse freely into the CNS, and their concentrations remain relatively constant (Feldman, 1989). The values for Ca, Mg, and K obtained before exposure in groups A and B agreed with previous reports published in the literature, with the exception of Mg in group B (Fankhauser, 1962; Coles, 1980; Welles **et al**, 1992). In group A, Ca concentration in the CSF increased 10% during exposure, and after exposure, the concentration did not return to a level similar to that measured before exposure. In group B, Ca concentration in CSF tended to increase (P = 0.059) 9.1% and after exposure returned to a concentration similar to that measured before exposure.

Calcium is a compound that plays an important role in nerve cell function, and slight variations in CSF Ca concentrations might have some physiological implications (Bawin and Adey, 1976). The increase in the concentration of Ca in the CSF in this experiment might have been due to an increased efflux of Ca from nerve cells, which agrees with the results obtained **in vitro** (Blackman, 1989). In that experiment, exposure to EMF increased the efflux of Ca from fresh chick brains cultured with radioactive Ca. However, in the chick brain preparation, the interaction between EMF exposure and the sample temperature might have confounded the results concerning the release of Ca (Blackman **et al**, 1991). Kavaliers and Ossenkopp (1987) have suggested that when mice are exposed to EMF, the functioning of their Ca channels and the distribution of Ca ions are altered. Marron **et al.**, (1988) have

demonstrated that the electric and magnetic components of EMF cause a response in cell membranes, increasing the surface charge density. The increase in the concentration of Ca in the CSF coincides with a tendency for a decrease ($P = 0.11$) in Ca in blood plasma in group A, but not in group B. However, because of the increase (group A) and the tendency for an increase (group B) in Ca concentrations in CSF, the blood-brain barrier might have been altered during the exposure period, allowing more Ca to enter the CSF. Stronger EMF associated with magnetic resonance techniques have been shown to temporarily increase the permeability of the blood-brain barrier in the rat (Shivers **et al.**, 1987). However, the tendency for a decrease in blood plasma Ca in group A could have resulted from an increased excretion of Ca, which was not measured in this study. Also, after exposure, concentrations of Ca in blood plasma returned to values similar to those measured before exposure. This result did not occur in the CSF after exposure; therefore, the changes in Ca in the CSF might have been due to an increased secretion of Ca by the choroid plexus and the epithelia associated with CSF production. Alternatively, it might possibly have been an increase of the efflux of Ca from the nerve tissues during exposure. After exposure, Ca did not return to values similar to those taken before exposure in group A; the observed changes could be related with gestation because, after exposure, the heifers that were not pregnant (group B) had Ca concentrations that were similar to those measured before exposure. Nevertheless, it could also be interpreted as if EMF generated an increase in Ca concentrations, but the 5 d between the deactivation of the EMF and the initiation of measurements

after exposure in group A were not long enough to allow CSF Ca to return to values that were similar to those measured before exposure. Disturbances of the blood-brain barrier may cause an increase of CSF Ca, mainly because protein-bound Ca enters into the CSF (Steinberg, 1969).

Inflammatory lesions and other insults to the CNS result in an increase in K and a decrease in Mg in CSF (Steinberg, 1969). A similar situation for Mg was observed in group A, but not in group B, during the exposure. The insertion of the subarachnoidal catheter might have caused a subclinical infection of the spinal membranes for cows in group A. However, the fact that K did not change in either group during or after exposure and that Mg did not change in group B suggested that there was no subclinical infection. Conversely, Feldman (1989) claimed that K concentration in the CSF is very stable and that there is no change associated with disease. Moreover, the results obtained for group C (Table 3), that the insertion of subarachnoidal catethers did not affect the concentration of minerals in CSF samples, support the idea that the changes observed in groups A and B were associated with EMF.

In group A, the 12-fold decreases in Fe and Mn that occurred during the exposure and the consecutive tendencies to reach values after exposure that were similar to those before exposure were, together with the change in P, the most significant changes attributable to the EMF exposure in this group. Iron in CSF

followed the same pattern as in blood plasma when cows were exposed to EMF and after exposure. However, Mn did not act in a similar manner as Fe. In group B, Fe tended to decrease, and Mn decreased in CSF during exposure, which was similar to the results obtained for group A. Therefore, it is still possible to confirm that Mn concentrations, when groups A and B were combined, decreased during exposure (Table 4). The concentration of Ca, P, and Mn in CSF in group B showed the same changes during exposure as those observed during exposure in group A, although the magnitude of the absolute values differed.

Calcium and Mn share a common carrier in the mitochondrial membrane (Shamoo, 1986). If the same is true for the cell membrane, the decrease in Mn concentration in the CSF migh have been associated with increase in Ca concentration in the CSF during exposure. This suggestion is not valid for the period after exposure when Mn tended to return to values similar to those measured before exposure. Calcium maintained higher concentrations after exposure when compared with values measured before exposure.

CONCLUSIONS

Overall, in mature cows (group A), trace minerals were much more affected by exposure to EMF than macro minerals. Disturbance of values taken before exposure were more substantial in the CSF than in plasma, during and after exposure.

This study demonstrated that when nonlactating, pregnant cows (group A) and nonpregnant nonlactating dairy heifers (group B) were exposed to EMF, similar to those under a 735 kV power line, some changes in the macro and trace elements contained in blood plasma and CSF did occur. The relationship between changes in Cu and Zn concentrations in plasma and CSF and EMF exposure in group A could be questionable because of the consistent tendency of the concentrations to increase or decrease when EMF were deactivated. However, in the case of Mg in blood plasma and Ca, P, Mn, and Fe in CSF, the possibility that the exposure to EMF caused concentration changes should be considered, even though some of the absolute values obtained in group A did not agree with those obtained in group B. These differences could have been caused by the smaller number of animals that finished group B or the different maturity stage of the animals in Group B, when compared to group A. Since Group B was formed in an attempt to support the results obtained in group A, a combined statistical analysis including groups A and B proceeded (Table 4). All the minerals that showed changes attributable to the EMF in groups A and B were analyzed with a statistical methodology similar to that used in groups A and B. These analyses were performed for Ca, Mg, and Cu in blood plasma and for Ca, Mn and P in CSF. The results indicated that there was a 4-fold increase in P concentration in CSF during exposure. However, this effect was more accentuated in group A becaused the ANOVA did not detect an increase in group B during exposure. Therefore, although there was an effect associated with EMF in groups A and B when the analyses were carried out by trial, when the data

were combined, the association between EMF and an increase in P in CSF became nondetectable in group B. Nevertheless, from the evidence presented, there is a necessity to clarify the effect of EMF on P in CSF. Also, Ca increased and Mn decreased in CSF during the exposure. This result was detectable when the statistical analyses were performed by group or with both groups combined (Table 4).

The variations observed in this study have not been reported previously; therefore, it is difficult to speculate about the physiological implications of such variations, if any. Because CSF is thought to be a very stable fluid of biological substances and because this liquid is in direct contact with the CNS, considerable attention should be dedicated to these variations in minerals in future research.

ACKNOWLEDGMENTS

The authors thank J. Carreño, B. Allard, R. I. Cue, B. R. Downey (McGill University); M. Misakian (National Institute of Standards and Technology), L-P. Rivest (University of Laval), J. Lee Jr (Department of Energy), P. Lamothe (University of Montreal); and S. Maruvada, F. Renaud, D. Goulet, and C. Cardinal (Hydro-Québec), for their contributions. This research was sponsored by the Environmental Branch of Hydro-Québec.

REFERENCES

1 Asociation of Analitical Chemist 1980. Official Methods of Analysis. 13th ed. AOAC, Washington, DC.

2 Bawin, S. M., and W. R. Adey. 1978. Sensitivity of calcium binding in cerebral tissue to weak environmental electric field oscillating at low frequency. Proc. Natl. Acad. Sci. USA 73:1999.

3 Blackman, C. F. 1989. ELF effects on calcium homeostasis. Page 187 in Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. B. W. Willson, R. G. Stevens, and L. E. Anderson ed. Battelle Press, Columbus, OH.

4 Blackman, C. F. 1991. The influence of temperature during electric-field-induced elteration of calcium-ion release from **in vitro** brain tissue. Bioelectromagnetics 12:173.

5 Coles, H. E. 1980. Cerebrospinal fluid. Page 267 in Veterinary Clinical Pathology. J. J. Kaneko, ed. Saunders Philadelphia, Pensylvania.

6 Fankhauser, R. 1962. The cerebrospinal fluid. Page 21 in Comparative Neuropathology. R. M. Innes, and L. Z. Sauders, ed. Academic press, New York, NY.

7 Feldman, B. F. 1989. Cerebrospinal fluid. Page 835 in Veterinary Clinical Biochemistry. J. J. Kaneko, ed. Academic Press, San Diego, CA.

8 Goodman, R., L. X. Wei, J. C. Xu, and A. Henderson. 1989. Exposure of human cells to low frequency electromagnetic fields results in quantitative changes in transcripts. Biochem. Biophys. Acta 1009:216.

9 Jimerson, D. C., J. H. Wood, R. M. Post. 1980. Cerebrospinal fluid calcium.Page 743 in Neurobiology of Cerebrospinal Fluid. J.H. Wood ed. Plenum press, New York, NY.

10 Kavaliers, M., and K. P. Ossenkopp. 1987. Calcium channel involvement in magnetic field inhibition of morphine-induced analgesia. Arch. Pharmacol. 336:308.

11 Marron, M. T., E. M. Goodman, P. T. Sharpe, B. Greenebaum. 1988. Low frequency electric and magnetic fields have different effects on the cell surface, FEBS Lett (FEd. Eur. Biochem. Soc. 230:13.

12 Nguyen, D. H., L. Richard, J. F. Burchard, E. Block. 1995. An electric and magnetic field exposure chamber for studies of biological effects on dairy cows. Page 15 in The annual review of research on biological effects of

electric and magnetic fields from the generation, delivery and use of electricity. Palm Springs, CA. W/L Associates, Ltd. Frederick, MD.

13 National Research Council. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.

14 Shamoo, A. E. 1986. Mn(II) and Ca(II) Transport in mitochondria. Page 65 in Manganese in Metabolism and Enzyme Function. V. L.Schramm, and F. C. Wedler, ed. Academic Press, Orlando, FL.

15 Shivers, R. R., M. Kavaliers, G. C. Teskey, F. S. Prato, and R. M. Pelletier. 1987. Magnetic resonance imaging temporarily alters blood barrier permeability in the rat. Neurosci. Lett. 776:21.

16 Steinberg, S. A. 1969. Cerebrospinal fluid. Page 166 in Veterinary Clinical Pathology. W. Medway, J. E. Prier, and J. S. Wilkinson ed. The Williams and Wilkins Co. Baltimore, MD.

17 Teskey, G. C., F. S. Prato, K. P. Ossenkopp, and M. Kavaliers. 1988. Exposure to time varying magnetic fields associated with magnetic resonance imaging reduces fentanyl-induced analgesia in mice. Bioelectromagnetics 9:167.

18 Vogt, M. 1975. Metabolites of cerebral transmitters entering the cerebrospinal fluid; their value as indicators of brain function.Page 225 in The Fluid Environment of the Brain. H. F. Cserr, J. D. Fenstermacher, and V. Fencl, ed. Academic Press, New York. NY.

19 Welles, E. G., J. W. Tyler, D. C. Sorjonen, and E. M. Whatley 1992. Composition an analysis of cerebrospinal fluid in clinically normal adult cattle. Am. J. Vet. Res. 53:2050.

Table 1. Macro-and trace element concentrations in nonlactating, pregnant, Holstein cows (n = 8) exposed to electric and magnetic fields (EMF). Samples were collected daily for three days before an exposure period of 30 d, at 27 d of exposure and at 5 d after the EMF were deactivated. Values are actual means (± SE). Contrasts between values taken before and during exposure (Pre x Exp), and before and after exposure (Pre x Post) and the probabilities associated with these contrasts are presented ($P > f$).[1]

	EMF Exposure			Contrast	
Plasma	Before	During	After	Pre x Exp	Pre x Post
Element	(mg / L)			($P > f$)	($P > f$)
Ca	93.96 ± 1.18	91.76 ± 0.77	94.12 ± 0.67	0.1108	0.9039
P	55.38 ± 2.77	53.80 ± 1.18	52.30 ± 1.76	0.6148	0.4095
Mg	22.49 ± 1.06	20.59 ± 0.68	21.87 ± 0.59	0.0232	0.6111
Na	2984.58 ± 15.43	2985.62 ± 39.40	2799.58 ± 25.00	0.9829	0.0006
K	152.79 ± 3.84	157.21 ± 3.36	143.54 ± 4.59	0.4569	0.2420
	(µg / L)				
Cu	1080.00 ± 47.7	980.00 ± 47.0	870.00 ± 50.7	0.0824	0.0024
Zn	1030.00 ± 79.0	890.00 ± 50.0	810.00 ± 69.7	0.2030	0.1220
Fe	2070.00 ±134.2	1390.00 ±344.0	2140.00 ±164.0	0.0590	0.7551
Mn	120.00 ± 11.7	100.00 ± 15.0	90.00 ± 7.6	0.6225	0.0934
CSF[2]					
Element	(mg / L)				
Ca	55.06 ± 0.85	61.18 ± 0.61	61.82 ± 1.06	0.0016	0.0047
P	4.42 ± 1.79	23.13 ± 1.17	19.56 ± 2.21	0.0001	0.0022
Mg	21.68 ± 0.29	19.65 ± 0.45	20.55 ± 0.25	0.0004	0.0025
Na	3119.79 ± 27.50	3032.31 ± 19.50	2888.85 ± 26.11	0.0062	0.0005
K	114.65 ± 3.15	121.21 ± 2.21	115.02 ± 2.25	0.1050	0.9125
	(µg / L)				
Cu	70.00 ± 10.5	90.00 ± 21.1	110.00 ± 29.2	0.6062	0.2837
Zn	60.00 ± 26.6	60.00 ± 16.8	170.00 ± 27.5	0.8733	0.0491
Fe	1030.00 ± 353.3	70.00 ± 73.8	430.00 ± 93.4	0.0352	0.1639
Mn	130.00 ± 13.0	10.00 ± 9.0	70.00 ± 6.7	0.0001	0.0024

[1] The EMF were 10 kV/m and 30 µT, respectively.

[2] Cerebrospinal fluid.

Table 2 . Macro-and trace element concentrations in nonlactating nonpregnant Holstein heifers (n = 4) exposed to electric and magnetic fields (EMF). Samples were collected daily for three days before an exposure period of 30 d, at 27 d of exposure and at 5 d after the EMF were deactivated. Values are actual means (± SE). Contrasts between values taken before and during exposure (Pre x Exp), and before and after exposure (Pre x Post) and the probabilities associated with these contrasts are presented ($P > f$).[1]

	EMF Exposure			Contrast	
Plasma	Before	During	After	Pre x Exp	Pre x Post
Element	(mg / L)			($P > f$)	($P > f$)
Ca	115.29 ± 5.29	120.84 ± 6.12	120.12 ± 5.59	0.090	0.372
P	41.23 ± 2.10	37.80 ± 1.70	37.14 ± 3.08	0.300	0.122
Mg	15.45 ± 1.05	17.36 ± 1.58	16.18 ± 1.25	0.109	0.207
Na	3053.85 ± 52.07	2974.00 ± 115.28	3076.17 ± 69.10	0.552	0.771
K	226.84 ± 19.85	211.91 ± 14.90	204.38 ± 13.47	0.294	0.329
Cu	10.76 ± 0.43	9.98 ± 0.32	10.16 ± 0.48	0.012	0.340
Zn	7.44 ± 0.27	7.38 ± 0.46	7.23 ± 0.42	0.900	0.965
Fe	36.76 ± 2.42	36.56 ± 1.95	35.30 ± 3.17	0.970	0.619
Mn	2.95 ± 0.10	2.99 ± 0.11	2.94 ± 0.10	0.814	0.938
CSF[2]					
Ca	77.83 ± 5.30	84.91 ± 5.86	80.58 ± 5.23	0.059	0.447
P	3.56 ± 0.21	3.78 ± 0.22	3.90 ± 0.17	0.004	0.387
Mg	24.91 ± 2.68	24.30 ± 2.21	23.99 ± 2.29	0.647	0.642
Na	3078.33 ± 67.50	3037.83 ± 75.85	2622.89 ± 355.87	0.638	0.238
K	276.83 ± 31.84	275.70 ± 36.07	248.98 ± 29.54	0.940	0.104
Cu	10.11 ± 0.35	9.93 ± 0.28	10.57 ± 0.53	0.535	0.594
Zn	6.04 ± 0.23	5.95 ± 0.18	6.49 ± 0.18	0.736	0.187
Fe	35.87 ± 1.39	34.17 ± 1.90	32.79 ± 1.26	0.130	0.952
Mn	3.42 ± 0.11	2.93 ± 0.12	3.23 ± 0.12	0.041	0.371

[1] The EMF were 10 kV/m and 30 μT, respectively.

[2] Cerebrospinal fluid

* Because of missing values for one heifer, only four observations were considered for statistical analysis.

Table 3. Macro and trace element concentrations in nonlactating, nonpregnant, Holstein heifers (n = 2) not exposed to electric and magnetic fields (EMF). Samples were collected daily for three days before the cows in groups A and B were exposed for 30 d to EMF, at 27 d of exposure and at 5 d after the EMF were deactivated for cows in groups A and B. Values are actual means (± SE). Contrasts between values taken before and during exposure (Pre x Exp), and before and after exposure (Pre x Post), for cows in groups A and B, and the probabilities associated with these contrasts are presented ($P > f$).[1]

	EMF Exposure			Contrast	
CSF[2]	Before	During	After	Pre x Exp	Pre x Post
Element	(mg / L)			($P > f$)	($P > f$)
Ca	74.03 ± 6.27	75.96 ± 6.14	68.40 ± 6.44	0.190	0.514
P	4.40 ± 0.09	4.28 ± 0.23	3.78 ± 0.19	0.295	0.523
Mg	20.23 ± 1.89	19.42 ± 1.19	19.33 ± 1.61	0.819	0.500
Na	3091.33 ± 79.20	3134.20 ± 105.00	3125.50 ± 46.50	0.512	0.497
K	271.60 ± 26.79	284.42 ± 26.94	287.30 ± 27.39	0.286	0.501
Cu	9.07 ± 0.32	8.26 ± 0.19	8.63 ± 0.41	0.238	0.579
Zn	5.20 ± 0.12	5.42 ± 0.27	5.68 ± 0.25	0.386	0.529
Fe	33.45 ± 2.02	33.16 ± 2.17	33.00 ± 1.77	0.808	0.500
Mn	3.17 ± 0.15	3.16 ± 0.10	2.90 ± 0.09	0.958	0.451

[1] The EMF were 10 kV/m and 30 μT, respectively.

[2] Cerebrospinal fluid.

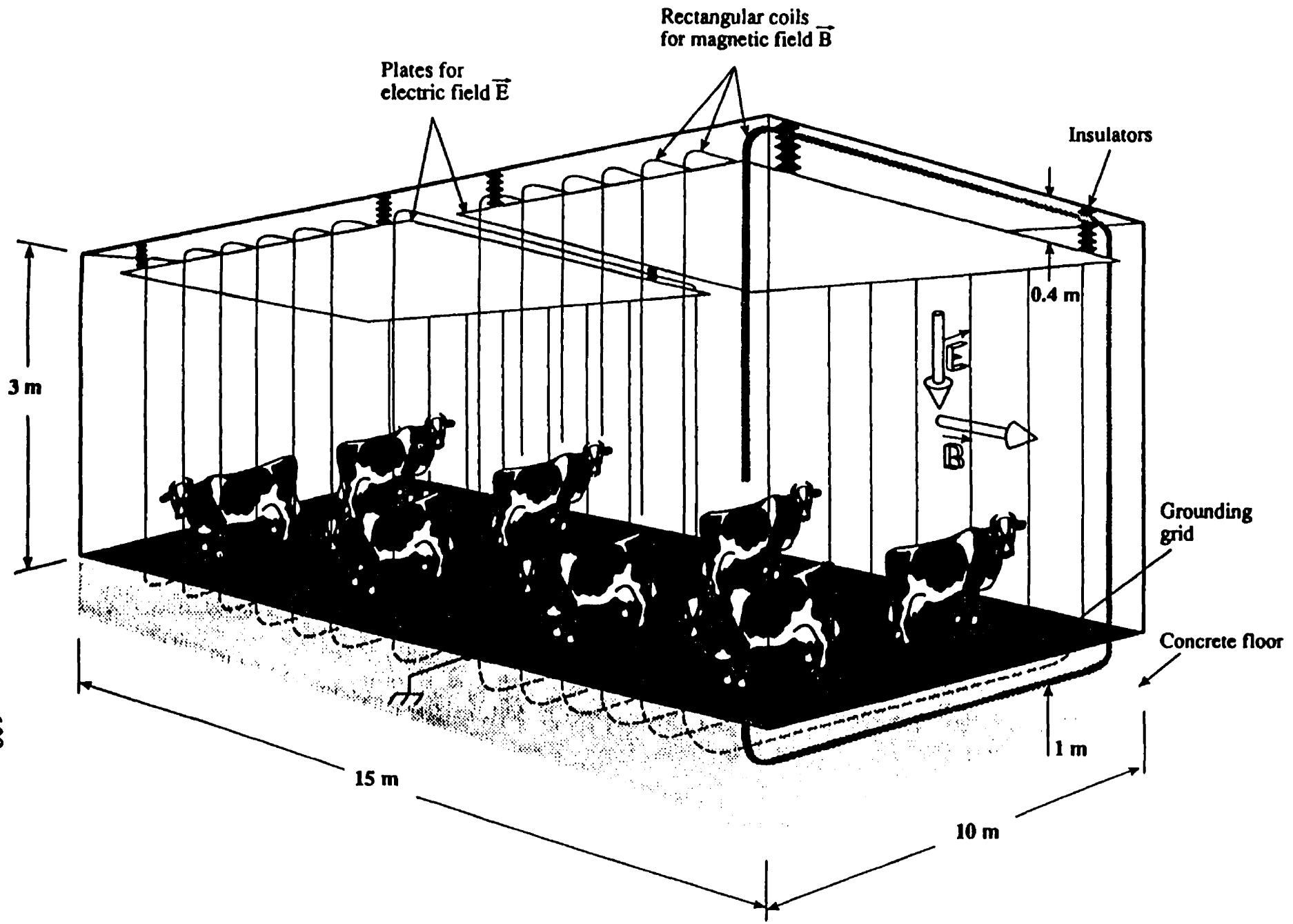
Table 4. Macro- and trace element concentrations in the combination of multiparous, nonlactating, pregnant Holstein cows (group A) and nonlactating, nonpregnant ovariectomized heifers (group B). Samples were collected daily for three days before a 30-d exposure period to electric and magnetic fields (EMF), at 27 d of exposure, and at 5 d after EMF were deactivated. Values are actual means (± SE). Contrasts between values taken before and during exposure (Pre x Exp) and after exposure (Pre x Post) and the probabilities associated with these contrasts are presented ($P > f$)[1].

	EMF Exposure			Contrast	
Plasma	Before	During	After	Pre x Exp	Pre x Post
Element	(mg / L)			($P > f$)	($P > f$)
Mg	20.09 ± 1.38	19.51 ± 1.08	19.98 ± 1.13	0.887	0.878
Ca	101.03 ± 4.11	101.46 ± 5.10	102.78 ± 4.66	0.165	0.190
Cu	4.31 ± 1.39	3.98 ± 1.29	3.96 ± 1.35	0.000	0.050
CSF[2]					
Ca	62.65 ± 4.27	69.09 ± 4.61	68.07 ± 3.98	0.000	0.000
P	4.14 ± 1.17	16.68 ± 2.86	14.34 ± 2.65	0.000	0.008
Mn	1.23 ± 0.47	0.98 ± 0.41	1.12 ± 0.45	0.000	0.060

[1] The EMF were 10 kV/m and 30 μT, respectively.

[2] Cerebrospinal fluid

Figure 1. Electric and Magnetic Exposure Chamber.


Rectangular coils for magnetic field $\vec{B}$
Plates for electric field $\vec{E}$
Insulators
0.4 m
3 m
E
B
Grounding grid
Concrete floor
1 m
15 m
10 m

CONNECTING STATEMENT

Since the central nervous system (CNS) has the majority of the body's electrical activity in mammals, it is plausible to expect that the electric and magnetic field (EMF) exposure could change the direction and the force of the magnetic and electric fields naturally occurring in vivo. This might generate a CNS response to EMF exposure.

In Chapter VI, this hypothesis that exposure of cows to EMF results in a CNS biological response is tested measuring some neurotrasnmitter metabolites in the cerebrospinal fluid.

VI. NEUROTRANSMITTER METABOLITES CONCENTRATIONS IN CEREBROSPINAL FLUID OF DAIRY COWS EXPOSED TO ELECTRIC AND MAGNETIC FIELDS.

To be submitted to the Brain Research

Running head: Electric and magnetic fields and neurotransmitters in cows

Neurotransmitter Metabolite Concentrations in Cerebrospinal Fluid of Dairy Cows Exposed to Electric and Magnetic Fields.

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ABSTRACT

Eight multiparous non-lactating pregnant Holstein cows at 198±35 d of gestation weighing 608 ± 24 kg were confined to wooden metabolic cages in an electric and magnetic field chamber with a 12:12 h light:dark cycle. Subarachnoidal catheters were installed 5 d before the activation of the electric and magnetic fields. The cows were exposed to EMF (60 Hz, 10 kV/m and 30 μT) continuously except for the feeding and cleaning time for an average of 21.44 ± 1.4 h per day for a period of 30 d. Cerebrospinal fluid samples were collected three consecutive days before an exposure period of 30 d, the last 3 d of the exposure period and for 3 d starting 5 d after exposure. The concentrations of ß-endorphin, tryptophan, 5-hydroxyindoleacetic acid, homovanillic acid and methoxy-hydroxy-phenyl-glycol in CSF were determined. Overall, no significant changes were found.

Key words: opioids, neurotransmitters, pregnant cows, electric field, magnetic field, heifers, ß-endorphin, tryptophan, 5-hydroxyindoleacetic acid, homovanillic acid and methoxy-hydroxy-phenyl-glycol

Abbreviations: EMF= electric and magnetic fields, **BE**=ß-endorphin, **TRY** = tryptophan, **5HVAA**= 5-hydroxyindoleacetic acid, **HVA** = homovanillic acid, **MHPG** = methoxy-hydroxy-phenyl-glycol.

INTRODUCTION

The central nervous system (CNS) has a considerable amount of electric and magnetic fields, so it seems fundamental to study the effects of EMF on the CNS.

The relationship between neurotransmitters and the body responses to stress are well established; therefore, it is reasonable to assume that variations in their concentrations and/or the metabolites of the neurotransmitters in the cerebrospinal fluid (CSF) may reflect neurophysiological changes (Vogt, 1975; Dowman et al., 1989). Exposure of pig-tailed macaques to EMFs ranging from 3 to 30 kV/m and 0.1 to 0.9 G (Gauss) for three 21-d periods resulted in a decrease of up to 20-30% in the cerebral spinal fluid concentration of homovanillic acid and 5-hydroxyindoleacetic acid (metabolites of dopamine and serotonin, respectively). These neurotransmitters are associated with higher functions of the central nervous system (Seegal et al., 1989). Exposure of these macaques to the above mentioned EMFs (highest exposure level was about three times the maximum intensity under high-voltage power lines) did not result in a significant change in blood chemistry, blood cell counts, and cerebellum and cerebrum macromorphology. Close observation with videotapes failed to detect any animal reaction to field activation (Wolpaw et al., 1989). Studies on the effect of 60 Hz EMFs on evoked potentials (specific neural activity in the CNS which, due to the conductivity of the body, can be detected on the surface of the skin as changes in voltages over time) did not show any difference in

the various potentials assessed in pig-tailed macaques. An exception was the cortical somatosensory evoked potentials; the amplitude decreased significantly during exposure to 10 to 30 kV/m and 0.3 to 0.9 G EMF. Post-exposure amplitudes were normal. The authors speculate that EMF could have an inhibitory effect over the endogenous opiate system (Dowman **et al.**, 1989). The antagonistic effects of EMFs upon the analgesic and behavioral effects of opiates have been documented previously (Kavaliers and Ossenkopp, 1984; Kavaliers and Ossenkopp, 1986; Ossenkopp and Kavaliers, 1987; Ossenkopp and Kavaliers, 1988-1989). Treatment of mice with EMF resulted in the potentiation of the opiate antagonistic effect of methylnatrexone (an opiate receptor blocker). It is argued that this potentiation is not the result of an alteration of receptor or cellular function, but an alteration of the permeability of the blood-brain barrier (Qouck **et al.**, 1986). This agrees with the temporary alteration of the blood-brain barrier permeability demonstrated in rats exposed to magnetic resonance imaging (Shivers **et al.**, 1987). Mice treated with magnetic resonance imaging magnetic fields (0.6 Tesla/sec) had reduced fentanyl-induced analgesia. This also indicates an interference of the EMF with the opioid system to abolish pain (Teskey **et al.**, 1988). Exposure of rats to vertical 60 Hz, 39 kV/m, 20 h/d for 4 wk resulted in a shift in the periodicity of the daily rhythm of norepinephrine, dopamine and serotonin's metabolite 5-hydroxyindoleacetic acid (5HIAA) in the hypothalamus but not in the striatum or hippocampus. One of the biological clocks (suprachiasmatic nucleus) is located in the hypothalamus and a phase shift in the periodicity of the neurotransmitter levels may alter a variety of

physiological systems (Vasquez **et al.**, 1988). One approach to the study of the CNS metabolism and activity **in vivo** is to rely heavily upon the analyses of cerebrospinal fluid (CSF) composition (Vogt, 1975). The CSF is a clear watery fluid produced by the CNS that is in intimate contact with the brain and the spinal cord. While concentrations of chemical substances in blood plasma (electrolytes, hormones, nutrients, etc) fluctuate over a wide range, their concentrations in the CSF remain relatively stable (Feldman, 1989).

Based on the above discussion, the following study had the objective to assess the effect of EMF on ß-endorphin (BE), tryptophan and the neurotransmitter metabolites, 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA) and methoxy-hydroxy-phenyl-glycol (MHPG) concentrations in CSF.

MATERIALS AND METHODS

Animals

Eight multiparous, nonlactating, Holstein cows (at 198±35 d of gestation and weighing 608 ± 24 kg) were confined to wooden metabolism crates (1.79 m wide, 1.62 m high, and 2.43 m long) in an electric and magnetic field chamber. The chamber was exposed to 12 h of light followed by 12 h of darkness. Mean room temperature and humidity was 15.6 ± 1.4°C and 33.6 ±6 %. Water and feed intake was offered for **ad libitum** consumption. The animals were offered total mixed diet twice daily according to the National Research Council requirements (NRC, 1988).

Exposure Chamber

The EMF exposure chamber was designed to resemble the EMF prevailing directly underneath an alternating current, high tension line of 735 kV. The chamber was 15 m long, 10 m wide, and 3 m high; it contains eight wooden metabolism crates, each capable of housing one cow, and was designed to allow simultaneous exposure of up to eight cows during long, uninterrupted periods. Wooden, rather than metal, metabolism crates were used to minimize disturbance of the electric field. The chamber was ventilated and heated artificially.

The magnetic field were generated by 14 rectangular coils (10 m long and 4 m high). A current of 1.03 A (ampere) generates a uniform horizontal magnetic field of 30 μT (micro-Tesla). The electric field was generated by two plates (9 m long and

6.5 m wide) that were suspended 0.4 m from the ceiling by synthetic isolators. The total electric capacity of the plate was 4.3 nanofarad. These plates produced a vertical electric field of 10 kV/m (Figure 1)(Nguyen **et al.**, 1995).

Insertion of the Subarachnoidal Catheters

The subarachnoidal catheters were inserted 5 d before activation of the EMF. Briefly, the cows were restrained in a chute and tranquilized with 4 mg of xylazine (Rompun^r, Bayvet; Etobicoke, ON, Canada) intravenously. The surgical area was shaved, cleaned with alcohol, and disinfected with 2% iodine. Anesthesia of local skin and subcutaneous tissues was achieved by injecting 20 ml of 2% lidocaine (Astra Pharma, Mississauga, ON, Canada). A 17 gauge x 9-cm Touhy needle (Becton & Dickinson, Rutheford, NJ) was inserted in the midline between the sixth lumbar and first sacral vertebrae, approximately 3 cm behind the lumbar vertebra with a 50° caudocranial angle. Once CSF was obtained, 35 cm of a 19 gauge x 91 cm-epidural catheter (Becton & Dickinson, Sandy, UT) were fed into the Touhy needle and then inserted into the subarachnoid space of the spinal cord. Once the catheter was in place and verified to be patent, the Touhy needle was withdrawn, and the catheter was fixed to the skin with a nonresorbable stitch. The plastic guide of the catheter was reinserted and left in place to provide further protection against bending and kinking. A piece of surgical tape was attached to the skin with contact glue in order to provide a surface to further secure the catheter.

Exposure and Sampling Schedule

For 30 d, the cows were continuously exposed to EMF except for time spent feeding and cleaning. Mean exposure time was 21.4 $\pm$ 1.4 h/d. Cerebrospinal fluid samples were collected daily for three days before exposure, the last 3 d of the exposure period and for 3 d starting 5 d after the exposure period.

Sample Analyses

Cerebrospinal fluid samples (20 ml) were divided into 4 ml aliquots and stored in liquid nitrogen until analyzed for BE and neurotransmitter metabolite concentrations. Cerebrospinal concentration of BE was determined using a specific radioimmunoassay (RIA). Briefly, it is a double antibody RIA with a standard curve ranging from 10 to 1000 pg. All samples were analyzed in one assay therefore, there was no inter-assay coefficient of variation and the intra-assay coefficient of variation was 6.44 %. The measurement of 5HIAA, HVA and MHPG in CSF is an indication of brain turnover of neurotransmitters. In this experiment, TRP and neurotransmitter metabolites were measured with a combined liquid chromatographic-fluorometric/amperometric method capable of determining all four compounds in about 9 minutes. The solvent was 94% sodium acetate and 6% methanol at a pH 4. Briefly, the samples were centrifuged at 400 x g for 10 min and then 50 to 100 μl were injected into a non-polar chromatographic column. The solvent system was delivered at a flow rate of 1.5 ml per minute. The compounds were quantified by

peak height measurements (Anderson **et al**, 1979).

Statistical Analyses

The data were analyzed with repeated measures analysis of variance using the SAS general linear models procedure (SAS Institute Inc., Cary, North Carolina , U.S.A). Comparisons between treatments were done using analysis of variance of the contrast variables.

RESULTS

Results pertaining to BE, 5HIAA, HVA, TRP and MHPG are presented in Table 1 as actual means ($\pm$ SE). No differences were detected in BE, 5-HIAA, HVA and MHPG between the PRE and EXP periods and between the PRE and POST periods. Tryptophan tended to increase ($P = 0.086$) 2.7 fold during EXP and then decreased but did not reach PRE levels.

DISCUSSION

The results obtained in this experiment do not support the conclusions of other authors (Kavaliers and Ossenkopp, 1984; Ossenkopp and Kavaliers, 1987; Teskey et al., 1988; Dowman et al., 1989; Ossenkopp and Kavaliers, 1988-1989) that EMF could in some way change the levels of BE secretion, affecting the capacity of this opiate to mitigate pain. On the other hand, these results do not preclude the possibility that EMF, without affecting the secretion of BE, may interfere with the capacity of this opiate to increase the threshold for pain. This could be achieved altering the functionality of the BE receptor as has been suggested by Ossenkopp and Kavaliers (1988-1989). It also has to be considered that the EMF used in the above mentioned studies (Kavaliers and Ossenkopp, 1984; Ossenkopp and Kavaliers, 1987; Teskey et al., 1988; Ossenkopp and Kavaliers, 1988-1989) were of a much higher intensity (0.02-2 T) than the EMF to which the cows of the experiment described here were exposed.

The concentration of 5-HIAA and HVA in CSF did not change during the EXP period. This does not agree with the results obtained previously in primates exposed to 10 kV/m and 30 μT (Seegal et al, 1989). These authors observed a 20% decrease in 5-HIAA and HVA when primates were exposed to EMF. By contrast, an increase in the concentration of serotonin and 5-HIAA was found in the pineal gland of rats and mice when they were exposed for 1 hour to a 40 μT magnetic field (Lerchl et al, 1991). The authors also found a decrease in N-acetyl transferase activity which is the

most important rate limiting enzyme for the synthesis of melatonin. This would increase the accumulation of substrate (serotonin) and would also increase the activity of monoamino oxidase (MAO) to catabolize serotonin to 5-HIAA. The increase in serotonin may also increase the amount of its precursor TRP which tended to be higher during the EXP period in the experiment reported here. However, serotonin was not measured in the CSF samples; therefore, we can not draw the same conclusion as Lerchl **et al** (1991). On the contrary, in this experiment there was a small tendency ($P = 0.22$) towards a decrease of 5-HIAA and HVA in the CSF. Yao **et al.,** (1992) found a decreased serotonin content in the hippocampus and hypothalamus exposed to a 4 kV/m or 40 kV/m 50 Hz electric field. This decrease in serotonin may also cause an increase in the concentration of its precursor TRP. There is evidence that 5-HIAA in the CSF originates from the serotonergic neurons primarily located in the brainstem and spinal cord (Young and Ervin, 1984). Similarly, HVA is produced in dopaminergic neurons located in the basal ganglia (Wood, 1980). Therefore, changes in 5-HIAA and HVA in CSF certainly reflect variations in the activity of the CNS. There is evidence that decreases in 5-HIAA are associated with hyperparathyroidism and increases in the blood-brain barrier permeability (Joborn **et al,** 1991); however, the blood-brain barrier in rats was not affected by exposure to magnetic resonance imaging at 1.5 T (Prato **et al.** 1992).

ACKNOWLEDGMENTS

The authors thank J. Carreño, B. Allard, R. I. Cue, B. R. Downey (McGill

University.); M. Misakian (National Institute of Standards and Technology), L-P. Rivest (University of Laval), J. Lee Jr (Department of Energy), P. Lamothe (University of Montreal), S. Maruvada, F. Renaud, D. Goulet and C. Cardinal (Hydro Québec), for their contributions. This research was sponsored by the Environmental Branch of Hydro-Québec.

REFERENCES

1 Anderson G M, J G Young, D J Cohen (1979): Rapid liquid chromatographic determination of tryptophan, tyrosine, 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid. J. Chromatography 164:501-505.

2 Dowman R, J R Wolpaw, R F Seegal, S Satya-Murti (1989): Chronic exposure of primates to 60 Hz electric and magnetic fields. III. Neurophysiological effects. Bioelectromagnetics 10:303-317.

3 Joborn C, J Hetta, F Niklasson, J Rastad, L Wide, H Agren, G Akerstrom, S Ljunghall (1991): Cerebrospinal fluid calcium, parathyroid hormone, and monoamine and purine metabolites and the blood-brain barrier function in primary hyperparathyroidism. Psychoneuroendocrinology 16:311-322.

4 Kavaliers M, K P Ossenkopp (1987): Calcium channel involvement in magnetic field inhibition of morphine-induced analgesia. Arch. Pharmacol. 336:308-315.

5 Kavaliers M, K P Ossenkopp (1986): Magnetic field inhibition of morphine-induced analgesia and behavioral activity in mice. Evidence for involvement of calcium ions. Brain Res. 379:30-38.

6 Kavaliers M, K P Ossenkopp (1984): Magnetic fields abolish the enhanced nocturnal analgesic response to morphine in mice. Physiology & Behaviour 32:261-264.

7 Lerchl A, R J Reiter, K A Howes, K O Nonaka, K A Stokkan (1991): Evidence that extremely frequency calcium ion cyclotron resonance depresses pineal melatonin synthesis in vitro. Neurosci. Lett. 124:213-215

8 Nguyen D H, L Richard, J F Burchard, E Block. (1995): An electric and magnetic field exposure chamber for studies of biological effects on dairy cows. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Cal. U.S.A.

9 National Research Council (NRC) (1988): Nutrient requirements of dairy cattle. Ntnl. Acad. Press, Washington DC.

10 Ossenkopp K, M Kavaliers (1988-1989): Clinical and applied aspects of magnetic field exposure: A possible role for the endogenous opioid system (review). J. Bioelectr. 7:189-208.

11 Ossenkopp K, M Kavaliers (1987): Morphine-induced analgesia and exposure to

low-intensity 60-Hz magnetic fields: inhibition of nocturnal analgesia in mice is a function of magnetic field intensity. Brain Res. 418:356-360.

12 Prato F S, R H Frappier, J M Wills, R R Shivers (1992): Blood-Brain barrier permeability in rats is not altered by exposure to to magnetic resonance imaging at 1.5 tesla. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 29

13 Qouck R M, F J Kouchich, T K Ishii, D G Lange (1986): Microwave facilitation of methylnaltrexone antagonism of morphine-induced analgesia in mice. J. Bioelectr. 5:35-46.

14 Seegal R F, J R Wolpaw, R Dowman (1989): Chronic exposure to 60-Hz electric and magnetic fields: II Neurochemical effects. Bioelectromagnetics 10:289-301.

15 Shivers R R, M Kavaliers, G C Teskey, F S Prato, R-M Pelletier (1987): Magnetic resonance imaging temporarily alters blood barrier permeability in the rat. Neurosci. Lett. 776: 21-31.

16 Teskey G C, F S Prato, K-P Ossenkopp, M Kavaliers (1988): Exposure to time varying magnetic fields associated with magnetic resonance imaging

reduces fentanyl-induced analgesia in mice. Bioelectromagnetics 9:167-174.

17 Vasquez B J, L E Anderson, C I Lowery, W R Adey (1988): Diurnal patterns in brain biogenic amines of rats exposed to 60 Hz electric fields. Bioelectromagnetics 9:229-236.

18 Vogt M (1975): Metabolites of cerebral transmitters entering the cerebrospinal fluid; their value as indicators of brain function In: Cserr H F, J D Fenstermacher, V Fencl (eds). The fluid environment of the brain. Academic Press New York pp 225-236.

19 Wolpaw, J R, R F Seegal, R Dowman (1989): Chronic exposure of primates to 60-Hz electric and magnetic fields: I Exposure system and measurements of general health and performance. Bioelectromagnetics 10:277-288.

20 Wood J H (1980): Sites of origin and cerebrospinal fluid concentration gradients: Neurotransmitters, their precursors and metabolites and cyclic nucleotides. In Wood JH (ed): Neurobiology of cerebrospinal fluid. Vol 1. New York:Plenum Press, pp. 53-62.

21 Yao G D, M Z Lou, Y D Fu, Q D Lu (1992): Effects of 50-Hz electric fields

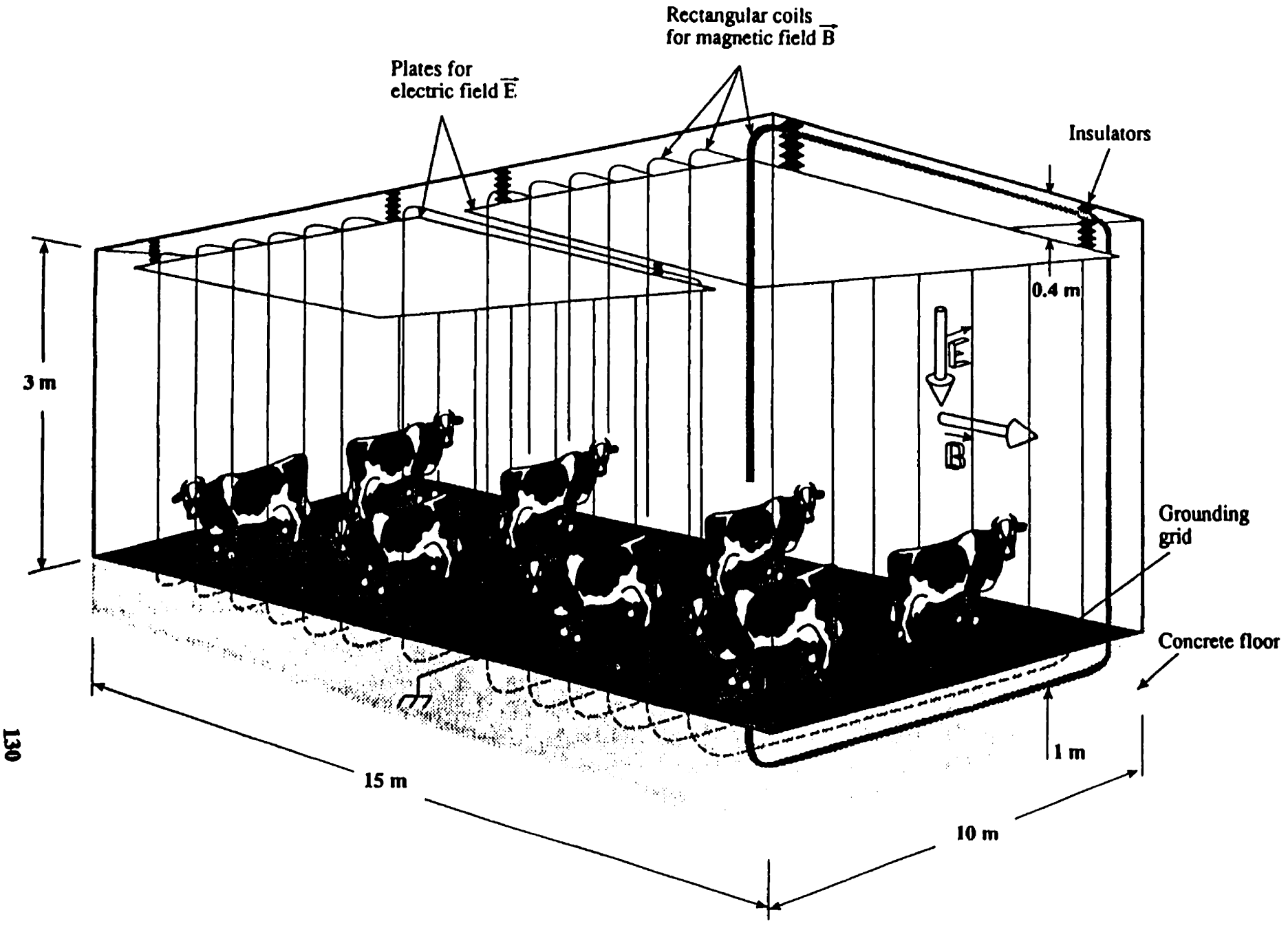
on monoamine neurochemestry and learning behaviour in rats. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. p 118.

22 Young S N, F R Ervin (1984): Cerebrospinal fluid measurements suggest precursor availability and sex are involved in the control of biogenic amine metabolism in a primate. J. Neurochem. 42:1570-1573.

Table 1.- ß-endorphin (BE), 5-hydroxyindolacetic acid (5HIAA), homovanillic acid (HVA), tryptophan (TRP) and methoxy hydroxy phenylglycol (MHPG) concentrations in cerebrospinal fluid (CSF) obtained from nonlactating, pregnant, Holstein cows (n = 8) exposed to EMF. Samples were collected daily for three days before an exposure period of 30 d, at 27 d of exposure and at 5 d after the EMF were deactivated. Values are actual means (± SE). Contrasts between values taken before and during exposure (Pre x Exp) and before and after exposure (Pre x Post) and the probabilities associated with these contrasts are presented ($P > f$).

	EMF Exposure			Contrast	
Variable	Before	During	After	Pre x Exp ($P > f$)	Pre x Post ($P > f$)
BE					
(pg/ 0.5 ml)	114.2 ± 21	134.5 ± 21	130.0 ± 23	0.5180	0.6525
5HIAA					
(ng / ml)	31.0 ± 3	26.3 ± 1	28.5 ± 2	0.2227	0.3816
HVA					
(ng / ml)	7.5 ± 2	4.8 ± 1	5.7 ± 1	0.2407	0.6602
TRP					
(ng / ml)	240.7 ± 59	652.6 ± 165	471.5 ± 94	0.0857	0.1539
MHPG					
(ng / ml)	2.5 ± 0.1	2.0 ± 0.3	3.1 ± 0.5	0.1854	0.3170

Figure 1. Electric and Magnetic Field Exposure Chamber.


Rectangular coils for magnetic field $\vec{B}$
Plates for electric field $\vec{E}$
Insulators
0.4 m
3 m
E
B
Grounding grid
Concrete floor
1 m
15 m
10 m

CONNECTING STATEMENT

In chapter III it was demonstrated that 4% fat corrected milk, feed consumption and plasma progesterone increased when cows where exposed to electric and magnetic fields.

In Chapter VII, the effect of EMF on the concentration of progesterone during the estrous cycle was evaluated.

VII. EFFECT OF ELECTRIC AND MAGNETIC FIELDS ON THE ESTROUS CYCLE IN DAIRY COWS.

Submitted to Bioelectromagnetics

Running head: Electric and magnetic fields and estrous in cows

Effects of Electric and Magnetic Fields on The Estrous Cycle in Dairy Cows.

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ABSTRACT

Sixteen multiparous non-pregnant lactating Holstein cows (weighing 662 $\pm$ 65 kg in 150.4$\pm$40 days of lactation) were confined to wooden metabolic cages during the experiment with 12:12 h light:dark cycle. The cows were divided into two replicates of eight cows each and exposed to electric and magnetic fields (EMF) in an exposure chamber. This chamber produced a vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 μT. One replicate was exposed for three periods. During the first period, the electric and magnetic fields were off; during the second period they were on; and during the final period, they were off. The second replicate was also exposed for three periods, but the activity of the fields was reversed (first period, on; second period, off; last period, on). The length of each exposure period varied according to the estrous cycle length. Overall, based on blood progesterone concentrations, calculated estrous cycle length were longer during EMF exposure.

(**Key words**: progesterone, electric field, magnetic field, cows, extremely low frequency, alternating current)

Abreviations: **EMF** = electric and magnetic fields, **NAT** = serotonin N-acetyl-tranferase, **MLT** = melatonin.

INTRODUCTION

Since electric and magnetic fields (EMF) are capable of inducing electric current and fields in the tissues of exposed subjects (Kaune and Gillis, 1981; Kaune and Forsythe, 1988, Tenforde, 1989), the hypothesis that EMF may represent an hazard to animal health has motivated research efforts toward a better understanding of the biological effects of EMF. Studies reported to date in the bovine species are farm surveys (Busby **et al.**, 1974; Ware, 1974; Amstutz and Miller, 1980), retrospective analyses (Williams and Beiler; 1979, Algers **et al.**, 1981; Algers and Hennichs, 1985; Martin **et al**, 1986) or prospective studies (Algers and Hultgren 1987). Rats exposed to 60 Hz, 39 kilovolts (kV)/m EMF for 3 wk had decreased levels of pineal N-acetyl transferase (NAT) activity and melatonin (MLT) concentrations (Wilson **et al.**, 1986; Wilson and Anderson, 1989). Rats exposed to inverted horizontal geomagnetic fields of 20 to 30 micro-Tesla (μT) for 30 to 60 minutes during a dark period showed depressed NAT activity and MLT concentrations in their pineal gland 45 minutes after the initiation of exposure (Yaga **et al.**, 1993; Lerchl **et al.**,1990; Lerchl **et al.**, 1991). Exposure to magnetic fields produces the isomerization of 11-cis to all-trans retinal in retinas of rats. This isomerization represents the initial step in the photostimulation of the visual system; therefore, as with light, magnetic fields may stimulate the retina and, hence, inhibit pineal activity (Reiter **et al.**, 1992). Conversely, when ewe lambs were exposed to the EMF produced by a 500-kV transmission line (4-7 kV/m, 0.1-5 μT), they did not show any significant variation

in the mean nighttime MLT concentration at 18 or 32 wk of age (Thompson **et al.**, 1991; Lee **et al.**, 1993, Lee **et al.** 1995). Rats exposed to alternating EMF with intensities similar to domestic EMF exposure as arising from neighboring power lines showed significantly reduced nocturnal MLT concentrations. A study of 55 dairy farms during a period of 4-6 years, before and after the construction of 765 kV AC power lines, revealed no effect of the lines on fertility, calf mortality and birth abnormalities (Williams and Beiler, 1979). Another retrospective study of cow fertility based on artificial insemination records of 106 farms in Sweden that were exposed to 400 kV AC lines for 15 or more days, revealed no effect on reproductive performance (Algers and Hennichs, 1985). Fifty eight heifers kept beneath a 400 kV, 50 Hz AC transmission line were exposed for 120 days to an EMF calculated to be 4 kV/m and 2 μT. No differences in regularity of the estrous cycle, mid-cycle plasma progesterone level, intensity of estrus, and conception index in response to EMF exposure was detected (Algers and Hultgren, 1987). Furthermore exposure of 774 beef cattle to an average of 5.6 kV/m under a 500 kV direct current (DC) transmission line over a period of 3 years did not affect feed consumption, health, mortality, breeding, number of calves weaned, calving, sexual development in bull calves and carcass weight (Raleigh, 1988). Free **et al.** (1981) found alterations in the secretion pattern of follicle-stimulating hormone (FSH) in rats exposed to a 80kV/m electric field for 20 to 56 days. Studying the effect of a 735-kV, 60 Hz transmission line upon dairy cattle, Williams and Beiler (1979) did not find differences in the number of services per conception. Exposure of cows to a 400kV, 50 Hz

transmission line with a 4kV/m and 2 μT EMF did not cause any change in the length of estrous cycles or mid-cycle blood progesterone levels, the expression of estrous, ovary weight, size of corpora lutea, or conception rate (Hultgren, 1989). Since MLT is a hormone that is antagonistic to the secretion of gonadotropins (Axelrod **et al.**, 1982), its nocturnal decrease caused by EMF exposure could result in an increased amount of circulating gonadotrophic hormones (Wilson and Anderson, 1989) affecting the estrous cycle. This experiment was designed to evaluate the effect of EMF exposure on the estrous cycle in lactating dairy cows.

MATERIALS AND METHODS

Sixteen multiparous non-pregnant lactating Holstein cows (weighing 662 $\pm$ 65 kg in 150.4$\pm$40 days of lactation) were confined to wooden metabolic cages during the experiment with 12:12 h light:dark cycle and an average room temperature and humidity of 18.2 $\pm$ 4.62° C and 57.1 $\pm$ 29.8%, respectively. Water consumption and feed intake were **ad libitum**. The cows were fed total mixed ration twice daily according to the U.S. National Research Council requirements (NRC, 1988). The cows were divided into two replicates of eight cows each. One replicate was exposed for three periods. During the first period, the electric and magnetic fields were off; during the second period they were on; and during the final period, they were off. The second replicate was also exposed for three periods, but the activity of the fields was reversed (first period, on; second period, off; last period, on). The length of

each exposure period varied according to the estrous cycle length. The magnetic field in the chamber was generated by 14 rectangular coils of 10 m long and 4 m high. A current of 1.03 A generated a uniform horizontal magnetic field of 30 μT. The electric field was generated by two plates 9 m long and 6.5 m wide that are suspended 0.4 m from the ceiling by synthetic isolators. The total electric capacity of the plate is 4.3 nanofarad. This produced a vertical electric field of 10 kV/m. The intensity of the EMF chosen for this experiment resembles a situation in which the cows are standing continuously under an 735 kV AC power line when the line has a maximun load of current. In reality, these conditions are found only for a few days during the winter in the Province of Québec.

Synchronization and Definition of Estrous Cycles

The beginning of the first estrous cycle was synchronized with two 25 mg $PGF_{2\alpha}$ (Lutalyse^r, Upjohn, Kalamazoo, MI) injections separated by 11 days. Thirteen days after the first dinopros-tromethamine injection was defined as day 1 of treatment. After 17 d of treatment, milk progesterone concentration was evaluated every other day in order to establish the presence of a new corpus luteum. Once the corpus luteum was detected in all cows, 25 mg dinopros-tromethamine were injected to synchronize the initiation of the second estrous cycle to be included in the replicate. This procedure was repeated twice in order to evaluate three consecutive estrous cycles per cow.

Determination of Progesterone in Milk Samples

Milk samples were collected in the morning and the presence of high or low levels of progesterone was determined using a qualitative immunoreactive commercial kit (Target^r, Biometallics, Princeton, NJ). This analysis detected the initiation of a new estrous cycle and the presence of a new corpus luteum that would allow estrous synchronization with $PGF_{2\alpha}$.

Determination of Progesterone in Blood Samples

Blood samples were collected in heparinized vacutainer tubes three times per week from the coccygeal vein. Samples were centrifuged immediately at 1000 x g for 15 min and stored at -20°C pending radioimmunoassay. This measurement was carried out to estimate the actual progesterone concentrations in blood throughout the experiment. This allowed the definition of length of the estrous cycles, area under the progesterone curve across estrous cycles and the detection of ovarian pathologies such as follicular and / or luteal cysts.

Plasma progesterone concentrations were estimated using a solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) not requiring extraction of the plasma. The standard curve ranged from 0 to 40 ng/ml with a maximum binding of 39% and an approximate sensitivity of 0.03 ng/ml and the sample size was 100 μl. Intra and inter assay coeficients of variation were 2.12 and 9.33 %, respectively.

Statistical Analysis

The animals were exposed according to a switch-back design (Cochran, 1957) for which the regression model is described below. This design was developed to test two treatments in the same experimental unit which results in a reduced number of experimental animals required and variability between cows. In this design each cow receives both treatments (treatment A=off, treatment B=on) in either of the sequences 1 or 2 (sequence $1=A_1 B_2 A_3$, sequence $2=B_1 A_2 B_3$ where the subscripts denote periods) (Cochran and Cox, 1957):

The data were analyzed with analysis of variance using the following linear regression model (SAS, Cary, NC):

$Y_{ijkl} = \mu + C_{ij} + MC_{ij} + P_k + T_l + E_{ijkl}$

Y_{ijkl} = dependent variable

μ = overall population mean

C_{ij} = effect of the j^{th} cow in the i^{th} sequence group, (i=1 or 2).

MC_{ij} = linear time trend of the j^{th} cow in the i^{th} sequence group, (j=1,...16),

P_k = effect of the k^{th} period, (k=1,2 or 3),

T_l = effect of the l^{th} treatment, (l=1 EMF off or 2 EMF on),

E_{ijkl} = random error, normally and independently distributed with zero mean.

A normality test (W) was carried out using the univariate statistical analysis system (SAS) procedure, to evaluate if the distribution of the experimental error complied with the normality assumptions. When the normality assumptions were not met, the non-parametric Kruskal-Wallis test (Steel and Torrie, 1980) was performed.

The power of the test (1-ß) for each variable was estimated using the following formula:

$$n = (Z\alpha + Z\beta)^2 \sigma^2 / D^2$$

where:

n = number of observations

D = Difference desired to detect

σ^2 = Variance

$Z\alpha$ = Value of t asociated with the α probability level

Zß = Value of t associated with the ß probability level

1-ß = Power of the test (Steel and Torrie, 1980).

RESULTS

Results are presented as least-squares means (LSM) $\pm$ standard error (SE) (Table 1). The dependent variables are mean progesterone concentration in plasma and progesterone area under the curve formed by progesterone concentrations on the y axis and estrous cycle length on the x axis. Estrous cycle length was determined using the blood and milk progesterone concentrations. Three cows were excluded from the statistical analyses since their progesterone concentration curves across the

estrous cycles indicated possible ovarian pathology. No differences were detected in progesterone concentrations and area under the progesterone curve between EMF exposed and non-exposed cows.

Cows exposed to EMF showed a longer estrous cycle ($P = 0.027$) (Table 1). The test of normality of the estrous cycle length data demonstrated that the variance of the experimental error did not have a normal distribution (Table 2). Consequently a non-parametric statistical analysis was also performed using the Kruskal-Wallis test (Steel and Torrie, 1980). With this methodology the null hypothesis was rejected ($P = 0.004$). The power of the test to detect the observed differences in estrous cycle length was less than 75% (Table 3).

DISCUSSION

This study demonstrated that EMF did not affect plasma progesterone concentration during estrous cycles. This finding agrees with that obtained in previous studies with dairy cows (Algers and Hultgren, 1987; Hultgren, 1989). However, cows exposed to EMF showed longer estrous cycles. Within the context of a normal dairy operation the difference in estrous cycle length observed in this experiment should not have any implications in the reproductive efficiency and/or health. Even though it is not statistically greater, the area under the progesterone curve of the estrous cycles is numerically superior. In turn, estrous cycle length, in this

experiment, is a direct function of the blood progesterone concentrations; therefore, it is possible that EMF increased or maintained the blood progesterone levels for a longer time. On the other hand, the data related to the estrous cycle length did not comply with the normality assumption since the variance of the experimental error did not have a normal distribution. Due to this, an additional non-parametric statistical analysis was performed. This methodology resulted in an even smaller probability ($P = 0.004$) that also led to rejection of the null hypothesis. Nevertheless, it should be noted that for most of the biological data the usual disturbances produced by the fact that the data do not comply with the normality assumptions are unimportant (Steel and Torrie, 1980). In this particular case, where calculated estrous cycle lengths were increased in the cows exposed to EMF, considerable attention should be given to the power of the test which is less than 75% (Table 3). This means that with this number of cows and the variance shown by the variable estrous cycle length there is a high probability of incurring in a type II error, which is falsely accepting the null hypothesis or falsely accepting that there is no difference in estrous cycle length between treatments. All the statistical considerations seem to indicate that there is a difference in the estrous cycle length. If the power of the test for the variable area under the progesterone curve was improved, the numerical difference shown in this experiment would become statistically significant. This is agreement with higher blood progesterone concentrations reported previously in EMF exposed cows (Burchard **et al.**, 1996).

CONCLUSIONS

The exposure of cows to 10 kv/m 30 μT EMF resulted in longer calculated estrous cycles; however, the implications of EMF exposure on the reproductive performance cannot be assessed in this experiment.

transmission lines. Bovine Pract. 15:51.

5 Axelrod, J., and F. Fraschini, and G. P. Velo, ed. 1982. The Pineal Gland and its Neuro-endocrine Role. Plenum Press, New York, NY.

6 Burchard, J.F., D.H. Nguyen, L. Richard, and E. Block. 1995. Biological effects of 60 Hz electric and magnetic fields (EMF) on productivity of dairy cattle. J. Dairy Sci. 74:1549.

7 Busby, K., D. Driscoll, and W. E. Washbon. 1974. A field survey of farmer experience with 765 kV transmission lines. Agric. Res. Commiss., State of New York, Albany, New York, NY.

8 Cochran, W. G., and G. M. Cox. 1957. Page 141 in Experimental designs. John Williams and Sons, New York, NY.

9 Dominique, B. M. F., P. R. Wilson, D. W. Dellow, and T. N. Barry. 1992. Effects of subcutaneous melatonin implants during long daylength on voluntary feed intake, rumen capacity and heart rate of red deer (Cervus elaphus) fed on a forage diet. British J. Nutr. 68:77.

10 Free, M. J., W ,T, Kaune, R. D. Phillips, H. Cheng. 1981. Endocrinological

effects of strong 60-Hz electric fields on rats. Bioelectromagnetics 2:105-121.

11 Haggren, W., S. M. Yellon, J. L. Phillips, and W. R. Adey. 1992. Effect of magnetic field exposure in adult djungarian hamsters on melatonin rhythms and molecular markers. Page 105 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San Francisco Press, San Francisco, CA.

12 Hultgren, J. 1989. Cows and electricity study, biological interaction and effects on cow behaviour, health and reproduction of stray voltage, electric cow-trainers and high voltage transmission lines. Rep No 24 Swed. Univ. Agric. Sci. Dep. Anim. Hygiene with Farrier School, Skara, Sweden.

13 Kato, M., K. Honma, T. Shigemitsu, and Y. Shiga. 1994. Circularly polarized 50-Hz magnetic field exposure reduces pineal gland and blood MLT concentrations of Long-Evans rats. Neurosci. Lett. 166:59.

14 Kaune, W. T., and W. C. Forsythe. 1988. Current densities induced in swine and rat models power-frequency electric fields. Bioelectromagnetics 9:1.

15 Kaune, W. T., and M. F. Gillis. 1981. General properties of the interaction between animals and ELF electric fields. Bioelectromagnetics 2:1.

16 Lee, J. M. Jr, F. Stormshak, J. M. Thompson, D. L. Hess, and D. L. Foster. 1995. Melatonin and puberty in female lambs exposed to EMF: A replicate study. Bioelectromagnetics 16:119.

17 Lee, J. M. Jr, F. Stormshak, J. M. Thompson, P. Thinesen, L. J. Painter, E. G. Olencheck, D. L. Hess, R. Forbes, and D. L. Foster. 1993. Melatonin secretion and puberty in female lambs exposed to environmental electric and magnetic fields. Biol. Reprod. 49:857.

18 Lerchl, A., K. O. Nonaka, K. A. Stokkan, and R. J. Reiter. 1990. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. Biochem. Biophys. Res. Com. 169:102.

19 Lerchl, A., R. J. Reiter, K. A. Howes, K. O. Nonaka, and K. A. Stokkan. 1991. Evidence that extremely frequency calcium ion cyclotron resonance depresses pineal melatonin synthesis **in vitro**. Neurosci. Lett. 124:213.

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

21 Martin, F. B., A. Bender, G. Steuernagel, R. A. Robinson, R. Revsbech, D. K. Sorensen, N. Williamson, and A. Williams. 1986. Epidemiological study of Holstein dairy cow performance and reproduction near a high-voltage direct-current powerline. J. Toxicol. Environ. Health. 19:303.

22 Martinez Soriano, F., M. Gimenez Gonzalez, E. Armanazas, and T. Ruiz. 1992. A Pineal "synaptic ribbons" and serum melatonin levels in the rat following the pulse action of 52-Gs (50-Hz) magnetic fields: An evolutive analysis over 21 days. Acta Anatomica 143:289.

23 NRC. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Nat. Acad. Sci., Washington DC.

24 Peters, R. R., L. T. Chapin, K. B. Leining, and H. A. Tucker. 1978.: Supplemental lighting stimulates growth and lactation in cattle. Science 199:911.

25 Raleigh, R. J. 1988. Rep. DOE/BP-21216-3. Joint HVDC agricultural study. Oregon State University, Boneville Power Administration Portland, Oregon. DOE. Portland, OR.

26 Reiter, R. J., K. Yaga, L. C. Manchester, J. S. Sun, L. D. Chen, M. Abdolsalami. 1992. Parametric and mechanistic studies on the perturbation

of the circadian melatonin rhythm by magnetic field exposure. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 5

27 Richardson, B. A., K. Yaga, R. J. Reiter, and D.J. Morton. 1992. Pulsed static magnetic field effects on in vitro pineal indoleamine metabolism. Biochim. Biophys. Acta 1137:59.

28 Rogers, W. R., R. J. Reiter, and J. L. Orr. 1992. 60-Hz electric and magnetic fields and primate melatonin. Page 29 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San Francisco Press, San Francisco, CA.

29 Sasser, L. B., J. E. Morris, D. L. Miller, and L. E. Anderson 1992. Effect of acute exposure to magnetic fields on pineal function in the rat. Page 28 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San Francisco Press, San Francisco, CA.

30 Schiffman, J. S., H. M. Lasch, D. M. Rollag, A. E. Flanders, G. C. Brainard, and D. L. Burk. 1992. Effect of magnetic resonance imaging (MRI) on plasma melatonin in normal humans. Page 74 in The First World Congress for Electricity and Magnetism in Biology and Medicine. Orlando, FL., San

Francisco Press, San Francisco, CA.

31 Silman, R. 1991. Melatonin and the human gonadotropin-releasing hormone pulse generator. J. Endocrinol. 128:7.

32 Steel, R. G. D, J. H .Torrie. 1980. Page 363 in Principles and Procedures of Statistics. McGraw-Hill Book Co. New York, NY..

33 Tenforde, T. S. 1989. Electroreception and magnetoreception in simple and complex organisms. Bioelectromagnetics 10:215.

34 Thompson, J. M., F. Stormshak, L. J. Painter, P. K. Thinesen, J. Corvallis, M. Lee, and D. L. Hess 1991. Endocrine responses of ewe lambs exposed to a 500 kV a-c transmission line. J. Anim. Sci. 69(suppl 1):229.

35 Ware, B. J. 1974. Effect of 765-kV transmission lines on animal grazing habits. American electric power service corporation. New York.

36 Williams, J. H., and J. E. Beiler. 1979. An investigation of dairy farm operations in association with 765-kV transmission in Ohio. Rep.Ohio Power Siting Commission and Cleveland Electric Illuminating Co. Cleveland, OH.

37 Wilson, B. W., C. W. Wright, J. E. Morris, R. L. Buschbom, D. P. Brown, D.

L. Miller, F. R. Sommers, and L. E. Anderson. 1990. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J. Pineal Res. 9:259.

38 Wilson, B. R., and L. E. Anderson. 1989. ELF Electromagnetic field effect on the pineal gland. Page 157 in Wilson, B. W., R. G. Stevens, and L. E. Anderson. Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. Battelle Press, Columbus, OH.

39 Wilson, B. W., E. K. Chess, and L. E. Anderson. 1986. 60Hz Electric-field effects on pineal melatonin Rhythms: Time course for onset and recovery. Bioelectromagnetics 7:239.

40 Yaga K., R. J. Reiter, L. C. Manchesten, H. Nieves, J. H. Sun, and L. D. Chen. 1993. Pineal sensitivity of pulsed magnetic field changes during the photoperiod. Brain Res. Bull. 30:153.

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

Table 1. Estrous cycle length and progesterone concentration in blood samples obtained from lactating non-pregnant dairy cows in three consecutive estrous cycles during non-exposure (OFF) and exposure (ON) periods. Results are least-squares means (±) SE and the probability of significance ($P > t$ and $P > X^2$).

Dependant variable	OFF	ON	*$P > T$	**$P > X^2$
Progesterone (ng / ml)	2.28±0.17	2.25±0.17	0.916	0.999
Area under the progesterone curve during each cycle	24.5±1.9	26.4±1.9	0.496	0.317
Estrous cycle length (d)	22.0±0.9	25.3±1.4	0.027	0.004

* Analysis of variance asumming that the data comply with the normality assumptions.

** Non-parametric statistical analysis (Kruskal-Wallis test)

Table 2. Variables, W statistic and probability associated with the W statistic and test of normality.

VARIABLE	W:NORMAL	$P < W$
Progesterone during estrous cycles	0.966405	0.3827
Progesterone area	0.933083	0.0293*
Estrous cycle length	0.886395	0.0007*

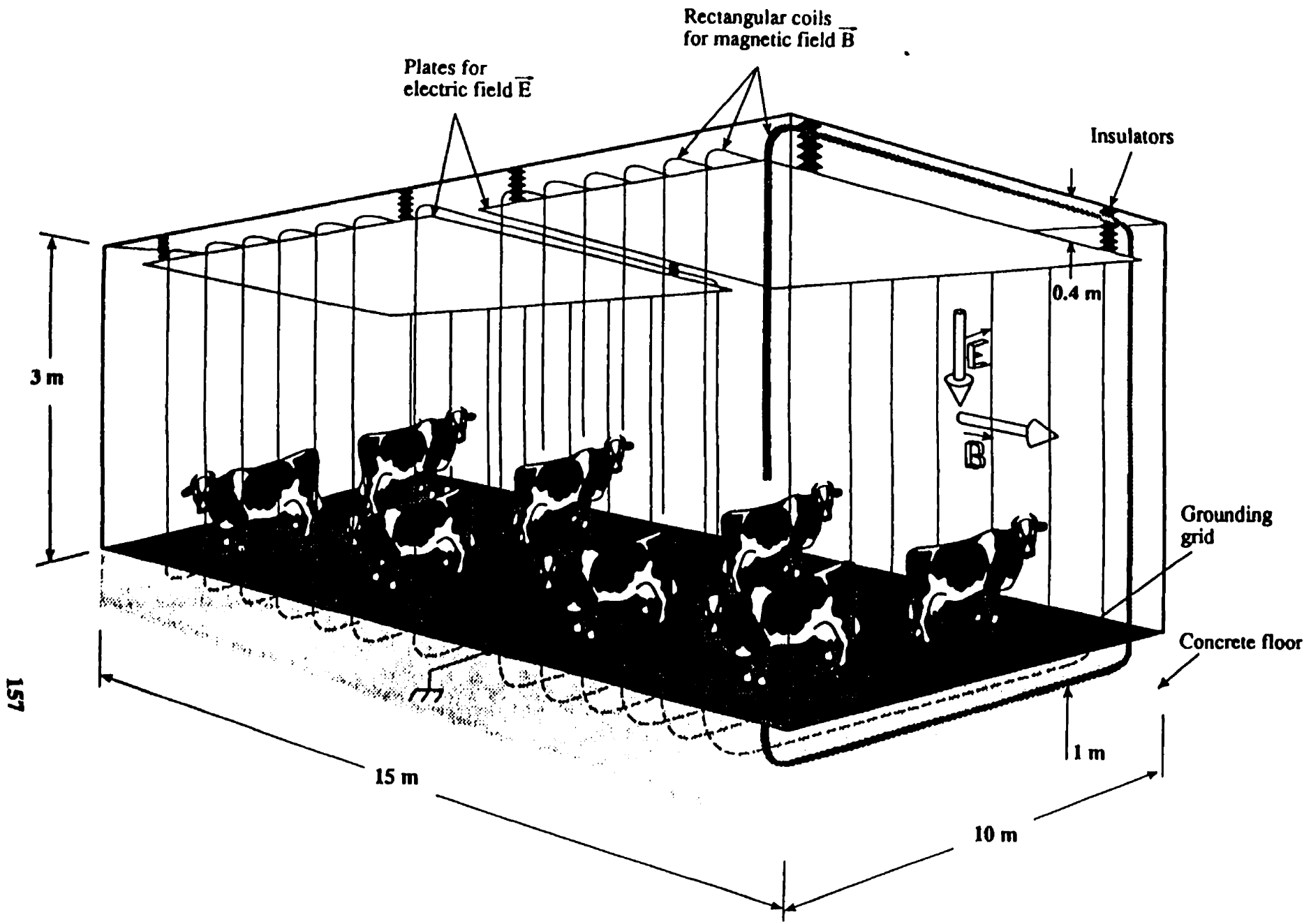
* Data do not comply with the normality assumption

Table 3. Variable, standard deviation (SD), degrees of freedom (df), differences obtainable with different test power (P75 etc.) and observed difference (OD).

Variable	S.D.	d.f.	P75**	P80	P90	O.D.***
Progesterone during estrus	0.723	11	0.71	0.76	0.90	0.026
Area under the P4 curve	8.076	11	7.90	8.46	10.01	1.940
Cycle length	3.720	11	3.64	3.90	4.61	3.245

** P75 = Minimum detectable difference with a power test of 75 %

Figure 1. Electric and Magnetic Field Exposure Chamber


Rectangular coils
for magnetic field $\vec{B}$
Plates for
electric field $\vec{E}$
Insulators
0.4 m
3 m
$\vec{E}$
$\vec{B}$
Grounding
grid
Concrete floor
1 m
15 m
10 m

VIII. GENERAL DISCUSSION

1.0 Endocrinology

The results of this research demonstrated that EMF did not affect progesterone concentrations during estrous cycles. This finding agrees with that obtained in previous studies with dairy cows (Algers and Hultgren,1987; Hultgren, 1989). However, in Chapter VII, cows exposed to EMF have shown a longer estrous cycle. Even though there was not a statistical difference, it can be observed that the area under the progesterone curve of the estrous cycle is numerically greater. In turn, estrous cycle length, in this experiment, is a direct function of the blood progesterone levels, therefore it may be possible that EMF increased or maintained for a longer time the blood progesterone concentration. All the statistical considerations indicate that there is a difference in the estrous cycle length. Probably, if the power of the test for the variable, area under the progesterone curve was improved, the numerical difference shown in this experiment would, probably, become statistically significant. This is in agreement with the fact that blood progesterone levels were higher in pregnant cows exposed to EMF (see Chapter III, Table 2).

Electromagnetic fields have been reported to decrease melatonin synthesis (Wilson **et al.**, 1986, 1990; Lerchl **et al.**, 1991; Rogers **et al.**, 1992; Haggren **et al.**, 1992; Yaga **et al.**, 1993) and also to have no effect on it (Thompson **et al.**, 1991; Sasser **et al.**, 1992; Schiffman **et al.**, 1992; Lee **et al.**, 1993; 1995). In the results

presented here, overall, EMF did not affect the nocturnal peak of melatonin blood concentrations. Nevertheless some ambiguity was detected in the melatonin results. If the melatonin values are separated by replicate, it can be observed that in replicate 2, melatonin had a tendency to decrease when cows were exposed to EMF. This was not observed in replicate 1 (See Chapter IV, Tables 1 and 2). Moreover, blood progesterone concentrations were increased significantly by EMF exposure, and a similar situation to that of melatonin was observed. Statistical analysis of progesterone values by replicate showed that there were not differences due to EMF in the first replicate. However, replicate 2 showed a significant increase in progesterone in plasma (See Chapter III, Table 4), paralleling melatonin in the same replicate. In this experiment, the decrease of plasma melatonin concentration observed during the EMF exposure of the second replicate is coincidental with the increased progesterone concentration obtained in the analogous period. Melatonin is an anti-gonadotropic hormone (Axelrod **et al.**, 1982; Wilson and Anderson, 1989; Silman, 1991) and its decreasing secretion may have resulted in an increased gonadotropin secretion that, in turn, could stimulate the secretion of steroidal hormones.

EMF did not have any significant effect on cortisol plasma levels. This agrees with previously reported work in female lambs (Thompson **et al.**, 1991).

2.0 Productivity

Average daily milk yield was not affected by the exposure of the cows to the EMF. However, milk fat content increased from 4.06 to 4.43 % during the exposure period. This increase in milk fat content during the exposure period corresponded to an increase in dry matter intake observed when the EMF were ON (See Chapter III, Table 1).

Intraventricular injections of noradrenaline in the brain induced anorexia in cattle (Baile **et al.**, 1972). However, no changes in MHPG (metabolite of adrenaline) were found in the CSF of cows exposed to EMF. The role of serotonin in feeding behaviour has been examined in rats by intracranial injections of this neurotransmitter which has been found to inhibit feed intake (Singer **et al,** 1971; Goldman **et al,** 1971). In contrast with the studies in rats, intracranial injections of serotonin dissolved in synthetic CSF in monkeys (Sharpe and Myers, 1969), sheep and cattle (Baile **et al.**, 1979) increased feed intake. The decrease in body temperature observed following intracranial serotonin injections in cattle (Findlay and Thompson, 1968) and sheep (Bligh **et al.**, 1971) could lead to an increased feeding response. If the trend in increased concentrations of tryptophan found in CSF of exposed cows (Chapter VI) resulted in increased serotonin levels in the CSF, this could result in an increased feed consumption. Furthermore, injections of calcium and magnesium into the third ventricle in sheep increased voluntary feed intake (Seoane and Baile, 1973).

In mature cows (Group A in Chapter V) the concentration of Ca increased in CSF, while Mg decreased. Nevertheless, this increase in Ca may have, by itself, affected feed intake. Intraventricular injections of Na or K blocked the intake stimulatory effect of Ca (Seoane and Baile, 1973). Sodium levels decreased in CSF of exposed mature cows. On the other hand, an increase in the length of days has been associated with an increase in voluntary dry matter intake (Dominique **et al**, 1992) body weight and prolactin in sheep (Forbes **et al**, 1979) and in red deer (Suttie and Kay, 1985). Melatonin decreases prolactin levels and, hence, decreases feed intake (Dominique **et al**, 1992). Conversely, immunization against melatonin tended to result in higher plasma prolactin levels in red deer (Ataja **et al**, 1992) and prolactin injections resulted in increases in feed intake and live weight gains in red deer (Ryg and Jacobsen, 1982). Photoperiods of 16 h light and 8 h darkness (16L:8D) increased 10 to 16% the rate of body growth in cattle (Peticlerc, 1983) and cows exposed to 16L:8D produced 3 kg of milk more than cows under natural photoperiod although, the percentage of milk fat was unaffected (Peters **et al**, 1978). In this experiment, the possible decrease in nocturnal MLT peak caused by the exposure to EMF, may have increased the prolactin plasma level leading to an increase in voluntary dry matter intake, in an analogous way to the effect of a longer day, since EMF may resemble light. However, altering the plasma concentrations of MLT
by infusion or feeding MLT did not affect prolactin plasma concentrations in calves (Stanisiewski **et al.**, 1988). Therefore the possibility exists that the diurnal MLT secretion is not a mediator of photoperiod-induced changes in serum prolactin in

cattle.

3.0 Minerals in blood plasma

In mature cows, the decrease in plasma Mg and Fe concentrations that occurred during the exposure period and the fact that the concentrations returned to values similar to those before exposure, 5 days after the EMF were deactivated, suggests an effect of EMF on the concentration of these minerals in plasma. The concentration of Cu also decreased during the exposure period, but its concentrations did not return to values similar to those before exposure after the EMF were deactivated. Although it could also be suggested that EMF may have had an effect on these elements, it should be pointed out that the values were maintained after exposure, suggesting an effect of gestation length. Alternatively, it may be that the period of 5 d after the deactivation of the EMF was not long enough to allow for the concentration in plasma to reach similar values to those before exposure. Concentrations of Mn and Na were not affected by EMF exposure and their values tended to decrease and decreased, respectively, after exposure. This result could be due to gestation length and might not have any relationship with EMF exposure unless there was a residual effect of the EMF. Conversely, in the experiment with non-mature cows (group B in Chapter V) this possible effect on Mn and Na was not detected. However, Ca and Mg showed an opposite situation to that observed in group A. In group A, Ca had a tendency to decrease, and in group B it had a tendency to increase during exposure. Magnesium decreased in group A and tended

to increase in group B . In both groups, after exposure, Ca and Mg returned to concentrations similar to those measured before exposure. In group A, Cu tended to decrease, and decreased, in group B during exposure; in group A, Cu concentrations in plasma decreased further after exposure.

Data from group A (mature cows) and group B (non-mature cows) were combined and analyzed statistically. Copper in plasma decreased during exposure and then remained at the same concentration after exposure. Because Cu did not return to concentrations similar to those measured before exposure, this additional statistical analysis did not support the hypothesis that EMF has an effect on the Cu concentration in blood plasma, unless the EMF effect on Cu levels is permanent or the time of recovery after exposure period precludes Cu concentrations from returning to concentrations that are similar to those measured before exposure. There was a clear association between EMF and Mg with a decrease in Mg in group A; however, in group B there was a tendency for Mg to increase. The combined statistical analysis of groups A and B did not support the hypothesis that EMF have an effect on Mg concentrations in blood plasma (Chapter V, Table 4).

4.0 Minerals in CSF

The values of Ca, Mg and K obtained before exposure in groups A and B agree with previous literature (Fankhauser, 1962; Coles, 1980; Welles **et al**, 1992). In Groups A and B, Ca concentration in the CSF increased during exposure. Calcium

is important in nerve cell function and slight variations in CSF may have some physiological implications (Bawin and Adey, 1976). The increase in the concentration of Ca in the CSF in this experiment might have been due to an increased efflux of Ca from nerve cells, which agrees with the results obtained in vitro using chick brains (Blackman, 1989). Kavaliers and Ossenkopp (1987) have suggested that, when mice are exposed to EMF, the functioning of their Ca channels and the distribution of Ca ions are altered. Marron **et al.**, (1988) have demonstrated that the electric and magnetic components of EMF cause a response in cell membranes, increasing the surface charge density. The increase in the concentration of Ca in the CSF coincides with a tendency towards a decrease in blood plasma Ca. It is possible that the blood-brain barrier might have been altered during exposure allowing more Ca to enter the CSF. Stronger EMF associated with magnetic resonance techniques have been shown to temporarily increase the permeability of the blood-brain barrier in the rat (Shivers **et al.**, 1987). However, the tendency of Ca to decrease in blood plasma could have been caused by an increase in it's excretion, which was not measured in this study. Also, after exposure, concentrations of Ca in blood plasma returned to values similar to those measured before exposure. This result did not occur in the CSF after exposure; therefore, the changes in Ca in the CSF might possibly have been due to an increased secretion of Ca by the choroid plexus and the epithelia associated with CSF production. Alternatively, there might possibly have been an increase in the efflux of Ca from the nerve tissues during the exposure period. Because there is no information in the literature reviewed regarding changes of Ca in the CSF at the end

of gestation in cows, the fact that after exposure, Ca did not return to values similar to those taken before exposure in group A could be related to gestation. This is speculated because, after exposure, the heifers that were not pregnant group B had Ca concentrations that were similar to those measured before exposure. Nevertheless, it could also be suggested that EMF generated an increase in Ca concentrations, but the 5 d between the deactivation of the EMF and the initiation of the measurents after exposure in group A were not long enough to allow CSF Ca to return to values that were similar to those measured before exposure. Disturbances of the blood-brain barrier may cause an increase in CSF Ca, mainly as protein-bound Ca enters into the CSF (Steinberg, 1969).

Inflammatory lesions and other insults to the CNS result in an increase in K and a Mg decline in CSF (Steinberg, 1969). A similar situation for Mg was observed in group A but not in group B during exposure. The insertion of the subarachnoidal catheter might have caused a subclinical infection of the spinal membranes for cows in group A. However, the fact that K did not change in either group during exposure or after exposure and that Mg did not change in group B suggested that a subclinical infection was not present. Conversely, Feldman (1989) claimed that K concentration in the CSF is very stable and that there is no change associated with diseases. Moreover, the results obtained in group C (Chapter V, Table 3) that the insertion of subarachnoidal catheters did not affect mineral concentrations in CSF samples, support the idea that the mineral and neurotransmitter changes observed in groups

A and B are associated with EMF.

In Group A, the 12-fold decreases in Fe and Mn that occurred during the exposure period and the consecutive tendencies to reach values after exposure that were similar to those before exposure were, together with the change in P, the most significant changes attributable to the EMF exposure in this trial. Iron in CSF follows the same pattern of that in blood plasma during exposure to EMF and after exposure. However Mn did not act in a manner similar to Fe. Conversely, in group B Fe tended to decrease, and Mn decreased, in CSF during exposure which was similar to the results obtained in group A. The concentration of Ca, P and Mn in CSF in group B showed the same changes during exposure as those observed during exposure in group A, although the magnitude of the absolute values differred.

Calcium and Mn share a common carrier in the mitochondrial membrane (Shamoo, 1986). If the the same is true for the cell membrane, it may be that the decrease in Mn concentration in the CSF is associated with the increase in Ca concentration in the CSF during exposure. This suggestion is not valid for the period after exposure when Mn tends to return to values similar to those measured before exposure. Calcium maintained higher concentrations after exposure when compared with values measured before exposure.

This study demonstrated that when nonlactating, pregnant cows (Group

A) and nonpregnant, nonlactating, dairy heifers (group B) were exposed to EMF, similar to those under a high power line, some changes in the macro- and trace elements contained in blood plasma and CSF did occur. The relationship between changes in Cu and Zn levels in plasma and CSF and EMF exposure in group A could be questionable because of a consistent tendency of the concentrations to increase or decrease when EMF were deactivated. However, in the case of Mg in blood plasma and Ca, P, Mn, and Fe in CSF, exposure to EMF caused concentration changes in these elements, in spite of the fact that some of the absolute values obtained in group A do not agree with those obtained in group B. These differences could have been caused by the smaller number of heifers that finished group B or the different maturity stage of the heifers in group B. Because group B was conceived in an attempt to support the results obtained in group A, a combined statistical analysis including group A and B was carried out. All the minerals that showed changes that could be attributable to the EMF in groups A and B were analyzed. The Statistical Analysis System (SAS) programs and the SAS output can be seen in Appendix 4. These analyses were performed for Ca, Mg, and Cu in blood plasma and Ca, Mn, and P in CSF (Table 4 Chapter V) and they indicated that there is a 4-fold increase in P concentration in CSF during exposure. However, this effect was more accentuated in group A because the ANOVA did not detect an increase in group B during exposure. Therefore, although there was an effect associated with EMF in groups A and B when the analyses were carried out by trial, when the data were combined, the association between EMF and an increase in P in CSF became

nondetectable in group B. Nevertheless, from the evidence presented, there is a necessity to clarify the effect of EMF on P in CSF. Also it has been shown that Ca increased and Mn decreased in CSF during exposure. This result was detectable when the statistical analysis is performed by group or both groups combined (Chapter V, Table 4).

The variations observed in this study have not been reported previously in dairy cattle; therefore it is difficult to speculate about the physiological implications of such variations, if any. Because the CSF is a very stable fluid of biological substances and because this liquid is in direct contact with the CNS, considerable attention should be dedicated to these variations in minerals in future research.

With regard to the effect of EMF upon the levels of ß-endorphin in the CSF, results obtained in this experiment do not support the suggestion of several authors (Kavaliers and Ossenkopp, 1984; Ossenkopp and Kavaliers, 1987; Teskey **et al.**, 1988; Dowman **et al.**, 1989; Ossenkopp and Kavaliers, 1988-1989) that EMF could in some way change the levels of ß-endorphin secretion, affecting the capacity of this opiate to mitigate pain. On the other hand, these results do not preclude the possibility that EMF, without affecting the secretion of ß-endorphin, may interfere with the capacity of this opiate to increase the threshold for pain. This could be achieved by altering the functionality of the ß-endorphin receptor as suggested by Ossenkopp and Kavaliers (1988-1989). It also must be considered that the EMF used in the above mentioned studies (Kavaliers and Ossenkopp, 1984; Ossenkopp and

Kavaliers, 1987; Teskey **et al.**, 1988; Ossenkopp and Kavaliers, 1988-1989) were of a much higher intensity (0.02-2 T) than the EMF to which the cows of the experiment described here were exposed.

The concentrations of 5-HIAA and HVA in CSF did not change during the EXP period. This does not agree with the results obtained previously in primates exposed to 10 kV/m and 30 μT (Seegal **et al**, 1989) where a 20% decrease in 5-HIAA and HVA was observed when primates were exposed to EMF. On the other hand, in rat and mouse pineal glands, an increase in the concentration of serotonin and 5-HIAA was found when exposed to EMF (Lerchl **et al**, 1991). The authors also found a decrease in NAT, the limiting enzyme for the synthesis of melatonin. This would accumulate serotonin and also increase the activity of monoaminoxidase (MAO) to catabolize the conversion of serotonin to 5-HIAA. The increase in serotonin may also increase the amount of its precursor tryptophan. The concentration of tryptophan tended to be higher during the EXP period in the experiment reported here. However, since serotonin was not measured in the CSF samples, conclusion similar to Lerchl **et al.** (1991) cannot be made. On the contrary, in this experiment the absolute values showed numerical decreases for 5-HIAA and HVA in the CSF. Yao **et al.** (1992) found decreased serotonin in the hippocampus and hypothalamus exposed to electric fields. This may also cause an increase in the concentration of its precursor tryptophan. There is evidence that 5-HIAA in the CSF originates from the serotonergic neurons located in the brainstem and spinal cord (Young and Ervin,

1984) and, similarly, HVA is produced in dopaminergic neurons located in the basal ganglia (Wood, 1980). Therefore, changes in 5-HIAA and HVA in CSF certainly reflect variations in the activity of the CNS. The literature cites decreases in 5-HIAA associated with hyperparathyroidism and an increase in the blood-brain barrier permeability (Joborn **et al**, 1991). However, the blood-brain barrier in rats was not affected by exposure to magnetic resonance imaging at 1.5 Tesla (Prato **et al.** 1992).

IX. GENERAL CONCLUSIONS

From the results obtained in chapters III and IV, it can be concluded that most of the variables associated with health and productivity (bicarbonate, pH, O_2 and CO_2 partial pressures, cortisol and melatonin concentration in blood, non-corrected milk yield, milk components, except milk fat) were unaffected by EMF exposure. An association between EMF exposure and increased estrous cycle length was found in non-pregnant cows. Also a relationship existed between EMF, dry matter intake, 4% fat corrected milk yield, milk fat content and plasma progesterone in pregnant lactating cows.

The experiments to assess the EMF effects on CNS neurotransmitter metabolites and opioid systems provide sufficient information to conclude that EMF caused no change in the concentration of neurotransmitter metabolites in CSF and most of the minerals in plasma and CSF. However, there are some mineral concentrations (Ca, P, and Mn), both in plasma and CSF, that are affected by the EMF exposure.

X. STATEMENTS OF ORIGINALITY

To the best of the author's knowledge, the following information contained in this thesis constitutes an original contribution to the scientific literature:

1. This is the first study which evaluates the effect of electric and magnetic field exposure of dairy cattle under a controlled environment where the electric and magnetic field intensity and light period evaluated are uniform, controllable and resemble EMF under a 735 kV AC power line.

2. This thesis contains the first demonstration that electric and magnetic field exposure increases: (A) dry matter intake,(B) milk fat percentage, (C) four percent fat corrected milk, (D) blood plasma progesterone in pregnant lactating cows and (E) estrous cycle length in non-pregnant lactating cows.

3. In this thesis was demonstrated for the first time that electric and magnetic field exposure increases calcium and phosphorus and decreases manganese concentrations in cerebrospinal fluid in dairy cattle.

4. This thesis contains the first papers related to the evaluation of the effects of electric and magnetic field exposure on blood plasma nocturnal melatonin, minerals, cerebrospinal fluid neurotransmitter metabolites, ß-endorphin and minerals

in dairy cattle.

5. This thesis contains information demonstrating that the installation of subarachnoidal chronic catheterizations are a valuable technique to study the composition of the cerebrospinal fluid.

XI. LITERATURE CITED

Adair, R. K. 1991. Constrains on biological effects of weak extremely-low-frequency electromagnetic fields. Phys. Rev. A. 43:1039:1044.

Adair, R. K. 1995. Limits on the biological effects of very weak magnetic fields through effects on radical formation. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A.

Adey, W.R. 1981. Tissue interactions with nonionizing electromagnetic fields. Physiol Rev 61:435-514.

Algers, B., and J. Hultgren. 1987. Effects of long-term exposure to 400-kV, 50 Hz transmission line on estrous and fertility in cows. Preventive Veterinary Medicine 5:21-36.

Algers, B., and K. Hennichs. 1985. The effects of exposure to 400 kV transmission lines on the fertility of cows. A retrospective cohort study. Preventive Veterinary Medicine 3:352-361.

Algers, B., I. Ekesbo, K. Hennichs. 1981. The effects of ultra high-voltage

transmission lines on the fertility of dairy cows. A preliminary study. Swedish University of agricultural sciences. Department of Animal Hygiene with Farrier school, Skara, Sweden. Report #5.

Amstutz, H. E., D. B. Miller. 1980. A study of farm animals near 765 kV transmission lines. Bovine practicioner 15:51-62.

Anderson, G. M., J. G. Young, D. J. Cohen. 1979. Rapid liquid chromatographic determination of tryptophan, tyrosine, 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid. J. Chromatography 164:501-505.

AOAC .(1980). Official methods of analysis (13th edition). Association of official analytical chemists, Washington, DC.

Ataja, A. M., P. R. Wilson, T. N. Barry, J. Hodgson, R. M. Hoskinson, W. J. Parker, R. W. Purchas. 1992. Early venison production from red deer (Cervus elaphus) as affected by grazing perenial or annual ryegrass pastures, pasture surface height and immunization against melatonin. J. Agric. Sci. 118:352-369.

Axelrod, J. F. Fraschini, G. P. Velo (eds). 1982. The pineal gland and its neuro-endocrine role. Plenum Press, New York

Axelrod,J., R.J. Wurtman, S.H. Snyder. 1965. Control of hydroxyindole-O-methyl tranferase activity in the rat pineal gland by environmental lighting. J. Biol. Chem. 240:949-954.

Baile, C. A.,C. W. Simpson, L. F. Krabill, F. H. Martin. 1972. Adrenergic agonist and antagonists and feeding in sheep and cattle. Life Science 11:661-668.

Baile, C. A.,J. S. Beyea, L. F. Krabill, M. A. Della Fera. 1979. Intracranial injections of 5-HT and db cAMP and feeding ion sheep and cattle. J. Anim. Sci. 49:1076-1084.

Bawin, S. M., W. R. Adey. 1978. Sensitivity of Calcium binding in cerebral tissue to weak environmental electric field oscillating at low frequency. Proc. Natl. Acad. Sci. USA. 73:1999-2003.

Basset, C. A. L. 1989. Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields. Crit. Rev Biomed. Eng. 17:451-529.

Bawin, S. M., W. R. Adey. 1976. Sensitivity of calcium binding in cerebral tissue to weak environmental electric field oscillating at low frequency. Proc. Natl. Acad. Sci. USA. 73:1999-2003.

Blackman, C. F. ELF effects on calcium homeostasis. 1989. In: Wilson B W, R G

Stevens, L E Anderson (eds) Extremely low frequency electromagnetic fields: The question of cancer. Battelle Press, Columbus Ohio, pp 187-208.

Bligh. J, W. H. Cole, M. Maskrey. 1971. Influence of ambient temperature on the thermoregulatory responses of 5-hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerbral ventricles of sheep, goats and rabbits. J. Physiol. 212:377-388.

Borgens, R.B., J.W. Vanable, L.F. Jaffe. 1977. Bioelectricity and regeneration: Large currents leave the stumps of regenerating new limbs. Proc. Natl. Acad. Sci. (USA) 74:4528-4532.

Bowers, C.W., C. Baldwin, R.E. Zigmond. 1984. Sympathetic reinnervation of the pineal gland after postganglionic nerve lesions does not restore normal pineal function. J. Neurosci. 4:2010-2015.

Blask, D. E., D. P. Pelletier, S. M. Hill, W. A. Lemus, D. S. Grosso, S. T. Wilson, M. E. Wise. 1991). Pineal melatonin inhibition of tumour promotion in the N-nitroso-N-methylurea model of mammary carcinogenesis: Potential involvement of antiestrogenic mechanism in vivo. J of Cancer Res and Clin Onc 117:526-532

Burchard, J.F., D.H. Nguyen, L. Richard, E. Block. 1995. Biological effects of 60 Hz

electric and magnetic fields (EMF) on productivity of dairy cattle. J. Dairy Sci. 79: (Submitted)

Busby, K., D. Driscoll, W.E. Washbon. 1974. A field survey of farmer experience with 765 kV transmission lines. Agricultural Resources Commission, State of New York, Albany, New york, report.

Brugère, H., F. Pupin, J. Lambrozo. 1995. Effects of short term exposure to 50 Hz EMFs on plasma zinc and copper in rats. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A. pp 112.

Chance, W.T., C.J. Grossman, R. Newrock, G. Bovin, S. Yerian, G. Schmitt. 1995. Effects of electromagnetic fields and gender on neurotransmitters and aminoacids in rats. Physiology & Behavior. 58(4):743-748 .

Chiang, H.,G.D. Yao, Q.S. Tang, D.Z. Lu and Y.K. Zhou. 1989. Health effects of environmental electromagnetic fields. J. Bioelectr. 8:127-131.

Cochran, W. G., G. M. Cox. 1957. Experimental designs. John Williams and Sons, New York, pp 141-142.

Coles, H. E. 1980. Cerebrospinal fluid. In: Kaneko J. J.(ed) Veterinary Clinical Pathology. Saunders Philadelphia, Pennsylvania, pp 267-278.

Cossarizza, A., D. Monti, F. Bersani, M. Cantini, R. Cadossi, A. Sachi, C. Franceschi. 1989). Extremely low-frequency pulsed electromagnetic fields increase cell proliferation in lymphocytes from young and aged subjects. Biochem. Biophys. Res. Acta 1009:216-220.

Coulton, L. A., A. T. Barker. 1993. Magnetic fields and intracellular calcium: effects on lymphocytes exposed to conditions for 'cyclotron resonance'. Physics in Medicine & Biology. 38:347-60

Cremer-Bartels, G.K. Krause, G. Mitoskas, D. Brodersen. 1984. Magnetic field of the earth as additional Zeitgeber for endogenous rhythms? Naturwissenschaften 71:567-574.

Demein, C., P. Semm. 1985. The avian pineal gland as an independent magnetic sensor. Neurosci Lett. 62:119-122

Dominique, B. M. F., P. R. Wilson, D. W. Dellow, T. N. Barry. 1992. Effects of subcutaneous melatonin implants during long daylength on voluntary feed intake, rumen capacity and heart rate of red deer (Cervus elaphus) fed on a forage diet.

British J. Nutr. 68:77-88.

Dowman, R.,J.R. Wolpaw, R.F. Seegal, and S. Satya-Murti. 1989. Chronic exposure of primates to 60 Hz electric and magnetic fields. III. Neurophysiological effects. Bioelectromagnetism 10:303-317.

Eichwald, C., F. Kaiser. 1995. Model for external influences on cellular signal transduction pathways including cytosolic calcium oscillations. Bioelectromagnetics. 16:75-85

Fankhauser, R. 1962. Chapter III In: Innes R M and L Z Sauders (eds) Comparative Neuropathology, Academic press, New York pp 21

Feldman, B. F. 1989. Cerebrospinal fluid. In: Kaneko J J (ed) Veterinary clinical biochemistry, Academic Press, San Diego California, pp 835-865

Findlay, J. D., G. E. Thompson. 1968. The effects of intraventricular injections of noradrenaline 5-hydroxytryptamine, acetylcholine and tranylcypromine on the ox at different environmental temperatures. J. Physiol. 194:809-815.

Forbes, D. M., P. M. Driver, W. B. Brown, G. G. Scannes, I. C. Hart. 1979. The effect of daylength on the growth of lambs. 2. Blood concentrations of growth

hormone prolactin, insulin and thyroxine, and the effect of feeding. Animal Production 29:43-51.

Fraser, S., P. Cowen, M. Franklin, C. Franey, and J. Arendt. 1983. Direct radioimmunoassay for melatonin in plasma. Clin. Chem. 29:396-397.

Goldman, H. W., D. Lehr, E. Friedman. 1971. Antagobistic effects of alpha and beta adrenergically codded hypothalamic neurons on consummatory behaviour in the rat. Nature 231:453-456.

Goodman, R., L. X. Wei, J. C. Xu, A. Henderson. 1989. Exposure of human cells to low frequency electromagnetic fields results in quantitative changes in transcripts. Biochem. Biophys. Acta 1009:216-220.

Graham, C., M. R. Cook, D. W. Riffle. 1995. Human melatonin in 60 Hz magnetic fields: continuous versus intermittent exposure. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A. pp 48.

Haggren, W., S. M. Yellon, J. L. Phillips, W. R. Adey. 1992. Effect of magnetic field exposure in adult djungarian hamsters on melatonin rhythms and molecular markers. The first world congress for electricity and magnetism in biology and medicine. Lake

Buena Vista Florida. pp 105.

Hojevik, P., J. Sandblom, S. Galt, Y. Hamnerius. 1995. Ca2+ ion transport through patch-clamped cells exposed to magnetic fields. Bioelectromagnetics. 16:33-40

Howell, D. M., E. Martz. 1988. Low calcium concentration support killing by some but not all cytotoxic T lymphocytes, and reveal inhibition of a postconjugation step by calcium antagonists. J. Immunol. 140:1982-1988.

Hultgren, J. 1989). Cows and electricity study, biological interaction and effects on cow behaviour, health and reproduction of stray voltage, electric cow-trainers and high voltage transmission lines. Swedish University of agricultural sciences. Department of Animal Hygiene with Farrier school, Skara, Sweden. Report #27.

Jairath, L. K., J. F. Hayes, R. I. Cue. 1995. Correlations between first lactation and lifetime performance tratits of canadian holsteins. J. Dairy Sci. 78:438-448.

Jimerson, D. C., J. H. Wood, R. M. Post. 1980. Cerebrospinal fluid calcium In: Wood J H (ed) Neurobiology of cerebrospinal fluid Plenum press New york pp 743-749

Joborn, C., J. Hetta, F. Niklasson, J. Rastad, L. Wide, H. Agren, G. Akerstrom, S. Ljunghall 1991. Cerebrospinal fluid calcium, parathyroid hormone, and monoamine

and purine metabolites and the blood-brain barrier function in primary hyperparathyroidism. Psychoneuroendocrinology 16:311-322.

Kato, M., K. Honma, T. Shigemitsu, Y. Shiga. 1994. Circularly polarized 50-Hz magnetic field exposure reduces pineal gland and blood MLT concentrations of Long-Evans rats. Neuroscience Letters 166:59-62.

Kaune, W.T. and Gillis, M.F., 1981. General properties of the interaction between animals and ELF electric fields. Bioelectromagnetics, 2:1-11.

Kaune, W.T. and Forsythe, W.C., 1988. Current densities induced in swine and rat models power-frequency electric fields. Bioelectromagnetics 9:1-24.

Kavaliers, M., M. Hirst, G.C. Teskey. 1983. Aging, opioid analgesia and the pineal gland. Life Sci 32:2279-2287.

Kavaliers, M., and K.P. Ossenkopp. 1984. Magnetic fields abolish the enhanced nocturnal analgesic response to morphine in mice. Physiology & Behaviour 32:261-264.

Kavaliers, M., and K.P. Ossenkopp. 1986. Magnetic field inhibition of morphine-induced analgesia and behavioral activity in mice. Evidence for involvement

of calcium ions. Brain Res. 379:30-38.

Kavaliers, M., K. P. Ossenkopp. 1987. Calcium channel involvement in magnetic field inhibition of morphine-induced analgesia. Arch Pharmacol 336:308-315.

Krause, K., G. Cremer-Bartels, G. Mitoskas. 1985. Effects of low magnetic field on human and avian retina. In Brown G.M., Wainwright S.D. (eds): "The Pineal Gland: Endocrine aspects." Ontario: Pergamon Press.

Lee, J. M. Jr., F. Stormshak, J. Thompson, P. Thinesen, L. Painter, B. Olencheck, D. Hess, R. Forbes. 1992. Endocrine responses of ewe lambs exposed to 60-Hz electric and magnetic fields from a 500-kV transmission line. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 30

Lee, J. M. Jr, F. Stormshak, J. M. Thompson, D. L. Hess, D. L. Foster. 1995. Melatonin and puberty in female lambs exposed to EMF: A replicate study. Bioelectromagnetics 16:119-123.

Lee, J. M. Jr, F. Stormshak, J. M. Thompson, P. Thinesen, L. J. Painter, E. G. Olencheck, D. L. Hess, R. Forbes, D. L. Foster. 1993. Melatonin secretion and

puberty in female lambs exposed to environmental electric and magnetic fields. Biol. Reprod. 49:857-864.

Lee, J. M. Jr, V. L. Chartier, D. P. Hartmann, G. E. Lee, K. S. Pierce, F. L. Shon, R. D. Stearns, M. T. Zeckmeister. 1989. Electrical and biological effects of transmission lines: a Review. U.S. Department of Energy Bonneville Power Administration, Portland, Oregon.

Lerchl, A., K. O. Nonaka, K. A. Stokkan, R. J. Reiter. 1990. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. Biochem. Biophys. Res. Com. 169:102-108.

Lerchl, A., R. J. Reiter, K. A. Howes, K. O. Nonaka, K. A. Stokkan. 1991. Evidence that extremely frequency calcium ion cyclotron resonance depresses pineal melatonin synthesis **in vitro.** Neurosci lett 124:213-215

Lindstrom, E., P. Lindstrom, A. Berglund, E. Lundgren, K. H. Mild. 1995. Intracellular calcium oscillations in a T-cell line after exposure to extremely-low-frequency magnetic fields with variable frequencies and flux densities. Bioelectromagnetics. 16:41-7

Lissoni, P., S. Barni, G. Cattaneo, G. Tancini, G. Esposti, D. Esposti, F. Fraschini.

1991. Clinical results with the pineal hormone melatonin in advanced cancer resistant to standard antitumor therapies. Oncology (Basel) 48:448-450.

Lovsund, P., P.A. Oberg, S. G. E. Nilsson, T. Reuter. 1980. Magnetosphosphenes: a quantitative analysis of thresholds. Med. Biol. Engng. Comput. 18:326:334.

Loscher, W., M. Mevissen. 1994). Animal studies on the role of 50/60-Hertz magnetic fields in carcinogenesis. Life Sciences. 54(21):1531-43

Loscher, W., U. Wahnschaffe, M. Mevissen, A. Lerchl, A. Stamm. 1994). Effects of weak alternating magnetic fields on nocturnal melatonin production and mammary carcinogenesis in rats. Oncology. 51:288-95

Maestroni, J. M., A. Conti, W. Pierpaoli. 1989. Melatonin, Stress and the immune system. Pineal Res Rev 7:203-226

Marron, M. T., E. M. Goodman, P. T. Sharpe, B. Greenebaum. 1988. Low frequency electric and magnetic fields have different effects on the cell surface, FEBS Lett. 230:13-16

Martin, F. B., A. Bender, G. Steuernagel, R. A. Robinson, R. Revsbech, D. K. Sorensen, N. Williamson, A. Williams. 1986. Epidemiological study of Holstein dairy

cow performance and reproduction near a high-voltage direct-current powerline. J. Toxicol. Environ. Health. 19:303-324.

Martinez Soriano, F., M. Gimenez Gonzalez, E. Armanazas, T. Ruiz. 1992. A Pineal "synaptic ribbons" and serum melatonin levels in the rat following the pulse action of 52-Gs (50-Hz) magnetic fields: An evolutive analysis over 21 days. Acta Anatomica 143:289-293.

Milin, J., M. Bajic, and V. Brakus. 1988. Morphodynamic reactive response of the pineal gland of rats chronically exposed to stable strong magnetic fields. Neuroscience 26:

Nguyen D.H., L. Richard, J.F. Burchard and E. Block. 1995. An electric and magnetic field exposure chamber for studies of biological effects on dairy cows. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A. pp 115.

NRC (1988): Nutrient requirements of dairy cattle. National Academy Press Washington DC

Olcese, J., and E. Hurlbut. 1989. Comparative studies on the retinal dopamine

response to altered magnetic fields in rodents. Brain Res. 498:145-148.

Ossenkopp, K., M. Kavaliers. 1987. Morphine-induced analgesia and exposure to low-intensity 60-Hz magnetic fields: inhibition of nocturnal analgesia in mice is a function of magnetic field intensity. Brain Res. 418:356-360.

Ossenkopp, K., M. Kavaliers. 1988-1989. Clinical and applied aspects of magnetic field exposure: A possible role for the endogenous opioid system (review). J. Bioelectr 7:189-208.

Peters, R. R., L. T. Chapin, K. B. leining, H. A. Tucker (1978): Supplemental lighting stimulates growth and lactation in cattle. Science 199:911-912.

Petitclerc, D., L. T. Chapin, R. S. Emery, H. A. Tucker. 1983. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in holstein heifers. J. Anim. Sci. 57:892-898.

Pickard, W.T. (1988). A model for the acute elctrosensitivity in cartilagenous fishes. IEEE Trans. BME-35:243-249.

Prato, F. S., R. H. Frappier, J. M. Wills, R. R. Shivers. 1992. Blood-Brain barrier permeability in rats is not altered by exposure to to magnetic resonance imaging at

1.5 tesla. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 29.

Qouck, R.M., F.J. Kouchich, T.K. Ishii, D.G. Lange. 1986. Microwave facilitation of methylnaltrexone antagonism of morphine-induced analgesia in mice. 1986. J. Bioelectr 5:35-46.

Raleigh, R.J. 1988. Joint HVDC agricultural study. Oregon State University, Boneville Power Administration Portland, Oregon. Report.

Reif, J. S, J. B. Burch, C. A. Pitrat, T. J. Keefe, M. G. Yost. 1995. Melatonin Levels in electric utility workers. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A. pp 48.

Reiter, R. J., K. Yaga, L. C. Manchester, J. S. Sun, L. D. Chen, M. Abdolsalami. 1992. Parametric and mechanistic studies on the perturbation of the circadian melatonin rhythm by magnetic field exposure. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 5

Richardson, B. A., K. Yaga, R. J. Reiter, D.J. Morton. 1992. Pulsed static magnetic field effects on in vitro pineal indoleamine metabolism. Biochim. Biophys. Acta

1137:59-64.

Rogers W R, R J Reiter, J L Orr (1992): 60-Hz electeric and magnetic fields and primate melatonin. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 29

Rosenthal, G., and G. Obe. 1989. Effects of 50-Hertz electromagnetic fields in proliferation and on chromosomal alterations in human peripheral lymphocytes untreated or pretreated with chemical mutagens. Mut Res 210:329-335.

Ryg, M., E. Jacobsen. 1982. Effects of thyroid hormones and prolactin on food intake and weight changes in young male reindeer (Rangifer tarandus tarandus). Can. J. Zool. 60:1562-1567.

Sasser, L. B., J. E. Morris, D. L. Miller, L. E. Anderson. 1992. Effect of acute exposure to magnetic fields on pineal function in the rat. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 28

Savitz, D. A., H. Wachtel, F. A. Barnes, E. M. Jhon, J. G. Tvrdik. 1988. Case-control study of the chilhood cancer and exposure to 60-Hz magnetic fields. Am. J. Epidemiol. 128:21-38.

Schiffman, J. S., H. M. Lasch, M. D. Rollag, A. E. Flanders, G. C. Brainard, D. L. Jr Burk. 1994. Effect of MR imaging on the normal human pineal body: measurement of plasma melatonin levels. J. Magnetic Resonance Imaging. 4:7-11

Schiffman, J. S., H. M. Lasch, D. M. Rollag, A. E. Flanders, G. C. Brainard, D. L. Burk. 1992. Effect of magnetic resonance imaging (MRI) on plasma melatonin in normal humans. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista, Florida. pp 74.

Sears, F. W., M. W. Zemansky, H. D. Young. 1987. Electrodynamics In: Sears F W, M W Zemansky, H D Young (eds). University Physics 7th edition. pp 681.

Seegal, R.F., J.R., Wolpaw, and R. Dowman. 1989. Chronic exposure to 60-Hz electric and magnetic fields: II Neurochemical effects. Bioelectromagnetics 10:289-301.

Selmaoui, B., Y. Touitou. 1995. Sinosoidal 50-Hz magnetic fields depress rat pineal NAT activity and serum melatonin - Role of duration and intensity of exposure. Life Sciences. 57:1351-1358.

Semm, P. 1983. Neurobiological investigations on the magnetic sensitivity of the pineal gland in rodents and pigeons. Comp Biochem Physiol 76A:683-689.

Semm, P., T. Schneider, L. Wollrath. 1980. Effects of an earth-strength magnetic field on electrical activity of pineal cells. Nature: 288:607-608.

Seoane, J. R., C. A. Baile. 1973. Feeding elicited by injections of Ca and Mg into the third ventricle of Sheep. Experientia 29:61-62

Shah, P. N., M. C. Mhatre, L.S. Kothari. 1984. Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rats in varying photoperiods. Cancer Res 44:3403-3407.

Shamoo, A. E. 1986. Mn(II) and Ca(II) transport in mitochondria In: Schramm V L, F C Wedler (eds) Manganese in metabolism and enzyme function. Academic press Orlando Florida pp-65-80

Sharpe, L. G., R. D. Myers. 1969. Feeding and drinking following stimulation of the diencephalon of the monkey with amines and other substances. Exp. Brain Res. 8:295-301.

Shivers, R. R., M. Kavaliers, G. C. Teskey, F. S. Prato, R. M. Pelletier. 1987. Magnetic resonance imaging temporarily alters blood barrier permeability in the rat. Neurosci lett 776: 21-31.

Silman, R. 1991. Melatonin and the human gonadotropin-releasing hormone pulse generator. J. Endocrinol. 128:7-11.

Singer, G., I. Sanghvi, S. Gershon. 1971. Exploration of certain behaviourial patterns induced by psychoactive agents in the rat. Comm. Behav. Biol. 6:307-313.

Stagg, R. B., P. T. Hardy, A. MacMurray, W. D. Adey. 1992. Electric an magnetic field interactions with microsomal membranes: a novel system for studying calcium flux across membranes. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 12

Stanisiewski, E. P., L. T. Chapin, N. K. Ames, S. A. Zinn, H. A. Tucker. 1988. Melatonin and prolactin concentrations in blood of cattle exposed to 8, 16, or 24 hours of daily light. J. Anim. Sci. 66:727-734.

Steel, R. G. D., J. H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill.pp 363-372.

Steinberg, S. A. 1969. Cerebrospinal fluid In: Medway W, J E Prier, J S Wilkinson (eds) Veterinary Clinical Pathology The Williams and Wilkins Co. Baltimore pp 166-180.

Suttie, J. M., R. N. B. Kay. 1985. Influence of plane of winter nutrition on plasma

concentrations of prolactin and testosterone and their association with voluntary feed intake in red deer stags (Cervus elaphus). Anim. Repr. Sci. 8:247-258.

Tenforde, T.S.,1989. Electroreception and magnetoreception in simple and complex organisms. Bioelectromagnetics, 10:215-221.

Teskey G C, F S Prato, K-P Ossenkopp, M Kavaliers (1988): Exposureto time varying magnetic fields associated with magnetic resonance imaging reduces fentanyl-induced analgesia in mice. Bioelectromagnetics 9:167-174.

Thomas, W. J., L. J. Kinne, R. A. Jones, W. R. Adey. 1995. Carbachol (100 μM) induced intracellular calcium responce in PC12 cells exposed to a 1 gauss magnetic field at frequencies of 1, 160 and 200 Hz. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A.

Thompson, J. M., F. Stormshak, L. J. Painter, P. K. Thinesen, J. Corvallis, M. Lee, D. L. Hess. 1991. Endocrine responses of ewe lambs exposed to a 500 kV a-c transmission line. J An Sci 69(suppl 1):229-230

Vasquez, B.J., L.E. Anderson, C.I. Lowery, and W.R. Adey. 1988. Diurnal patterns in brain biogenic amines of rats exposed to 60 Hz electric fields. Bioelectromagnetics 9:229-236. @39

Vogt, M. 1975. Metabolites of cerebral transmitters entering the cerebrospinal fluid; their value as indicators of brain function In: Cserr H F, J D Fenstermacher, V Fencl (eds). The fluid environment of the brain. Academic press New York pp 225-236.

Walleczek, J., R. P. Liburdy. 1990. Combined DC/CA Magnetic fields alter Ca^{+2} metabolism in activated rat thymocytes. 12th Annual meeting of the Bioelectromagnetics Society June 1990 San Antonio Texas (Abstract E-3-2)

Walleczek, J. 1992. Electromagnetic field effects on cells of the immune system: the role of calcium signalling. FASEB J. 6:3177-3185.

Wallezec, J., P. L. Miller, W. R. Adey. 1992. simultaneous dual-sample fluorometric detection of real time-effects of ELF electromagnetic fields on cytosolic free calcium and divalent cation flux in human leukemic T-cells (JURKAT). The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 12.

Ware, B. J. 1974. Effect of 765-kV transmission lines on animal grazing habits. American electric power service corporation. New York.

Weaver, J.C., R.D. Astumian. 1990. The response of living cells to very weak electric fields: the thermal noise limit. Science 247:459-462.

Weiss, A., J. B. Imboden. 1987. Cell surfaces molecules and early events involved in human T lymphocytes activation. Adv. Immunol. 41:1-38

Welker, H.A., P. Semm, R.P. Willig, J.C. Commentz, W. Wiltschko, L. Wollrath. 1983. Effects of an artificial magnetic field on serotonin N-actetyltransferase activity and melatonin content of the rat pineal gland. Experimental Brain Research 50:426-432.

Welles, E. G., J. W. Tyler, D. C. Sorjonen, E. M. Whatley. 1992. Composition an analysis of cerebrospinal fluid in clinically normal adult cattle. Am. J. Vet. Res. 53:2050-2057.

Wertheimer, N., E. Leeper. 1979. Electrical wiring configurations and childhood cancer. Am J. Epidemiol. 109:273-284

Wertheimer N., E. Leeper. (1986): Possible effects of electric blankets and heated water beds on fetal development. Bioelectromagnetics 7:13-22.

Williams, J.H., and J.E. Beiler. 1979. An investigation of dairy farm operations in association with 765 765-kV transmission in Ohio. Ohio Power Siting Commission and Cleveland Electric Illuminating Company. Cleveland, Ohio, Report.

Wilson, B.W., L.E. Anderson, D.I. Hilton, R.D. Phillips. 1981. Chronic exposure to 60-Hz electric fields: Effects on pineal function in the rat. Bioelectromagnetics 17:371-380.

Wilson, B.W., 1988. Chronic exposure to ELF may induce depression. Biolectromagnetics. 9:195-205.

Wilson, B. R., L. E. Anderson. 1989. ELF Electromagnetic field effect on the pineal gland In: Wilson B W, R G Stevens, L E Anderson (eds) Extremely low frequency electromagnetic fields: The question of cancer. Battelle Press, Columbus Ohio, pp 157-186.

Wilson, B. W., E. K. Chess, L. E. Anderson. 1986. 60Hz Electric-field effects on pineal melatonin Rhythms: Time course for onset and recovery. Bioelectromagnetics 7:239-242

Wilson, B. W., C. W. Wright, J. E. Morris, R. L. Buschbom, D. P. Brown, D. L. Miller, F. R. Sommers, L. E. Anderson. 1990. Evidence for an effect of ELF electromagnetic fields on human pineal gland function. J Pineal Res 9:259-270

Winters, W. D., V. Schirf, N. Hagino. 1992, Influence of cyclic, rotating magnetic fields of extremely low ferquency on Na+/K+ transport in rat embrionic neuronal

cells. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. pp 37.

Wolpaw, J.R., R.F Seegal, and R. Dowman. 1989. Chronic exposure of primates to 60-Hz electric and magnetic fields: I Exposure system and measurements of general health and performance. Bioelectromagnetics 10:277-288.

Wood, A. W. 1993. Possible health effects of 50/60Hz electric and magnetic fields: review of proposed mechanisms. Australian Physical & Engineering Sciences in Medicine. 16(1):1-21

Wood, J. H. 1980. Sites of origin and cerebrospinal fluid concentration gradients:Neurotransmitters, their precursors and metabolites and cyclic nucleotides. In Wood JH (ed): Neurobiology of cerebrospinal fluid. Vol 1. New York:Plenum Press, pp. 53-62.

Yaga K, R J Reiter, L C Manchester, H Nieves, J H Sun, L D Chen (1993): Pineal sensitivity of pulsed magnetic field changes during the photoperiod. Brain Res. Bill. 30:153-156.

Yao, G. D., M. Z. Lou, Y. D. Fu, Q. D. Lu. 1992. Effects of 50-Hz electric fields on monoamine neurochemestry and learning behaviour in rats. The first world congress for electricity and magnetism in biology and medicine. Lake Buena Vista Florida. p

118.

Yellon, S. M. 1994. Acute 60 Hz magnetic field exposure effects on the melatonin rhythm in the pineal gland and circulation of the adult Djungarian hamster. Journal of Pineal Research 16: 136-144

Young, S. N., F. R. Ervin. 1984. Cerebrospinal fluid measurements suggest precursor availability and sex are involved in the control of biogenic amine metabolism in a primate. J. Neurochem. 42:1570-1573.

Zecca L, P Cerretelli, E Raschi, C Mantegazza. 1995. Serotonin system in brain cortex is affected by exposure to 50 Hz electromagnetic fields. The annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity. November 12-16, 1995. Palm Springs, Ca. U.S.A. pp 45.

XI. APPENDICES

Appendix 1: Exposure chamber design and Electric and magnetic field mapping

Electromagnetic Field Exposure Chamber for Studies on Biological Effects on Dairy Cattle.

Designed by D.H. Nguyen and L. Richard Institut de recherche d'Hydro-Québec (IREQ)

1. The exposure chamber

The electric and magnetic field (EMF) exposure chamber designed by Hydro Québec is constructed in the metabolism room at the Cattle Teaching and Research Complex on the Macdonald Campus of McGill University.

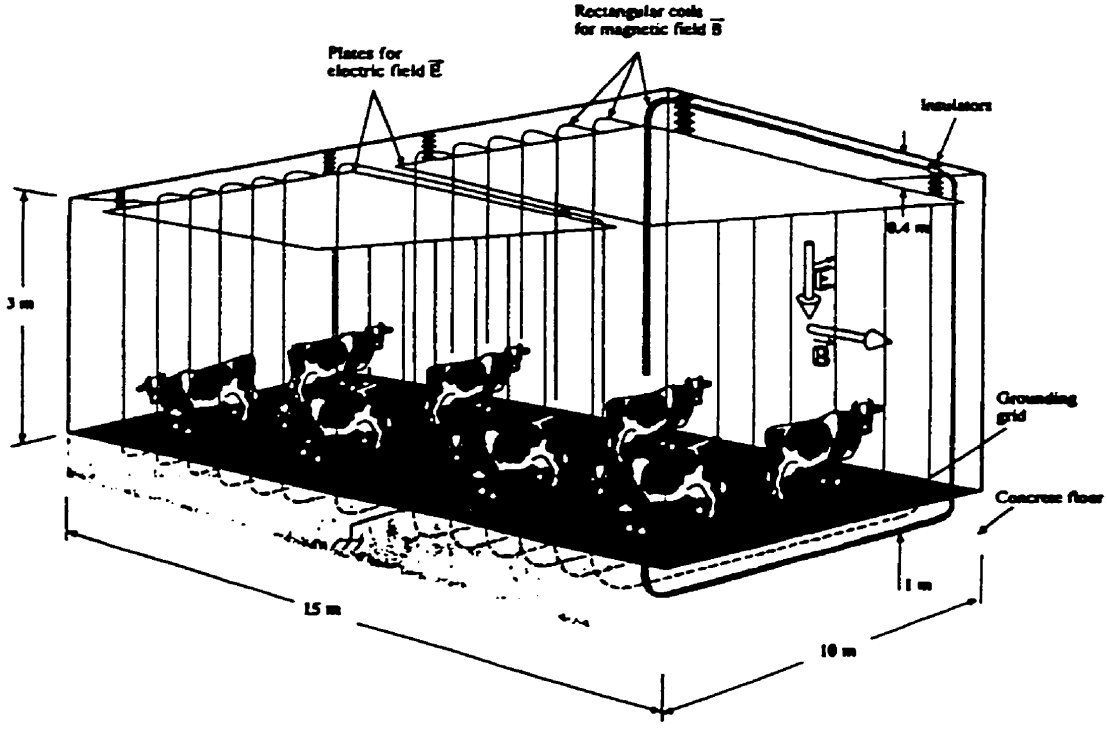


Figure 1. Electric and Magnetic Exposure Chamber

The chamber is 15 m long, 10 m wide by 3 m high (Figure 6). It contains eight wooden stalls, each holding one cow, and is designed to allow simultaneous exposure of eight animals during long uninterrupted periods. Wooden (rather than metal) box stalls are used to minimize disturbance of the electric field. The chamber is ventilated and heated artificially and the light and dark cycle is divided into two equal phases of 12 h.

2. EMF intensity of Hydro-Quebec 735-kV lines

For these studies the chamber is operated in such a way to generate the maximum value of the EMF prevailing directly underneath a 735 kV AC line at ground level. In normal operation, the maximum electric field is constant at a given point, whereas, the amplitude of the magnetic field, which is a function of the current, varies contnuously with the power demand. The EMF distribution at ground level and beneath this line, together with typical line energy flows coming down from James Bay, are shown in Figures 2 and 3. These Statistics are compiled from data records of three 735 kV lines.

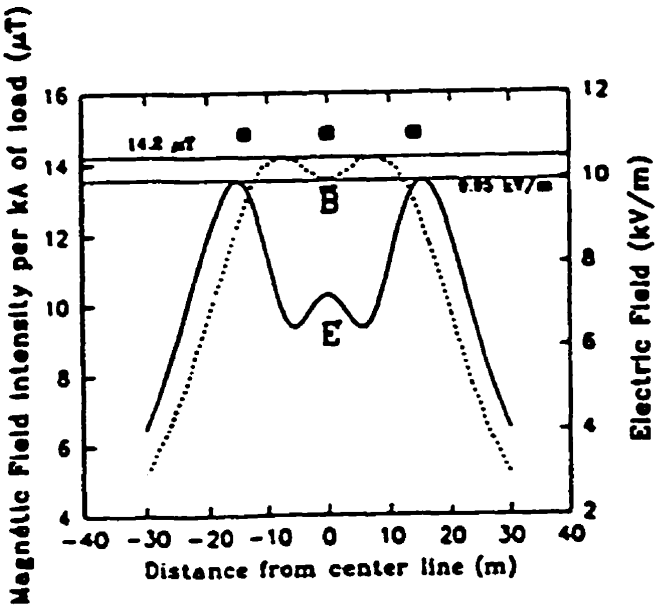


Figure 2. EMF distribution beneath of a Hydro Quebec 735-kV line at ground level.

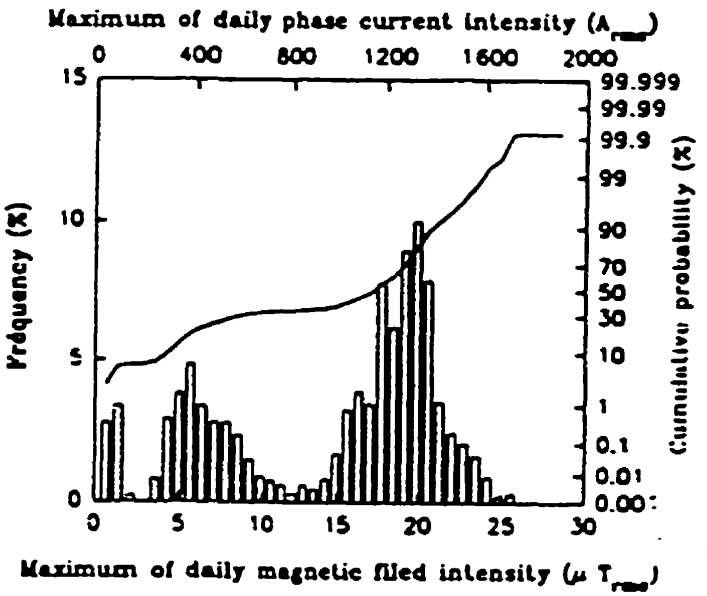


Figure 3. Daily magnetic field intensity of a typical 735 kV line

3. EMF generation in the exposure chamber

A magnetic field of 30 μT is generated by 14 rectangular coils, 10 m long and 4 m height. To ensure field uniformity (Figures 4 and 5), a section of the coils is embedded in the ground (Figure 1). An undistorted uniform electric field of 10 kV/m is generated by two plates, each 9 m long and 6.5 m wide, which are suspended from the ceiling by synthetic insulators (Figure 1). A view of the livestock inside the chamber is presented in Figure 1.

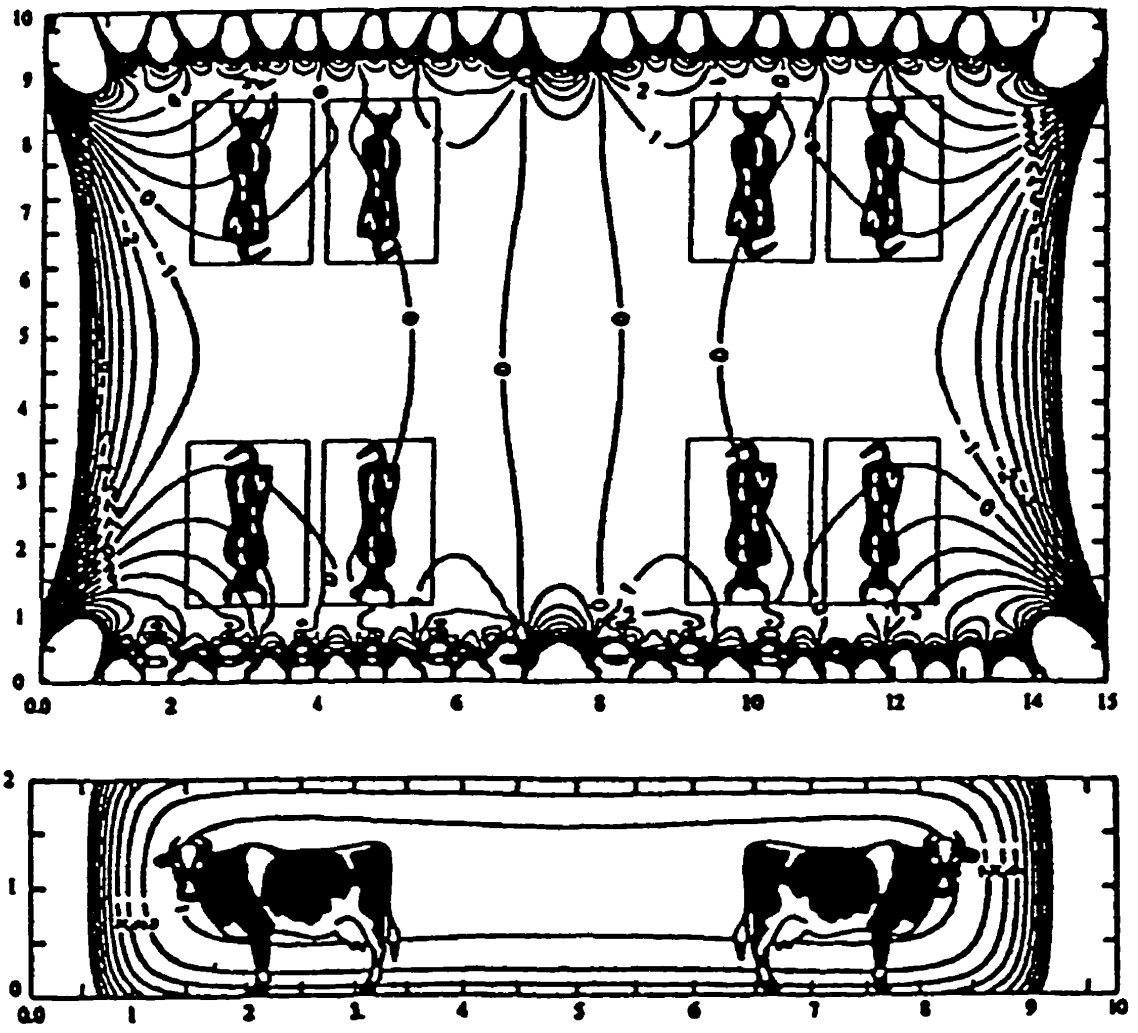


Figure 4. Magnetic field distribution in the EMF Chamber in terms of deviation (%) from 30 μT.

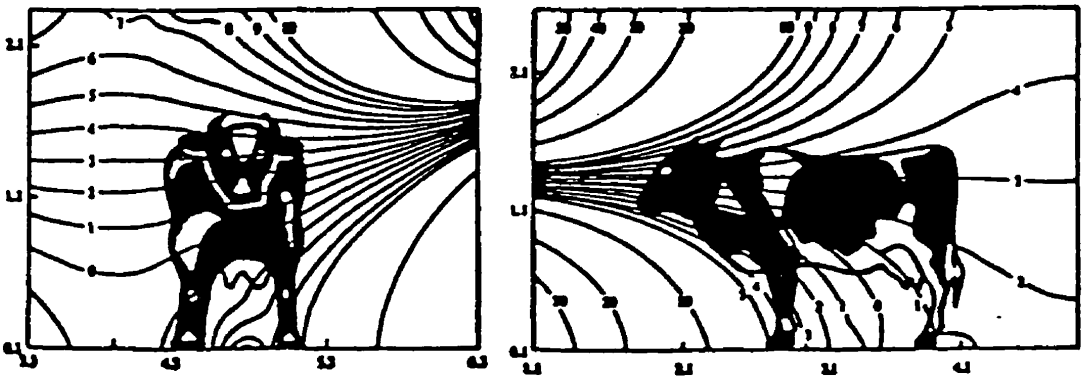


Figure 5. Undistorted electric field in terms of deviation from 10 kV/m.

4. Control and safety systems

The control system is fully computerized so that the field intensities can be altered and monitored continuously. The telephone link also allows remote monitoring. The components are shown in Figure 7. The system is equipped with integrated safety devices which automatically cut off the power and ground the plates when someone enters the room accidentally.

5. Chamber characteristics

electric field:	O-30 kV/m
magnetic field:	0-100 μT
frequency:	45-45,000 Hz
harmonic distortion:	0.1 %
Ozone level (at 30 kV/m)	less than 0.01 ppm
ambient electric field:	2.5 V/m
ambient magnetic field:	0.15 μT
maximum output:	3 kVA

The details of the exposure and control systems are presented in Nguyen **et al.**, 1989,

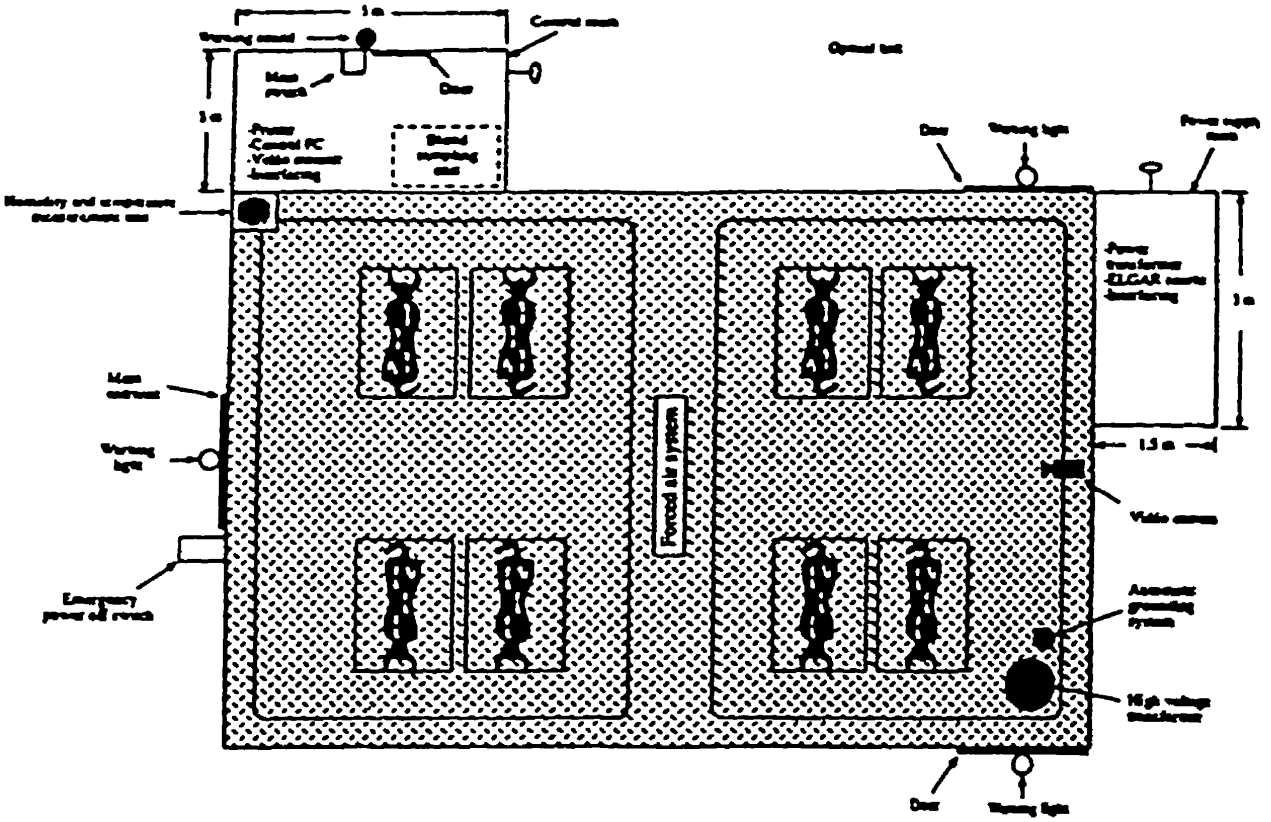


Figure 6. Overview of the control system

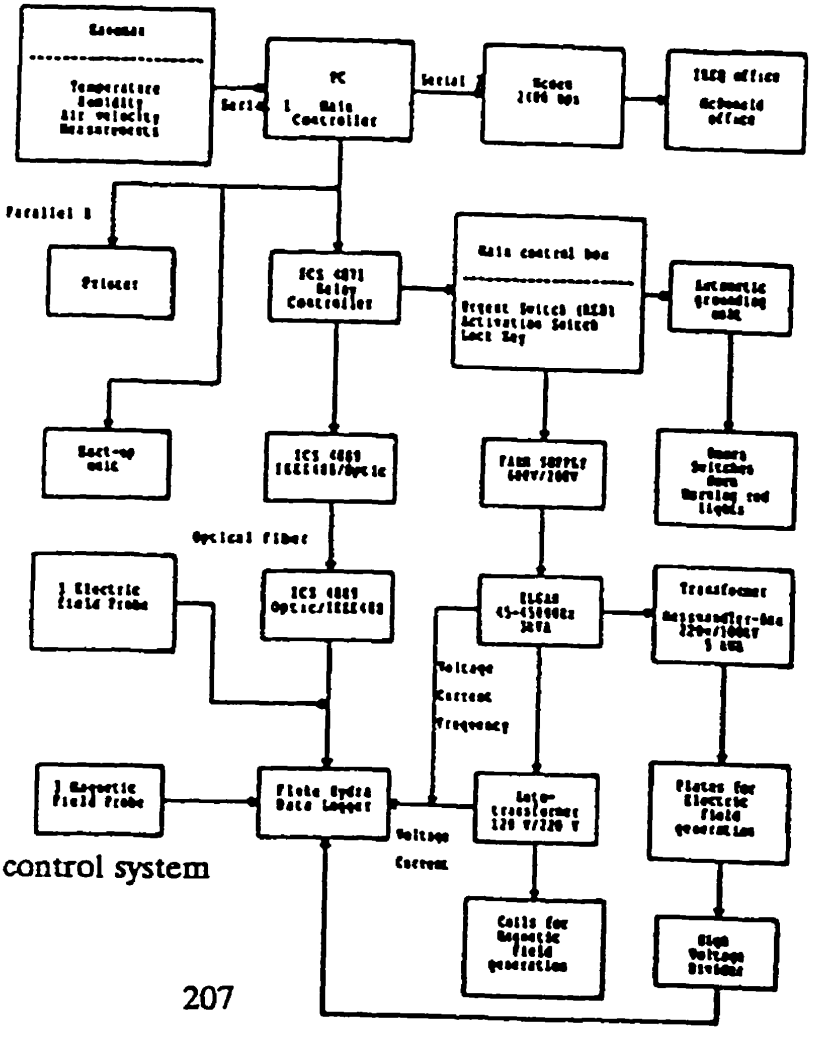


Figure 7. Schematic of the control system

6. Electric field shielding

The shielding and enhancing effect of the electric field were investigated during the design of the chamber by field intensity computations using the Charge Simulation Method (Singer **et al.**, 1974). If the body of the cow is modeled by a cylinder 0.6 m in diameter and terminated at both ends by a hemisphere; the total length is 2.3 m. The computed field intensity E on the back of the "animal" assuming an unperturbed field Eo = 10 kV/m is represented schematized in Figure 8.

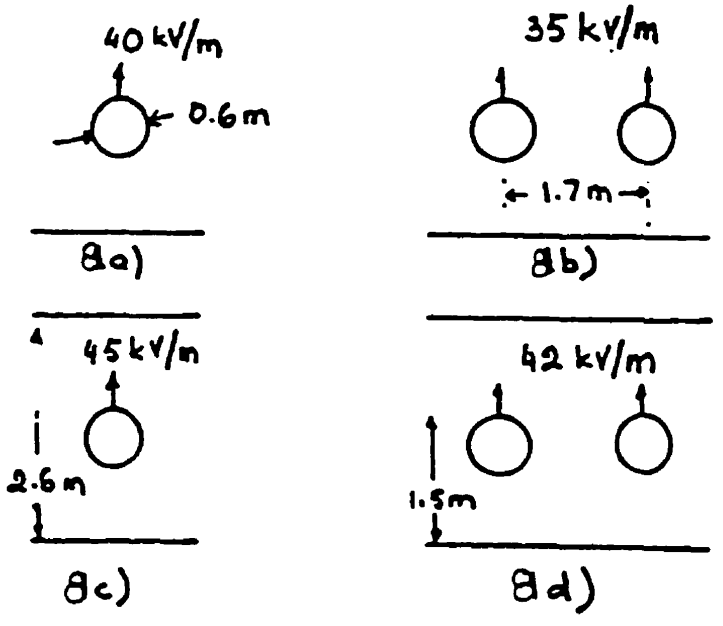


Figure 8. Field computations results

Figure 8a shows that the field enhancement factor f = E/Eo in a real uniform field is 4.0. With the proximity effect of the second animal (Figure 8b) this factor is reduced to only 3.5. In the exposure chamber, with one animal, the factor is increased to 4.5 (Figure 8c), i.e. 12.5% more than the geometric enhancement. When a the

animal is added the field factor is reduced only to 4.2 (Figure 8d) i.e. only 5% more than in a real uniform field.

To validate the field computations, we used a Holaday model 3602 fixed on the back of an animal to measure the field intensity. The data is monitored with a fiber optic cable. The measured field is 45 kV/m in the presence of one animal but decreases to 43 kV/m in presence of the second animal. The measured shielding effect is very close to the computation. At the ground level without any animals the field is 10 kV/m. These measurements were performed at 64% relative humidity after one day of animal occupancy. In the extreme case, when the stall is completely soaked with a hose, the field decreases to 25 kV/m. In this case, without any animals the field on the ground also decreases to 4.5 kV/m. In both cases the field does not change with the proximity of the second animal. Because of the stall geometry (no top, large meshing, height) and the fact that the cows are relatively immobile and soil the stall on the ground directly below, we expect the shielding effect as a function of relative humidity to be less compared to the case of exposure systems for small animals (Patterson and Dietrich , 1987).

7. References

Nguyen Duc Hai, Louis Richard, Gilbert Turmel " Salle d 'exposition aux champs electrique et magnetique en courant alternatif pour le betail: Description and

specifications", IREQ Novembre 1991 (tel 514 652 8053).

Patterson R.C., Dietrich F.M. 1987. Shielding effects in small animal exposure systems. Power Delivrery, Vol. PWRD-2, No 4, October 1987).

Singer H., H. Steinbigler, P. Weiss. 1974. A charge simulation method for the calculation of high voltage fields, IEEE Trans. PAS 93, No 5, 74:1660-1668)

Appendix 2: Experimental data

Table 13 Demographic data about the cows used in this study.

Obs.	Cow	Parity	Date of birth	Date of calving	Trial	Exp.	Rep.	Beginning date	Days in lactation
1	873	2	16SEP1987	14APR1992	1	1	1	15SEP1992	154
2	876	3	14OCT1986	16FEB1992	1	1	1	15SEP1992	212
3	897	2	21FEB1989	22FEB1992	1	1	1	15SEP1992	206
4	898	2	13OCT1988	06MAR1992	1	1	1	15SEP1992	193
5	899	2	13NOV1988	19FEB1992	1	1	1	15SEP1992	209
6	907	2	08FEB1989	10JUN1992	1	1	1	15SEP1992	97
7	908	2	08DEC1988	02MAR1992	1	1	1	15SEP1992	197
8	910	2	10JAN1989	24APR1992	1	1	1	15SEP1992	144
9	905	2	15JUN1989	31AUG1992	1	1	2	02DEC1992	93
10	911	4	15JUN1986	16JUL1992	1	1	2	02DEC1992	139
11	912	4	01MAR1986	24JUL1992	1	1	2	02DEC1992	131
12	937	2	01AUG1989	01AUG1992	1	1	2	02DEC1992	123
13	939	2	17DEC1988	18JUL1992	1	1	2	02DEC1992	137
14	940	2	20JUL1989	20JUL1992	1	1	2	02DEC1992	135
15	960	2	01FEB1989	01AUG1992	1	1	2	02DEC1992	123
16	9742	2	23APR1989	10AUG1992	1	1	2	02DEC1992	114
17	846	5	24FEB1985	08SEP1991	1	2	1	24FEB1992	169
18	847	4	07MAR1986	20OCT1991	1	2	1	24FEB1992	127
19	848	3	01OCT1986	10NOV1991	1	2	1	24FEB1992	106
20	849	3	21JAN1987	21SEP1991	1	2	1	24FEB1992	156
21	850	2	03JAN1988	12SEP1991	1	2	1	24FEB1992	165
22	851	2	01MAY1988	26SEP1991	1	2	1	24FEB1992	151
23	852	2	22MAR1988	29SEP1991	1	2	1	24FEB1992	148
24	853	2	13OCT1988	31OCT1991	1	2	1	24FEB1992	116
25	676	3	06APR1987	21OCT1991	1	2	2	18MAY1992	210
26	712	2	23MAY1988	23NOV1991	1	2	2	18MAY1992	177
27	713	2	30MAY1988	12AUG1991	1	2	2	18MAY1992	280
28	722	2	04JUL1988	14NOV1991	1	2	2	18MAY1992	186

Table 13. Demographic data about the cows used in this study (cont.)

Obs.	Cow	Parity	Date of birth	Date of calving	Trial	Exp.	Rep.	Beginning date	Days in lactation
29	879	2	03JAN1988	21SEP1991	1	2	2	18MAY1992	240
30	880	2	05JUN1988	13SEP1991	1	2	2	18MAY1992	248
31	881	2	21JUN1988	16SEP1991	1	2	2	18MAY1992	245
32	885	2	27NOV1988	29SEP1991	1	2	2	18MAY1992	232
33	843	3	08MAR1986	15NOV1990	2	-	-	29DEC1991	-
34	684	2	29JUN1987	17OCT1990	2	-	-	24DEC1991	-
35	346	1	08JUN1988	11SEP1990	2	-	-	24DEC1991	-
36	730	1	22SEP1988	19APR1991	2	-	-	24DEC1991	-
37	691	2	22SEP1987	16NOV1990	2	-	-	24DEC1991	-
38	845	2	22SEP1988	11OCT1990	2	-	-	24DEC1991	-
39	679	2	01MAY1987	17JUL1990	2	-	-	24DEC1991	-
40	844	2	19JUN1987	25JUL1990	2	-	-	24DEC1991	-
41	A	0	-	-	3	-	-	-	-
42	B	0	-	-	3	-	-	-	-
43	C	0	-	-	3	-	-	-	-
44	D	0	-	-	3	-	-	-	-
45	E	0	-	-	3	-	-	-	-
46	F	0	-	-	3	-	-	-	-
47	H	0	-	-	3	-	-	-	-

Table 14. Length of three consecutive estrous cycles in non-pregnant lactating dairy cows exposed to EMF. Values are actual days of estrous cycle obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	873	23.0000	21.0000	21.0000
2	1	876	23.0000	23.0000	23.0000
3	1	897	14.0000	38.0000	23.0000
4	1	898	23.0000	27.0000	28.0000
5	1	899	30.0000	27.0000	25.0000
6	1	907	21.0000	21.0000	23.0000
7	1	908	23.0000	37.0000	23.0000
8	1	910	35.0000	27.0000	35.0000

OBS	Rep.	Cow	ON	OFF	ON
9	2	905	26.0000	26.0000	28.0000
10	2	911	26.0000	23.0000	26.0000
11	2	912	23.0000	23.0000	26.0000
12	2	937	23.0000	16.0000	26.0000
13	2	960	23.0000	16.0000	26.0000
14	2	9742	23.0000	19.0000	23.0000

Table 15. Progesterone concentrations during three consecutive estrous cycles in non-pregnant lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	873	1.3873	2.2160	1.7180
2	1	876	2.0391	2.2664	1.3264
3	1	897	1.2429	3.1344	1.9664
4	1	898	4.7227	1.6233	2.2223
5	1	899	2.9000	2.1867	2.3183
6	1	907	3.5490	2.4750	1.6545
7	1	908	3.5518	2.5835	2.2791
8	1	910	4.0953	2.9958	3.5019
OBS	Rep.	Cow	ON	OFF	ON
9	2	905	2.7550	2.8550	3.7608
10	2	911	2.0194	2.6209	3.0483
11	2	912	2.2236	2.9873	1.9942
12	2	937	1.8945	2.2662	1.6917
13	2	960	1.3755	1.4875	1.3700
14	2	9742	1.4373	1.0000	2.1491

Table 16. Area under the curve formed by progesterone concentrations during three consecutive estrous cycles in non-pregnant lactating dairy cows exposed to EMF. Actual values obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	873	15.26	22.16	17.18
2	1	876	22.43	24.93	14.59
3	1	897	8.70	28.21	21.63
4	1	898	51.95	19.48	28.89
5	1	899	37.70	26.24	27.82
6	1	907	35.49	24.75	18.20
7	1	908	39.07	43.92	25.07
8	1	910	61.43	35.95	56.03

OBS	Rep.	Cow	ON	OFF	ON
9	2	905	33.06	34.26	48.89
10	2	911	24.23	28.83	36.58
11	2	912	24.460	32.86	23.93
12	2	937	20.840	18.13	20.30
13	2	960	15.130	11.90	16.44
14	2	9742	15.810	9.00	23.64

Table 17. Concentration of melaton in (pg/0.5ml) in lactating pregnant dairy cows exposed to EMF. Values are actual means obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	18.332	174.52	45.72
2	1	847	14.461	15.38	22.23
3	1	848	4.431	14.49	12.65
4	1	849	4.969	8.55	9.13
5	1	850	8.799	25.53	22.04
6	1	851	11.236	14.75	18.35
7	1	852	13.835	23.57	30.94
8	1	853	16.706	28.15	45.58

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	33.860	42.48	27.26
10	2	712	34.418	60.73	21.15
11	2	713	49.868	43.28	39.84
12	2	722	66.745	79.76	36.96
13	2	879	39.834	50.69	35.56
14	2	880	41.879	34.93	55.73
15	2	881	30.587	58.29	20.90
16	2	885	64.019	46.51	23.84

Table 18. Area under the curve of the nocturnal peak of melatonine concentration in lactating pregnant dairy cows exposed to EMF. Values are actual means of the area under the curve obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	485.27	4766.53	1260.88
2	1	847	401.79	408.72	606.89
3	1	848	120.89	400.35	345.13
4	1	849	134.96	234.16	248.96
5	1	850	240.22	697.41	597.55
6	1	851	308.99	400.30	494.14
7	1	852	379.40	646.14	855.86
8	1	853	456.06	756.32	1257.22

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	922.87	1150.43	751.71
10	2	712	941.93	1664.10	580.12
11	2	713	1371.67	1189.95	1100.39
12	2	722	1838.79	2220.30	1019.78
13	2	879	1095.61	1407.95	988.21
14	2	880	1153.01	967.24	1552.19
15	2	881	838.66	1612.60	572.00
16	2	885	1760.23	1268.27	660.15

Table 19. Progesterone concentrations in pregnant lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	4.72250	5.16750	5.39250
2	1	847	5.27875	5.59375	6.06125
3	1	848	4.26000	4.54875	4.63375
4	1	849	6.41125	6.36125	5.69625
5	1	850	6.49125	7.06250	5.40875
6	1	851	5.48750	6.54250	6.21875
7	1	852	6.96875	5.47875	4.99375
8	1	853	6.48250	7.54625	6.86000

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	6.51500	5.27875	6.84875
10	2	712	6.33375	5.00375	7.52500
11	2	713	6.25375	5.15625	5.95750
12	2	722	5.10750	3.93375	5.25375
13	2	879	6.61500	5.79500	5.95625
14	2	880	4.31000	4.00500	5.39375
15	2	881	5.46000	5.49125	6.07125
16	2	885	8.09750	8.58875	8.35375

Table 20. Cortisol concentrations in pregnant lactating dairy cows exposed to EMF. Values are actual means (µg/dl) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	0.91375	1.38750	1.01750
2	1	847	0.46375	0.51125	0.70750
3	1	848	0.77875	1.06250	2.23500
4	1	849	2.05750	3.58125	1.35500
5	1	850	1.21125	2.32625	1.14250
6	1	851	1.37475	1.41250	1.00375
7	1	852	2.16875	1.21750	1.28875
8	1	853	2.22625	1.21750	0.81875

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	0.80750	1.71500	0.60125
10	2	712	0.61125	1.12950	1.17250
11	2	713	0.49250	0.39825	0.81500
12	2	722	0.45750	0.35875	0.67250
13	2	879	0.67250	0.30625	1.00000
14	2	880	1.00375	1.08375	1.21500
15	2	881	0.77750	1.51500	0.61125
16	2	885	0.32625	1.63600	0.41500

Table 21. Milk yield in pregnant lactating dairy cows exposed to EMF. Values are actual means (kg of milk per day) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	28.8786	29.1464	28.0480
2	1	847	29.1536	28.9357	27.1840
3	1	848	29.1893	29.9464	26.1840
4	1	849	30.0643	27.6500	26.5560
5	1	850	24.6536	25.0821	24.0040
6	1	851	20.9321	20.8500	19.2760
7	1	852	25.8143	27.1464	27.3720
8	1	853	33.0571	31.8679	30.1560

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	15.1571	11.2929	7.7038
10	2	712	13.7179	10.657	16.5077
11	2	713	18.2643	16.1607	11.4423
12	2	722	21.1071	16.5500	12.8769
13	2	879	14.3857	8.3429	2.7231
14	2	880	15.5643	12.0786	9.2846
15	2	881	10.2714	4.1964	1.5692
16	2	885	14.3036	11.4286	6.4269

Table 22. Milk yield corrected for fat content produced by pregnant lactating dairy cows exposed to EMF. Values are actual means (kg milk with 4% of fat matter per day) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	21.9555	29.8763	26.9497
2	1	847	27.9899	27.7512	25.8732
3	1	848	19.8873	29.7783	27.1235
4	1	849	31.6860	32.3859	26.8119
5	1	850	22.9471	28.4847	26.1532
6	1	851	20.9526	26.1303	21.7602
7	1	852	26.3982	28.3840	25.3482
8	1	853	31.4506	30.6324	27.2017

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	16.8406	13.247	78.2830
10	2	712	11.4618	9.481	76.1018
11	2	713	18.5986	16.7495	13.1563
12	2	722	22.1801	17.5531	15.5273
13	2	879	16.3844	9.3706	2.9027
14	2	880	17.6942	13.5220	11.4333
15	2	881	11.0625	4.2066	1.6248
16	2	885	14.5003	13.969	17.3779

Table 23. Feed consumption (Dry matter intake) in pregnant lactating dairy cows exposed to EMF. Values are actual means (kg per day) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	24.9931	22.4099	20.7527
2	1	847	21.9318	21.6048	19.8178
3	1	848	23.4458	23.9203	19.6104
4	1	849	24.3969	20.7592	19.5375
5	1	850	22.3500	21.6817	22.4917
6	1	851	20.8957	22.0697	21.1037
7	1	852	22.2395	20.9656	21.5599
8	1	853	22.8004	21.0462	21.6312

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	16.2475	12.1837	14.3152
10	2	712	15.3595	13.4434	14.0731
11	2	713	17.7233	15.4498	15.6156
12	2	722	17.5769	13.9828	15.4214
13	2	879	18.4589	15.1889	14.9466
14	2	880	17.8604	13.7291	15.4492
15	2	881	16.8934	13.4162	14.6837
16	2	885	14.7007	13.3636	15.1043

Table 24. Milk fat percentage in pregnant lactating dairy cows exposed to EMF. Values are actual means (%) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	2.4475	4.1600	3.7688
2	1	847	3.7450	3.7200	3.6888
3	1	848	1.9150	3.9625	4.3836
4	1	849	4.3825	5.1700	4.0456
5	1	850	3.5525	4.9000	4.6520
6	1	851	4.0025	5.6675	4.9252
7	1	852	4.1700	4.3125	3.5012
8	1	853	3.6925	3.7500	3.3516

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	4.7425	5.1200	4.4119
10	2	712	2.9000	3.2700	3.5608
11	2	713	4.1250	4.2475	5.0054
12	2	722	4.3325	4.3550	5.3446
13	2	879	4.9000	4.6300	4.4623
14	2	880	4.9025	4.7400	5.5531
15	2	881	4.3900	3.9075	4.3765
16	2	885	4.0725	5.4500	4.9938

Table 25. Protein content in milk produced by pregnant lactating dairy cows exposed to EMF. Values are actual means (% of protein) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	3.2375	3.0775	3.0388
2	1	847	2.9325	2.9425	2.8784
3	1	848	3.3150	3.1350	2.9808
4	1	849	3.2650	3.1275	3.0820
5	1	850	3.420	3.275	3.512
6	1	851	3.643	3.702	3.564
7	1	852	2.967	3.003	2.894
8	1	853	3.010	2.895	2.856

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	3.385	3.473	3.617
10	2	712	2.808	2.990	3.347
11	2	713	2.900	3.105	3.394
12	2	722	2.968	3.188	3.464
13	2	879	3.180	3.540	4.281
14	2	880	3.362	3.772	3.528
15	2	881	3.520	3.950	4.603
16	2	885	3.993	4.173	4.231

Table 26. Lactose content in pregnant lactating dairy cows exposed to EMF. Values are actual means (%) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	5.0125	4.7875	4.6520
2	1	847	4.6875	4.6875	4.5600
3	1	848	4.9650	4.7925	4.6588
4	1	849	4.9200	4.8600	4.7460
5	1	850	4.7500	4.5875	4.6664
6	1	851	4.7550	4.5425	4.4648
7	1	852	4.7500	4.7825	4.7880
8	1	853	4.8750	4.7325	4.5996

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	4.2650	4.1400	3.8404
10	2	712	4.3425	4.2075	4.1973
11	2	713	4.6775	4.7375	4.7158
12	2	722	4.5825	4.5425	4.4065
13	2	879	4.2200	3.8775	3.7488
14	2	880	4.3500	3.8750	3.9615
15	2	881	3.8750	3.6625	3.8154
16	2	885	4.5050	4.1550	4.2112

Table 27. Solids non-fat content in milk produced by pregnant lactating dairy cows exposed to EMF. Values are actual means (% of protein) obtained during the exposure (ON) or non-exposure (OFF) periods.

	OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	8.76	8.37	8.25	
2	1	847	8.15	8.14	8.02	
3	1	848	8.78	8.43	8.21	
4	1	849	8.69	8.49	8.39	
5	1	850	8.67	8.36	8.72	
6	1	851	8.89	8.72	8.58	
7	1	852	8.25	8.29	8.25	
8	1	853	8.42	8.14	8.03	

	OBS	Rep.	Cow	ON	OFF	ON
9	2	676	8.16	8.10	8.23	
10	2	712	7.69	7.72	8.16	
11	2	713	8.10	8.33	8.70	
12	2	722	8.07	8.22	8.47	
13	2	879	7.92	7.91	8.62	
14	2	880	8.22	8.12	8.10	
15	2	881	7.91	8.08	8.99	
16	2	885	8.96	8.76	9.02	

Table 28. Somatic cell count in milk produced by pregnant lactating dairy cows exposed to EMF. Values are actual means (thousand cell per ml milk) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	48.500	36.250	44.680
2	1	847	318.250	345.500	417.960
3	1	848	37.500	39.500	232.160
4	1	849	79.750	63.750	116.640
5	1	850	82.250	46.500	60.560
6	1	851	240.500	540.50	478.840
7	1	852	63.250	46.00	76.360
8	1	853	45.750	79.75	46.000

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	397.500	336.00	448.538
10	2	712	185.250	233.75	508.885
11	2	713	114.750	132.25	614.231
12	2	722	49.250	41.25	86.731
13	2	879	160.500	382.00	878.769
14	2	880	142.500	1680.00	397.154
15	2	881	186.750	305.25	397.154
16	2	885	36.750	50.75	93.577

Table 29. Blood pH in venous blood collected from pregnant lactating dairy cows exposed to EMF. Values are actual means obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	7.4450	7.3972	7.4012
2	1	847	7.4310	7.4196	7.4380
3	1	848	7.4099	7.3922	7.4195
4	1	849	7.4550	7.4442	7.4477
5	1	850	7.4064	7.4305	7.4150
6	1	851	7.4459	7.4055	7.4260
7	1	852	7.4354	7.4214	7.3936
8	1	853	7.4259	7.4234	7.4134

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	7.4406	7.3884	7.4083
10	2	712	7.4027	7.3934	7.4209
11	2	713	7.4079	7.3972	7.4074
12	2	722	7.3739	7.3351	7.3615
13	2	879	7.4275	7.4127	7.4127
14	2	880	7.4190	7.3671	7.0079
15	2	881	7.3829	7.3759	7.3611
16	2	885	7.3984	7.4085	7.3921

Table 30. Partial pressure of carbon dioxide (CO_2) in venous blood collected from pregnant lactating dairy cows exposed to EMF. Values are actual means (mmHg) obtained during the exposure (ON) or non-exposure (OFF) periods.

	OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	39.2500	42.5250	41.1875	
2	1	847	42.1500	45.0250	42.6000	
3	1	848	40.6750	41.3000	41.1625	
4	1	849	37.6375	40.5250	42.2375	
5	1	850	43.8875	41.0750	40.6250	
6	1	851	39.1750	40.5000	40.9750	
7	1	852	41.6375	41.7500	42.8000	
8	1	853	42.3714	42.3000	42.7750	

	OBS	Rep.	Cow	ON	OFF	ON
9	2	676	39.2625	41.9250	38.6125	
10	2	712	44.3000	40.1375	34.7500	
11	2	713	43.6875	41.3000	38.2500	
12	2	722	43.9250	46.0250	40.9125	
13	2	879	40.2125	37.9375	38.7375	
14	2	880	43.2125	47.0875	41.4625	
15	2	881	46.2000	42.3000	45.9625	
16	2	885	41.9125	37.8250	37.2375	

Table 31. Oxygen partial pressure in venous blood collected from pregnant lactating dairy cows exposed to EMF. Values are actual means (mmHg) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	65.125	43.125	35.125
2	1	847	51.500	37.500	32.571
3	1	848	66.000	49.625	43.250
4	1	849	93.875	41.125	48.750
5	1	850	52.750	39.000	42.250
6	1	851	71.125	56.875	65.375
7	1	852	62.125	47.875	35.250
8	1	853	59.142	43.375	58.125
OBS	Rep.	Cow	OFF	ON	OFF
9	2	676	74.000	40.250	54.375
10	2	712	52.000	66.625	77.125
11	2	713	46.625	56.375	62.875
12	2	722	57.625	38.125	44.625
13	2	879	66.125	64.500	67.250
14	2	880	63.875	35.625	67.000
15	2	881	42.250	62.000	37.500
16	2	885	56.000	66.375	43.125

Table 32. Bicarbonate concentration in blood collected from pregnant lactating dairy cows exposed to EMF. Values are actual means (mEq/l) obtained during the exposure (ON) or non-exposure (OFF) periods.

OBS	Rep.	Cow	OFF	ON	OFF
1	1	846	27.0500	26.4625	25.8625
2	1	847	28.2875	29.2750	28.3714
3	1	848	25.9625	25.3875	26.9500
4	1	849	26.6250	28.0750	29.4875
5	1	850	27.1625	27.5000	26.2000
6	1	851	27.1750	25.7250	27.0375
7	1	852	28.1500	27.4375	26.4125
8	1	853	27.8857	27.9250	27.4375

OBS	Rep.	Cow	ON	OFF	ON
9	2	676	26.9625	25.3750	24.5125
10	2	712	27.7250	24.6375	22.7750
11	2	713	27.6250	25.3625	24.2250
12	2	722	25.4000	24.6125	23.2500
13	2	879	26.6625	24.0750	24.4250
14	2	880	28.1125	27.2750	24.7875
15	2	881	27.5750	25.0500	26.1250
16	2	885	26.0750	24.0750	22.8875

Table 33. Calcium concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	94.1000	87.2700	94.1667
2	167	98.5000	93.7367	94.7067
3	287	88.3500	90.4867	92.2000
4	346	92.9667	91.9367	92.7533
5	679	93.2500	92.1533	91.8333
6	684	98.5000	91.9033	93.7333
7	691	93.8167	94.4000	96.5333
8	730	92.2167	92.2200	97.0000

Table 34. Cooper concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	1.14000	1.20333	0.97667
2	167	1.31000	0.96000	0.98000
3	287	0.93000	0.85000	0.82333
4	346	1.02667	1.07667	0.98667
5	679	1.20000	0.97667	0.97667
6	684	0.93667	0.76667	0.62333
7	691	0.98000	0.96667	0.87000
8	730	1.10333	1.01667	0.69000

Table 35. Iron concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	1.63333	0.13333	1.97333
2	167	2.36667	0.40000	1.77333
3	287	1.73333	0.43333	2.47333
4	346	1.93333	1.86667	1.79333
5	679	2.76667	2.20000	1.64000
6	684	1.76667	1.26667	1.94000
7	691	2.20000	2.07333	2.67333
8	730	2.13333	2.73333	2.87333

Table 36. Potassium concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	160.667	145.667	116.667
2	167	163.333	173.667	144.000
3	287	136.667	167.000	148.667
4	346	148.333	154.667	148.000
5	679	138.667	158.667	150.667
6	684	161.000	147.000	132.667
7	691	163.000	158.000	150.000
8	730	150.667	153.000	157.667

Table 37. Magnesium concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	19.5300	17.2400	19.1533
2	167	19.2800	19.0567	21.1367
3	287	21.4300	21.3733	21.4200
4	346	28.4800	22.5900	20.1400
5	679	24.5800	21.9233	23.5367
6	684	22.6133	21.3900	22.9533
7	691	23.1467	22.2167	23.5667
8	730	20.8967	18.9400	23.0833

Table 38. Manganese concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	0.13667	0.04667	0.07667
2	167	0.16333	0.07333	0.09667
3	287	0.11000	0.08333	0.07333
4	346	0.15333	0.10333	0.04667
5	679	0.11667	0.14667	0.12167
6	684	0.09667	0.14000	0.09333
7	691	0.08333	0.07833	0.09167
8	730	0.07000	0.16333	0.08667

Table 39. Sodium concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	2960.00	2978.33	2728.33
2	167	2960.00	2981.67	2825.00
3	287	2990.00	2951.67	2760.00
4	346	2953.33	3045.00	2945.00
5	679	2930.00	3101.67	2741.67
6	684	3046.67	2998.33	2763.33
7	691	3050.00	2773.33	2791.67
8	730	2986.67	3055.00	2841.67

Table 40. Phosphorus concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	55.4533	54.9900	46.9800
2	167	51.4667	55.5433	56.0933
3	287	56.7200	54.6167	59.3833
4	346	64.3300	58.1233	52.6100
5	679	65.2367	50.3800	51.3000
6	684	52.3733	47.4333	44.2567
7	691	40.4133	55.0600	56.0000
8	730	57.0833	54.2500	51.7700

Table 41. Zinc concentration in blood plasma collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	1.30667	0.82667	0.86000
2	167	0.98000	1.00667	0.88000
3	287	0.68000	0.80000	0.87333
4	346	0.97000	0.93667	0.53000
5	679	1.35000	0.87333	0.57667
6	684	1.15667	0.66667	0.71000
7	691	0.92667	1.14000	1.12000
8	730	0.90667	0.87000	0.95667

Table 42. Calcium concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	53.2500	61.7533	63.2667
2	167	54.7667	61.4533	63.0667
3	287	55.2333	57.3200	56.5333
4	346	52.4000	62.8950	65.8633
5	679	54.3667	61.5867	64.0000
6	684	55.8000	61.4367	59.0333
7	691	54.3250	62.5667	62.3000
8	730	60.3750	60.4367	60.5000

Table 43. Cooper concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	0.06667	0.14000	0.16667
2	167	0.06000	0.08667	0.14667
3	287	0.04667	0.00000	0.06333
4	346	0.03000	0.20000	0.13333
5	679	0.09333	0.05667	0.27000
6	684	0.08000	0.09000	0.06000
7	691	0.10000	0.08000	0.03667
8	730	0.12000	0.06000	0.02667

Table 44. Iron concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	0.50000	0.00	0.30667
2	167	0.53333	0.00	0.47333
3	287	3.46667	0.00	0.27667
4	346	0.76667	0.00	0.06333
5	679	0.46667	0.00	0.66500
6	684	0.73333	0.00	0.30667
7	691	0.85000	0.59	0.42333
8	730	0.90000	0.00	0.92333

Table 45. Potassium concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	120.000	126.000	114.333
2	167	122.333	122.333	126.333
3	287	127.000	117.000	107.000
4	346	112.333	116.000	118.667
5	679	115.667	122.333	118.500
6	684	109.333	133.000	111.667
7	691	98.000	113.333	107.667
8	730	112.500	119.667	116.000

Table 46. Magnesium concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	20.9200	19.1067	20.0700
2	167	20.9800	18.8400	19.5033
3	287	21.6133	19.0900	20.2700
4	346	22.9800	21.9900	20.4333
5	679	22.8967	20.0567	21.8200
6	684	21.4300	18.9567	20.6533
7	691	21.4300	20.9867	21.2533
8	730	21.2300	18.2067	20.4367

Table 47. Manganese concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	0.12667	0.000000	0.061667
2	167	0.10667	0.000000	0.066667
3	287	0.20667	0.000000	0.083333
4	346	0.14000	0.000000	0.023333
5	679	0.09333	0.000000	0.070000
6	684	0.12000	0.000000	0.076667
7	691	0.16000	0.071667	0.066667
8	730	0.10500	0.000000	0.081667

Table 48. Sodium concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	3153.33	3098.00	2905.00
2	167	3166.67	3043.00	2850.00
3	287	3176.67	3088.00	2823.33
4	346	3063.33	3065.50	3045.00
5	679	3003.33	2991.33	2812.50
6	684	3100.00	2988.00	2885.00
7	691	3055.00	2938.33	2865.00
8	730	3240.00	3046.33	2925.00

Table 49. Phosphorus concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	1.2100	20.1233	14.9367
2	167	2.3667	26.1167	27.1500
3	287	6.7133	19.5700	11.1767
4	346	0.0000	28.8300	28.4200
5	679	5.2633	24.2700	21.7950
6	684	0.6100	23.9000	14.1867
7	691	3.6350	19.8200	21.7200
8	730	15.5900	22.4267	17.0967

Table 50. Zinc concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	0.00333	0.06333	0.24333
2	167	0.03333	0.08000	0.28667
3	287	0.00000	0.00100	0.12000
4	346	0.04667	0.10500	0.07667
5	679	0.00000	0.01667	0.22000
6	684	0.13667	0.06333	0.08667
7	691	0.07000	0.12667	0.11667
8	730	0.21000	0.00000	0.17667

Table 51. ß-endorphin concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (pg/0.5ml) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	60.860	120.812	97.084
2	167	121.702	111.826	71.170
3	287	114.496	242.026	112.172
4	346	78.664	120.974	85.779
5	679	102.426	210.204	243.348
6	684	35.862	64.790	190.768
7	691	197.464	94.221	67.452
8	730	201.995	111.028	172.430

Table 52. 5-Hydroxyindoleacetic acid (HIAA) concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	24.1000	24.3333	29.4000
2	167	47.2667	26.3000	38.7667
3	287	34.1333	27.9333	29.0000
4	346	20.0667	30.8000	31.8667
5	679	32.5667	29.1000	22.5667
6	684	28.9333	19.1667	20.5000
7	691	36.7333	24.8667	32.0667
8	730	24.1667	28.1000	23.6333

Table 53. Homovanillic acid concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	2.6000	2.83333	3.56667
2	167	11.5000	3.50000	7.16667
3	287	7.3333	7.06667	6.36667
4	346	5.5000	3.30000	7.90000
5	679	5.3000	6.60000	7.03333
6	684	7.5000	5.20000	4.40000
7	691*	16.0000	.	4.50000
8	730	4.5333	4.93333	4.7666

* Cow 691 not considered in statistical analyses.

Table 54. Methoxy-hydroxy-phenyl-glycol (MHPG) concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	2.33333	1.56667	3.43333
2	167	2.30000	2.60000	3.76667
3	287	2.80000	1.70000	2.06667
4	346	2.23333	3.13333	3.80000
5	679	2.56667	2.70000	5.80000
6	684	2.90000	1.63333	2.06667
7	691	2.33333	2.10000	2.33333
8	730	2.36667	0.86667	1.20000

Table 55. Tryptophan concentration in cerebrospinal fluid collected from pregnant non-lactating dairy cows exposed to EMF. Values are actual means (ng/ml) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	96	59.467	997.47	591.200
2	167	155.900	1017.50	869.167
3	287	505.433	372.53	305.867
4	346	145.467	1474.73	607.233
5	679	37.233	437.80	682.167
6	684	322.700	335.47	398.300
7	691	396.867	58.10	41.300
8	730	302.833	527.03	277.067

Table 56. Calcium concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	114.100	114.500	133.033
2	B	116.825	128.167	117.867
3	C	92.967	97.400	94.733
4	E	180.200	170.800	.
5	F	136.767	143.300	134.833

Table 57. Cooper concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	12.2667	11.4000	12.6333
2	B	10.6000	9.7667	9.2333
3	C	10.9000	9.8000	9.1667
4	E	12.1667	12.5000	.
5	F	9.3333	8.9333	9.6000

Table 58. Iron concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	44.7000	43.7667	48.4667
2	B	37.9000	32.3333	30.8667
3	C	33.9333	33.1667	30.1333
4	E	37.6000	42.1000	.
5	F	30.1333	36.9667	31.7333

Table 59. Potassium concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	253.600	211.633	249.200
2	B	167.750	148.900	168.733
3	C	322.367	279.767	224.567
4	E	315.033	331.300	.
5	F	183.333	207.367	175.033

Table 60. Magnesium concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	9.4333	9.3000	10.0667
2	B	17.4250	19.6000	17.5667
3	C	15.7667	17.6667	16.0333
4	E	12.3667	13.4000	.
5	F	18.5333	22.8667	21.0667

Table 61. Manganese concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	3.10000	3.33333	3.20000
2	B	2.90000	2.93333	2.43333
3	C	3.20000	2.86667	3.03333
4	E	2.66667	3.40000	.
5	F	2.63333	2.83333	3.10000

Table 62. Sodium concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	2879.33	2436.67	2827.00
2	B	3015.75	2972.33	3057.00
3	C	3065.00	3138.00	3244.33
4	E	2817.67	3006.00	.
5	F	3268.00	3351.33	3176.33

Table 63. Phosphorus concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	52.5667	40.6667	49.7000
2	B	37.2000	32.4000	26.7667
3	C	40.6333	40.9333	39.7000
4	E	43.9333	47.3000	.
5	F	35.9000	37.2000	32.4000

Table 64. Zinc concentration in blood plasma collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	8.26667	9.03333	9.30000
2	B	7.47500	6.83333	6.43333
3	C	6.56667	5.80000	6.66667
4	E	5.53333	5.60000	.
5	F	7.43333	7.86667	6.50000

Table 65. Calcium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	90.2333	103.167	86.8333
2	B	77.8000	86.800	89.2333
3	C	91.1000	94.100	93.8667
4	E	87.2333	.	.
5	F	52.1667	55.567	52.4000

Table 66. Cooper concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	10.5667	9.7000	10.7000
2	B	10.0000	9.6667	12.5333
3	C	11.1000	11.1000	10.0000
4	E	8.7667	.	.
5	F	8.8000	9.2333	9.0333

Table 67. Iron concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	33.0667	32.7000	30.4667
2	B	34.4333	36.6667	34.2667
3	C	38.2000	40.4667	37.1667
4	E	40.1667	.	.
5	F	25.8000	26.8333	29.2667

Table 68. Potassium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	418.933	439.1	392.467
2	B	324.633	324.1	264.933
3	C	135.633	134.2	132.533
4	E	212.000	.	.
5	F	226.333	205.4	206.000

Table 69. Magnesium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	22.1333	23.9667	21.8333
2	B	34.1667	31.4667	34.2000
3	C	31.1333	28.5000	25.1667
4	E	15.8333	.	.
5	F	12.2333	13.3000	14.7667

Table 70. Manganese concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	3.76667	3.16667	3.16667
2	B	3.20000	3.13333	3.23333
3	C	3.56667	2.96667	3.23333
4	E	3.36667	.	.
5	F	3.13333	2.46667	3.30000

Table 71. Sodium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	3023.67	2752.67	3109.33
2	B	3369.67	3379.00	3447.33
3	C	3075.33	3113.67	2065.40
4	E	2964.33	.	.
5	F	2844.67	2906.00	1869.50

Table 72. Phosphorus concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	2.40000	2.63333	3.40000
2	B	3.96667	4.23333	4.53333
3	C	3.90000	4.06667	4.23333
4	E	3.96667	.	.
5	F	4.03333	4.20000	3.46667

Table 73. Zinc concentration in cerebrospinal fluid collected from ovariectomized dairy heifers exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	A	5.50000	6.03333	6.26667
2	B	6.50000	6.56667	7.36667
3	C	6.33333	5.76667	6.03333
4	E	5.36667	.	.
5	F	5.83333	5.43333	6.30000

Table 74. Calcium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	87.5333	90.5500	87.5000
2	H	60.5333	66.2333	62.0333

Table 75. Cooper concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	9.56667	8.5	9.6
2	H	8.56667	8.1	8.3

Table 76. Iron concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	35.8	37.9500	35.8000
2	H	31.1	29.9667	32.0667

Table 77. Potassium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	217.967	221.400	218.0
2	H	325.233	326.433	310.4

Table 78. Magnesium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	23.6000	21.6500	23.6000
2	H	16.8667	17.9333	17.6333

Table 79. Manganese concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	3.03333	3.15000	3.00000
2	H	3.30000	3.16667	2.86667

Table 80. Sodium concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	3138.67	3137.00	3139
2	H	3044.00	3132.33	3121

Table 81. Phosphorus concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	4.26667	4.20000	4.3
2	H	4.53333	4.33333	3.6

Table 82. Zinc concentration in cerebrospinal fluid collected from ovariectomized dairy heifers not exposed to EMF. Values are actual means (mg/l) obtained during the pre-exposure (PRE), exposure (EXP) and post-exposure (POST).

OBS	Cow	PRE	EXP	POST
1	D	5.33333	5.40000	5.3
2	H	5.06667	5.43333	5.8

Appendix 3: Power test and Normality test data

Table 83. Variables, α and ß values associated with the power of the statistical test.

Variable	Zα5%	Zß75%*	Zß80%	Zß90%	N
Progesterone during estrus	1.796	0.697	0.876	1.363	13
Area under the P4 curve	1.796	0.697	0.876	1.363	13
Cycle length	1.796	0.697	0.876	1.363	13
Cortisol	1.761	0.692	0.868	1.345	16
Total milk yield	1.761	0.692	0.868	1.345	16
4% Fat corrected	1.761	0.692	0.868	1.345	16
Milk fat	1.761	0.692	0.868	1.345	16
Milk protein	1.761	0.692	0.868	1.345	16
Milk lactose	1.761	0.692	0.868	1.345	16
Solids non fat	1.761	0.692	0.868	1.345	16
Somatic cell count	1.761	0.692	0.868	1.345	16
Feed intake	1.761	0.692	0.868	1.345	16
Dry matter intake	1.761	0.692	0.868	1.345	16
Pregnant progestr	1.761	0.692	0.868	1.345	16
Blood pH	1.761	0.692	0.868	1.345	16
PCO2	1.761	0.692	0.868	1.345	16
PO2	1.761	0.692	0.868	1.345	16
HCO3	1.761	0.692	0.868	1.345	16
TCO2	1.761	0.692	0.868	1.345	16
BEB	1.761	0.692	0.868	1.345	16
MLT	1.645	0.674	0.842	1.282	16
Area under the MLT curve	1.761	0.692	0.868	1.345	16

* Zß75%=Z value of ß in a power test of 75 %

Table 84. Variables, α and ß values associated with the power of the statistical test.

Group A

Variable	Zα5%	ZB75%*	ZB80%		ZB90% N
CSF Ca	1.943	0.718	0.906	1.44	8
CSF P	1.943	0.718	0.906	1.44	8
CSF Mg	1.943	0.718	0.906	1.44	8
CSF Cu	1.943	0.718	0.906	1.44	8
CSF Zn	1.943	0.718	0.906	1.44	8
CSF Fe	1.943	0.718	0.906	1.44	8
CSF Mn	1.943	0.718	0.906	1.44	8
CSF Na	1.943	0.718	0.906	1.44	8
CSF Ca	1.943	0.718	0.906	1.44	8
CSF Endorphin	1.943	0.718	0.906	1.44	8
CSF HIAA	1.943	0.718	0.906	1.44	8
CSF HVA	1.943	0.718	0.906	1.44	8
CSF Tryptophan	1.943	0.718	0.906	1.44	8
CSF MHPG	1.943	0.718	0.906	1.44	8
Plasma Ca	1.943	0.718	0.906	1.44	8
Plasma P	1.943	0.718	0.906	1.44	8
Plasma Mg	1.943	0.718	0.906	1.44	8
Plasma Cu	1.943	0.718	0.906	1.44	8
Plasma Zn	1.943	0.718	0.906	1.44	8
Plasma Fe	1.943	0.718	0.906	1.44	8
Plasma Mn	1.943	0.718	0.906	1.44	8
Plasma Na	1.943	0.718	0.906	1.44	8
Plasma K	1.943	0.718	0.906	1.44	8

* ZB75%=Z value of ß in a power test of 75 %

Table 85. Variable, standard deviation (SD), degrees of freedom (df), differences obtainables with different test power (P75 etc.) and observed difference (OD).

Variable	S.D.	d.f.	P75**	P80	P90	O.D.**
Progesterone during estrus	0.723	11	0.71	0.76	0.90	0.026
Area under the P4 curve	8.076	11	7.90	8.46	10.01	1.940
Cycle length	3.720	11	3.64	3.90	4.61	3.245
Cortisol	0.617	14	0.54	0.57	0.68	0.001
Total milk yield	0.723	14	0.63	0.67	0.79	0.332
4% Fat corrected	1.457	14	1.26	1.35	1.60	1.728
Milk fat	0.387	14	0.34	0.36	0.43	0.365
Milk protein	0.103	14	0.09	0.10	0.11	0.002
Milk lactose	0.084	14	0.07	0.08	0.09	0.032
Solids non fat	0.105	14	0.09	0.10	0.12	0.050
Somatic cell count	312.100	14	270.74	290.16	342.81	50.520
Feed intake	1.547	14	1.34	1.44	1.70	1.970
Dry matter intake	0.869	14	0.75	0.81	0.95	1.008
Pregnant progesterone	0.514	14	0.45	0.48	0.57	0.587
Blood pH	0.033	14	0.03	0.03	0.04	0.008
PCO2	1.871	14	1.62	1.74	2.06	0.057
PO2	11.78	14	10.22	10.95	12.94	3.522
HCO3	0.681	14	0.59	0.63	0.75	0.241
TCO2	0.718	14	0.62	0.67	0.79	0.237
BEB	0.638	14	0.55	0.59	0.70	0.273
MLT	24.48	1237	20.07	21.53	25.33	3.094
Area under the MLT curve	835.5	14	724.66	776.66	917.57	81.604

** P75= Minimum detectable difference with a power test of 75 %

Table 86. Variable, standard deviation (SD), degrees of freedom (df), differences obtainables with different test power (P75 etc.) and observed difference (OD).

Group A

Variable	S.D.	d.f.	P75**	P80	P90	O.D.**
CSF Ca	2.244	7	2.99	3.20	3.80	6.120
CSF P	2.742	7	6.31	6.76	8.02	18.710
CSF Mg	0.755	7	1.01	1.08	1.28	2.030
CSF Cu	0.027	7	0.04	0.04	0.05	0.020
CSF Zn	0.007	7	0.01	0.01	0.01	0.000
CSF Fe	0.934	7	1.24	1.33	1.58	0.960
CSF Mn	0.034	7	0.05	0.05	0.06	0.120
CSF Na	72.75	7	96.80	103.64	123.07	7.480
CSF Ca	8.341	7	11.10	11.88	14.11	6.560
CSF Endorphin	55.99	7	74.50	79.76	94.71	20.310
CSF HIAA	8.122	7	10.81	11.57	13.74	4.680
CSF HVA	4.030	7	5.36	5.74	6.82	2.750
CSF Tryptophan	247.600	7	329.53	352.82	418.95	411.840
CSF MHPG	0.693	7	0.92	0.99	1.17	0.440
Plasma Ca	3.107	7	4.13	4.43	5.26	2.200
Plasma P	7.334	7	9.76	10.45	12.41	1.580
Plasma Mg	2.815	7	3.75	4.01	4.76	1.900
Plasma Cu	0.126	7	0.17	0.18	0.21	0.100
Plasma Zn	0.209	7	0.28	0.30	0.35	0.140
Plasma Fe	0.355	7	0.47	0.51	0.60	0.680
Plasma Mn	0.030	7	0.04	0.04	0.05	0.020
Plasma Na	40.82	7	54.31	58.15	69.05	1.040
Plasma K	10.17	7	13.54	14.50	17.22	4.420

** P75= Minimum detectable difference with a power test of 75 %

Table 87. Variables, α and ß values associated with the power of the statistical test.

Group B

Variable	Zα5%	Zß75%*	Zß80%	Zß90%	N
CSF CA	2.92	0.816	1.061	1.886	4
CSF P	2.92	0.816	1.061	1.886	4
CSF Mg	2.92	0.816	1.061	1.886	4
CSF Na	2.92	0.816	1.061	1.886	4
CSF K	2.92	0.816	1.061	1.886	4
CSF Cu	2.92	0.816	1.061	1.886	4
CSF Zn	2.92	0.816	1.061	1.886	4
CSF Fe	2.92	0.816	1.061	1.886	4
CSF Mn	2.92	0.816	1.061	1.886	4
Plasma Ca	2.92	0.816	1.061	1.886	4
Plasma P	2.92	0.816	1.061	1.886	4
Plasma Mg	2.92	0.816	1.061	1.886	4
Plasma Na	2.92	0.816	1.061	1.886	4
Plasma K	2.92	0.816	1.061	1.886	4
Plasma Cu	2.92	0.816	1.061	1.886	4
Plasma Zn	2.92	0.816	1.061	1.886	4
Plasma Fe	2.92	0.816	1.061	1.886	4
Plasma Mn	2.92	0.816	1.061	1.886	4

* Zß75%=Z value of ß in a power test of 75 %

Table 88. Variable, standard deviation (SD), degrees of freedom (df), differences obtainables with different test power (P75 etc.) and observed difference (OD).

Group B

Variable	S.D.	d.f.	P75**	P80	P90	O.D.***
CSF CA	9.776	2	20.54	21.89	26.43	5.20
CSF P	0.294	2	0.78	0.83	1.00	0.13
CSF Mg	4.035	2	10.66	11.36	13.71	1.20
CSF Na	97.18	2	256.74	273.58	330.27	17.70
CSF K	44.69	2	123.36	131.45	158.69	12.19
CSF Cu	0.658	2	1.74	1.85	2.24	0.08
CSF Zn	0.346	2	0.92	0.98	1.18	0.04
CSF Fe	2.407	2	6.36	6.78	8.18	0.16
CSF Mn	0.155	2	0.41	0.44	0.53	0.48
Plasma Ca	13.54	2	35.78	38.13	46.03	2.77
Plasma P	3.048	2	8.05	8.58	10.36	3.21
Plasma Mg	1.576	2	4.16	4.44	5.36	2.17
Plasma Na	86.06	2	227.36	242.27	292.48	32.50
Plasma K	32.70	2	86.39	92.05	111.13	22.28
Plasma Cu	0.710	2	1.88	2.00	2.41	0.86
Plasma Zn	0.502	2	1.33	1.41	1.71	0.17
Plasma Fe	3.464	2	9.15	9.75	11.77	0.06
Plasma Mn	0.155	2	0.41	0.44	0.53	0.12

** P75= Minimum detectable difference with a power test of 75 %

Table 89. Variables, W statistic and probability associated with the W statistic and test of normality.

VARIABLE	W:NORMAL	p < W
Progesterone during estrous Cycles	0.966405	0.3827
Progesterone area	0.933083	0.0293*
Estrous cycle length	0.886395	0.0007*
Cortisol in pregnant cows	0.957381	0.1334
Total milk yield	0.947860	0.0531
4% Fat corrected milk yield	0.980963	0.7729
Milk fat	0.977212	0.6386
Milk protein	0.898063	0.0003*
Milk lactose	0.989443	0.9751
Solids non fat	0.929823	0.0084
Somatic cell count	0.688757	0.0001*
Feed intake	0.889835	0.0001*
Dry matter intake	0.912353	0.0014*
Progesterone in pregnant cows	0.990872	0.9875
Blood pH	0.780186	0.0001*
Blood PCO_2	0.981411	0.7880
Blood PO_2	0.966539	0.2992
Blood HCO_3	0.964551	0.2534
Blood TCO_2	0.963372	0.2290
Blood BEB	0.979199	0.7108
Area of plasma melatonin	0.916165	0.0020*

* Data do not comply with the normality assumption

Table 90. Variables, W statistic and probability associated with the W statistic and test of normality.

Group A

VARIABLE	W:NORMAL	p < W
CSF CA	0.979771	0.8825
CSF P	0.981356	0.9101
CSF Mg	0.957587	0.3991
CSF Cu	0.975679	0.7986
CSF Zn	0.940061	0.1683
CSF Fe	0.854911	0.0021*
CSF Mn	0.983939	0.9474
CSF Na	0.979373	0.8751
CSF K	0.946664	0.2355
CSF Endorphin	0.945844	0.2260
CSF HIAA	0.964841	0.5471
CSF HVA	0.973471	0.7617
CSF Tryptophan	0.954703	0.3489
CSF MHPG	0.974826	0.7795
Plasma CA	0.965042	0.5516
Plasma P	0.974764	0.7781
Plasma Mg	0.883275	0.0087*
Plasma Cu	0.958855	0.4228
Plasma Zn	0.96116	0.4683
Plasma Fe	0.940162	0.1692
Plasma Mn	0.973472	0.7484
Plasma Na	0.967336	0.6039
Plasma K	0.984076	0.9490

* Data do not comply with the normality assumption

Table 91. Variables, W statistic and probability associated with the W statistic and test of normality.

Group B

VARIABLE	W:NORMAL	p < W
CSF Ca	0.97499	0.9072
CSF P	0.954046	0.6180
CSF Mg	0.97303	0.8851
CSF Cu	0.978333	0.9400
CSF Zn	0.971724	0.8694
CSF Fe	0.926966	0.2972
CSF Mn	0.953845	0.6151
CSF Na	0.945202	0.4958
CSF K	0.933786	0.3623
Plasma Ca	0.938444	0.3828
Plasma P	0.933919	0.3340
Plasma Mg	0.927886	0.2770
Plasma Cu	0.928982	0.2867
Plasma Zn	0.917739	0.2005
Plasma Fe	0.902877	0.1233
Plasma Mn	0.927106	0.2703
Plasma Na	0.962353	0.7194
Plasma K	0.984023	0.9773

Appendix 4: Statistical Analysis System (SAS) programs and outputs

List of the SAS programs used in this study

1.- Trial 1: Progesterone, Area under the progestrone curve and estrous cycle length

```
libname hq 'C:\worksas\';
proc sort data=hq.Progeshq;
        by cow cyclnumb cycIday;
        run;

data hq.Progeshq;
        set  hq.Progeshq;
** this calculates area under the progesterone cycle curve**;
        retain;
        if pcyclnb=cyclnumb then do;
                curvarea=(prog*(cycIday-pcycIday))-
                (((prog-pprog)*(cycIday-pcycIday))/2);
        end;
        else do;
                curvarea='.';
        End;
        pcyclnb=cyclnumb;
        pprog=prog;
        pcycIday=cycIday;
        run;

data one;set hq.Progeshq;
        if cow=940 then delete;
        if cow=939 then delete;
        if cow=910 then delete;
** this eliminates cow 940 (fol. cyst) 910 (luteal cyst) & 939 = They did not
        cycle**;

        run;

proc means noprint;
        by cow  period  cyclnumb;
        var prog curvarea;
        output out=hq.P4avghq mean=prog sum=progarea;
        run;
proc sort data=hq.P4avghq;by cow period;

proc glm data=hq.P4avghq;
        classes   cow  trt period ;
        model prog progarea cyclenth=cow  cow*timetr  period trt ;
        lsmeans trt/stderr pdiff;
        estimate 'off vs on' trt 1 -1;
        contrast 'off vs on' trt 1 -1;
```

```
        title 'progesterone blood levels during estrous cycles in cows exposed to emf';
        title2 ' in a switch-back design trt1=off trt2=off';
        title3 ' sqce1=off-on-off sqce2=on-off-on in periods 1 2 & 3';
        footnote 'mcgill university/hq/block/burchard';
        run;
data one; set hq.P4avghq;
        drop exp begdate timetr trial;
        run;
proc sort ; by cow rep;

proc transpose  data=hq.P4avghq out=hq.P4avgtr
        (rename= (_1=period1 _2=period2 _3=period3));
        by cow rep;
        id period;
        run;

data hq.P4avgtr1; set hq.P4avgtr;
** this performs quadratic contrasts**  ;
        if  _name_= 'prog' then p4= period1 - 2*period2 + period3;
        if  _name_= 'progarea' then p4area= period1 - 2*period2 + period3;
        if  _name_= 'cyclenth' then p4cylent= period1 - 2*period2 + period3;
        run;

data one  ;set hq.P4avgtr1;
        if p4='.' Then delete;

        run;

proc npar1way wilcoxon;
class rep;
var p4;
run;

data one  ;set hq.P4avgtr1;
        if p4cylent='.' Then delete;
        run;

proc npar1way wilcoxon;
        class rep;
        var p4cylent;
        run;

proc sort ; by cow rep;
        data one  ;set hq.P4avgtr1;
        if p4area='.' Then delete;
        run;
```

```
proc npar1way wilcoxon;
        class rep;
        var p4area;
        run;
```

2.- Trial I : Progesterone, Cortisol and production variables.

```
libname hq 'C:\worksas\';
data hq.cortishq;
        infile 'c:\data\proprehq.prn';
        input prog cow date;
proc means noprint;
        by cow trial exp rep period ;
        var prog;
        output out= hq.progvghq mean=prog;
proc glm data=hq.progvghq;
        classes   cow  trt period ;
        model prog=cow  cow*timetr  period trt /p clm;
        lsmeans trt/stderr pdiff;
        estimate 'off vs on' trt 1 -1;
        contrast 'off vs on' trt 1 -1;
        title 'PROGESTERONE BLOOD LEVELS DURING PREGNANCY IN COWS
        EXPOSED TO EMF';
        title2 ' in a switch-back design trt1=off trt2=off';
        title3 ' sqce1=off-on-off sqce2=on-off-on in periods 1 2 & 3';
run;
```

OUTPUT:

```
 PROGESTERONE BLOOD LEVELS DURING PREGNANCY IN COWS EXPOSED TO EMF IN A SWITCH-BACK DESIGN TRT1=OFF
TRT2=OFF
        SQCE1=OFF-ON-OFF SQCE2=ON-OFF-ON IN PERIODS 1 2 & 3


                        General Linear Models Procedure
                           Class Level Information

Class     Levels    Values

COW           16    676 712 713 722 846 847 848 849 850 851 852 853 879 880
                    881 885

TRT            2    1 2

PERIOD         3    1 2 3


                    Number of observations in data set = 48
```

PROGESTERONE BLOOD LEVELS DURING PREGNANCY IN COWS EXPOSED TO EMF IN A SWITCH-BACK DESIGN TRT1=OFF TRT2=OFF
SQCE1=OFF-ON-OFF SQCE2=ON-OFF-ON IN PERIODS 1 2 & 3

General Linear Models Procedure

Dependent Variable: PROG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	33	50.61199775	1.53369690	5.79	0.0005
Error	14	3.70656862	0.26475490		
Corrected Total	47	54.31856637			

R-Square	C.V.	Root MSE	PROG Mean
0.931762	8.728047	0.514543	5.89528646

Source	DF	Type I SS	Mean Square	F Value	Pr > F
COW	15	40.64283512	2.70952234	10.23	0.0001
TIMETR*COW	16	5.57457969	0.34841123	1.32	0.3058
PERIOD	1	0.71976725	0.71976725	2.72	0.1214
TRT	1	3.67481569	3.67481569	13.88	0.0023

Source	DF	Type III SS	Mean Square	F Value	Pr > F
COW	15	40.38815313	2.69254354	10.17	0.0001
TIMETR*COW	15	5.46992656	0.36466177	1.38	0.2775
PERIOD	1	0.71976725	0.71976725	2.72	0.1214
TRT	1	3.67481569	3.67481569	13.88	0.0023

PROGESTERONE BLOOD LEVELS DURING PREGNANCY IN COWS EXPOSED TO EMF IN A SWITCH-BACK DESIGN TRT1=OFF TRT2=OFF
SQCE1=OFF-ON-OFF SQCE2=ON-OFF-ON IN PERIODS 1 2 & 3

16:36 Friday, December 3, 1993

General Linear Models Procedure
Least Squares Means

TRT	PROG LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	Pr > |T| HO: LSMEAN1=LSMEAN2
1	5.60180990	0.10826320	0.0001	0.0023
2	6.18876302	0.10826320	0.0001	

General Linear Models Procedure

Dependent Variable: PROG

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
OFF VS ON	1	3.67481569	3.67481569	13.88	0.0023

Parameter	Estimate	T for HO: Parameter=0	Pr > |T|	Std Error of Estimate
OFF VS ON	-0.58695313	-3.73	0.0023	0.15754609

Similar SAS programs were used to analyses the following variables:

Total milk production, 4% fat corrected milk, percentages of milk fat, protein, Lactose, solids non-fat,somatic cell counts, total feed intake, dry matter intake, progesterone levels during pregnancy, blood gases and blood pH and area under the melatonin curve.

3.- Trial I: Nocturnal melatonin

```
options pagesize=60;
libname hq 'b:\';
proc glm data=hq.Melaton;
        class sqce cow  trt sample;
        model mlt=sqce cow(sqce)  trt pi*cow(sqce)
        cow*trt(sqce) sample sample*trt;
        test h=trt e=cow*trt(sqce);
        lsmeans trt / stderr e=cow*trt(sqce) pdiff;
        ** sample means hour** ;

TITLE1 'GLM ANOVA Effect of EMF on nocturnal Melatonin secretion';
```

OUTPUT:

```
        GLM ANOVA Effect of EMF on nocturnal Melatonin secretion          7
                        General Linear Models Procedure
                            Class Level Information

Class     Levels    Values

SQCE           2    1 2

COW           16    676 712 713 722 846 847 848 849 850 851 852 853 879
                    880 881 885

TRT            2    OFF ON

SAMPLE        28    1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
                    22 23 24 25 26 27 28

                    Number of observations in data set = 1339
```

General Linear Models Procedure

Dependent Variable: MLT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	101	1075620.09	10649.70	17.77	0.0001
Error	1237	741332.01	599.30		
Corrected Total	1338	1816952.10			

R-Square	C.V.	Root MSE	MLT Mean
0.591991	71.34289	24.4806	34.3140

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SQCE	1	108864.818	108864.818	181.65	0.0001
COW(SQCE)	14	351139.958	25081.426	41.85	0.0001
TRT	1	2843.242	2843.242	4.74	0.0296
PI*COW(SQCE)	16	74971.262	4685.704	7.82	0.0001
COW*TRT(SQCE)	15	443202.572	29546.838	49.30	0.0001
SAMPLE	27	80682.617	2988.245	4.99	0.0001
TRT*SAMPLE	27	13915.621	515.393	0.86	0.6723

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SQCE	1	90281.531	90281.531	150.65	0.0001
COW(SQCE)	14	92402.757	6600.197	11.01	0.0001
TRT	1	2851.545	2851.545	4.76	0.0293
PI*COW(SQCE)	16	75273.430	4704.589	7.85	0.0001
COW*TRT(SQCE)	15	443133.786	29542.252	49.29	0.0001
SAMPLE	27	80719.387	2989.607	4.99	0.0001
TRT*SAMPLE	27	13915.621	515.393	0.86	0.6723

Tests of Hypotheses using the Type III MS for COW*TRT(SQCE) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	1	2851.54488	2851.54488	0.10	0.7603

General Linear Models Procedure

Least Squares Means

Standard Errors and Probabilities calculated using the Type III MS for COW*TRT(SQCE) as an Error term

TRT	MLT LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	Pr > |T| HO: LSMEAN1=LSMEAN2
OFF	35.4163315	7.0475320	0.0002	0.7603
ON	38.5095396	7.0325610	0.0001	

4.- Group A and B: Minerals and neurotransmitters

```
data one;set hq.csfmint3;
        if sample= 'PL' then delete;
proc sort;
        by cow trt;
proc means noprint;
        var ca p mg na k cu zn fe mn;
        by cow trt;
        output out=hq.avcsftr3
        mean =ca p mg na k cu zn fe mn;
proc sort data=hq.avcsftr3;
        by cow;
proc transpose data=hq.avcsftr3
        out=hq.travcst3 (rename =(_name_=varcsf _1=preexp _2=exp _3=posexp));
        by cow;
        id trt;
proc sort data=hq.travcst3;
  by varcsf;
proc glm data=hq.travcst3;
  by varcsf;
        model preexp exp posexp= /nouni;
        repeated trt 3 contrast(1) / summary;
Title1 ' CSF minerals in ovariectomized heifers exposed to EMF';
        run;

proc sort data=hq.csfmint3;
        by type  sample trt;
proc means data=one noprint;
        var ca p mg na k cu zn fe mn;
        by type sample trt;
        output out=dos
        mean =ca p mg na k cu zn fe mn
        stderr= stdca stdp stdmg stdna stdk stdcu stdzn stdfe stdmn ;
        run;
proc print data=dos;
        var type sample trt trtca stdca p stdp mg stdmg na stdna k stdk cu stdcu
        zn stdzn fe stdfe mn stdmn;
title1 ' minerals in ovariectomized heifers exposed to emf';
        run;
```

OUTPUT:

CSF minerals in ovariectomized heifers exposed to EMF 795
17:28 Friday, January 6, 1995

---------- NAME OF FORMER VARIABLE=CA ----------

General Linear Models Procedure

Number of observations in by group = 5

NOTE: Observations with missing values will not be included in this analysis.
Thus, only 4 observations can be used in this analysis.

---------- NAME OF FORMER VARIABLE=CA ----------

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	PREEXP	EXP	POSEXP
Level of TRT	1	2	3

Manova Test Criteria and Exact F Statistics for
the Hypothesis of no TRT Effect
H = Type III SS&CP Matrix for TRT E = Error SS&CP Matrix

S=1 M=0 N=0

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.22533807	3.4378	2	2	0.2253
Pillai's Trace	0.77466193	3.4378	2	2	0.2253
Hotelling-Lawley Trace	3.43777648	3.4378	2	2	0.2253
Roy's Greatest Root	3.43777648	3.4378	2	2	0.2253

---------- NAME OF FORMER VARIABLE=CA ----------

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source: TRT

DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
2	101.98351852	50.99175926	2.32	0.1793	0.2035	0.1793

Source: Error(TRT)

DF	Type III SS	Mean Square
6	131.88388889	21.98064815

Greenhouse-Geisser Epsilon = 0.7229
Huynh-Feldt Epsilon = 1.2169

---------- NAME OF FORMER VARIABLE=CA ----------

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

TRT.N represents the contrast between the nth level of TRT and the 1st

Contrast Variable: TRT.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	200.69444444	200.69444444	8.84	0.0589
Error	3	68.13666667	22.71222222		

Contrast Variable: TRT.3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	30.43361111	30.43361111	0.76	0.4465
Error	3	119.55638889	39.85212963		

5.- Test of normality for all the variables

```
proc glm data=hq.csfmint3;
        class cow trt period;
        model  CA cow cow*timetr period trt;
        ** this saves the residuals to the file stat1**;
        output out=stat1
              r= rca;
        ** this tests the normality of the data **;
        proc univariate data=stat1 normal;
        var rca;
        run;
```

OUTPUT:

General Linear Models Procedure
Class Level Information

Class	Levels	Values
COW	5	A B C E F
TRT	3	1 2 3

Number of observations in data set = 13

General Linear Models Procedure

Dependent Variable: CA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3360.301410	560.050235	25.48	0.0005
Error	6	131.883889	21.980648		
Corrected Total	12	3492.185299			

R-Square	C.V.	Root MSE	CA Mean
0.962235	5.747155	4.688352	81.5769231

Source	DF	Type I SS	Mean Square	F Value	Pr > F
COW	4	3258.317892	814.579473	37.06	0.0002
TRT	2	101.983519	50.991759	2.32	0.1793

Source	DF	Type III SS	Mean Square	F Value	Pr > F
COW	4	3294.470056	823.617514	37.47	0.0002
TRT	2	101.983519	50.991759	2.32	0.1793

UNIVARIATE PROCEDURE

Variable=RCA

Moments

N	13	Sum Wgts	13
Mean	0	Sum	0
Std Dev	3.315166	Variance	10.99032
Skewness	0.178974	Kurtosis	0.125193
USS	131.8839	CSS	131.8839
CV	.	Std Mean	0.919462
T:Mean=0	0	Prob>|T|	1.0000
Sgn Rank	-2.5	Prob>|S|	0.8926
Num ^= 0	13		
W:Normal	0.97499	Prob<W	0.9072

Quantiles(Def=5)

100% Max	5.952778	99%	5.952778
75% Q3	1.366667	95%	5.952778
50% Med	1.42E-14	90%	5.144444
25% Q1	-1.61389	10%	-3.53056
0% Min	-6.05556	5%	-6.05556
		1%	-6.05556
Range	12.00833		
Q3-Q1	2.980556		
Mode	-6.05556		

Extremes

Lowest	Obs	Highest	Obs
-6.05556(	3)	1.358333(	7)
-3.53056(	4)	1.366667(	9)
-2.725(	8)	2.069444(	11)
-1.61389(	12)	5.144444(	6)
-1.61389(	5)	5.952778(	2)

6.- Combined analysis of Group A and B to detect changes in mineral concentrations.

```
* ------------------------------------------------------------;
* lines 17 and 21 should be changed to the corresponding situation:
*       locali='PLASMA'   or localis='CSF'
*                    and
*        chimique='Mg','Ca','Cu','Mn','P'
* ------------------------------------------------------------;
options pagesize=60;
data lecture;
infile 'trial3a.dat';
attrib localis length=$8.;
attrib trial label='Numero de l''experience' length=$8.;
attrib vache label='Numero de la vache' length=$8.;
attrib chimique label='Element chimique' length=$8.;
input localis $ trial $ vache $ chimique $ pre exp post;

title1 '-------------------------------------------------------------------';
title2 '                          Ca (CSF) Analysis                        ';
title3 '-------------------------------------------------------------------';
data un;
set lecture;
if localis='CSF' and chimique='Ca';
if pre=. or exp=. or post=. then delete;
lpre=log(pre);
lexp=log(exp);
lpost=log(post);
```

```
data temps;
set un;
temps=1; mesure=pre; lmesure=log(mesure); output;
temps=2; mesure=exp; lmesure=log(mesure); output;
temps=3; mesure=post; lmesure=log(mesure); output;
drop pre exp post;

* analysis for non-transformed data;
* ------------------------------------;
proc glm data=un;
class trial;
model pre exp post=trial/ss3 nouni;
repeated time 3 contrast(1)/printe summary;

proc glm data=temps;
class trial temps vache;
model mesure=trial vache(trial) temps trial*temps/ss3;
test h=trial e=vache(trial);
lsmeans temps trial*temps/pdiff stderr;
output out=stat p=predite r=residus;
run;

* analysis for log-transformed data;
* ------------------------------------------------;
proc glm data=un;
class trial;
model lpre lexp lpost=trial/ss3 nouni;
repeated time 3 contrast(1)/printe summary;

proc glm data=temps;
class trial temps vache;
model lmesure=trial vache(trial) temps trial*temps/ss3;
test h=trial e=vache(trial);
lsmeans temps trial*temps/pdiff stderr;
output out=stat p=predite r=residus;
run;
```

OUTPUT:

```
------------------------------------------------------------------------ 87
                          Ca (CSF) Analysis
------------------------------------------------------------------------

                  General Linear Models Procedure
                     Class Level Information

                    Class     Levels    Values

                    TRIAL          2   A B

                Number of observations in data set = 12


------------------------------------------------------------------------ 88
                          Ca (CSF) Analysis
------------------------------------------------------------------------

                  General Linear Models Procedure
              Repeated Measures Analysis of Variance
               Repeated Measures Level Information

          Dependent Variable        PRE       EXP      POST

               Level of TIME          1         2         3
```

Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance

Partial Correlation Coefficients from the Error SS&CP Matrix / Prob > |r|

DF = 9	PRE	EXP	POST
PRE	1.000000 0.0	0.941282 0.0001	0.875611 0.0004
EXP	0.941282 0.0001	1.000000 0.0	0.907700 0.0001
POST	0.875611 0.0004	0.907700 0.0001	1.000000 0.0

E = Error SS&CP Matrix

TIME.N represents the contrast between the nth level of TIME and the 1st

	TIME.2	TIME.3
TIME.2	152.1961237	97.4867940
TIME.3	97.4867940	273.4722816

Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance

Partial Correlation Coefficients from the Error SS&CP Matrix
of the Variables Defined by the Specified Transformation / Prob > |r|

DF = 9	TIME.2	TIME.3
TIME.2	1.000000 0.0	0.477845 0.1371
TIME.3	0.477845 0.1371	1.000000 0.0

Test for Sphericity: Mauchly's Criterion = 0.7090261
Chisquare Approximation = 3.0947665 with 2 df Prob > Chisquare = 0.2128

Applied to Orthogonal Components:
Test for Sphericity: Mauchly's Criterion = 0.8946184
Chisquare Approximation = 1.0022221 with 2 df Prob > Chisquare = 0.6059

Manova Test Criteria and Exact F Statistics for
the Hypothesis of no TIME Effect
H = Type III SS&CP Matrix for TIME E = Error SS&CP Matrix

S=1 M=0 N=3.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.24587782	13.8018	2	9	0.0018
Pillai's Trace	0.75412218	13.8018	2	9	0.0018

Hotelling-Lawley Trace	3.06706059	13.8018	2	9	0.0018
Roy's Greatest Root	3.06706059	13.8018	2	9	0.0018

Manova Test Criteria and Exact F Statistics for
the Hypothesis of no TIME*TRIAL Effect
H = Type III SS&CP Matrix for TIME*TRIAL E = Error SS&CP Matrix

S=1 M=0 N=3.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.77775948	1.2859	2	9	0.3227
Pillai's Trace	0.22224052	1.2859	2	9	0.3227
Hotelling-Lawley Trace	0.28574454	1.2859	2	9	0.3227
Roy's Greatest Root	0.28574454	1.2859	2	9	0.3227

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Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	3784.58990	3784.58990	11.61	0.0067
Error	10	3260.41457	326.04146		

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Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source: TIME

DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
2	247.41624356	123.70812178	11.31	0.0005	0.0009	0.0005

Source: TIME*TRIAL

DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
2	36.94382255	18.47191127	1.69	0.2101	0.2136	0.2101

Source: Error(TIME)

DF	Type III SS	Mean Square
20	218.78774082	10.93938704

Greenhouse-Geisser Epsilon = 0.9047
Huynh-Feldt Epsilon = 1.2033

Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

TIME.N represents the contrast between the nth level of TIME and the 1st

Contrast Variable: TIME.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	464.63736000	464.63736000	30.53	0.0003
TRIAL	1	2.49376407	2.49376407	0.16	0.6942
Error	10	152.19612368	15.21961237		

Contrast Variable: TIME.3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	241.38413393	241.38413393	8.83	0.0140
TRIAL	1	42.61335000	42.61335000	1.56	0.2404
Error	10	273.47228156	27.34722816		

Ca (CSF) Analysis

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRIAL	2	A B
TEMPS	3	1 2 3
VACHE	12	096 167 287 346 679 684 691 730 A B C F

Number of observations in data set = 36

General Linear Models Procedure

Dependent Variable: MESURE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	7369.555943	491.303730	44.91	0.0001
Error	20	218.787741	10.939387		
Corrected Total	35	7588.343684			

R-Square	C.V.	Root MSE	MESURE Mean
0.971168	4.965772	3.307474	66.6054361

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	3784.589901	3784.589901	345.96	0.0001
VACHE(TRIAL)	10	3260.414567	326.041457	29.80	0.0001
TEMPS	2	247.416244	123.708122	11.31	0.0005
TRIAL*TEMPS	2	36.943823	18.471911	1.69	0.2101

Tests of Hypotheses using the Type III MS for VACHE(TRIAL) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	3784.589901	3784.589901	11.61	0.0067

General Linear Models Procedure
Least Squares Means

TEMPS	MESURE LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	LSMEAN Number
1	66.4447937	1.0127031	0.0001	1
2	73.0447750	1.0127031	0.0001	2
3	71.2018688	1.0127031	0.0001	3

Pr > |T| HO: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3
1	.	0.0002	0.0034
2	0.0002	.	0.2129
3	0.0034	0.2129	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

TRIAL	TEMPS	MESURE LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	LSMEAN Number
A	1	55.0645875	1.1693688	0.0001	1
A	2	61.1810500	1.1693688	0.0001	2
A	3	61.8204125	1.1693688	0.0001	3
B	1	77.8250000	1.6537372	0.0001	4
B	2	84.9085000	1.6537372	0.0001	5
B	3	80.5833250	1.6537372	0.0001	6

Pr > |T| HO: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6
1	.	0.0014	0.0006	0.0001	0.0001	0.0001
2	0.0014	.	0.7031	0.0001	0.0001	0.0001
3	0.0006	0.7031	.	0.0001	0.0001	0.0001
4	0.0001	0.0001	0.0001	.	0.0066	0.2521
5	0.0001	0.0001	0.0001	0.0066	.	0.0792
6	0.0001	0.0001	0.0001	0.2521	0.0792	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRIAL	2	A B

Number of observations in data set = 12

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	LPRE	LEXP	LPOST
Level of TIME	1	2	3

General Linear Models Procedure
Repeated Measures Analysis of Variance

Partial Correlation Coefficients from the Error SS&CP Matrix / Prob > |r|

DF = 9	LPRE	LEXP	LPOST
LPRE	1.000000	0.930519	0.867000
	0.0	0.0001	0.0005
LEXP	0.930519	1.000000	0.937974
	0.0001	0.0	0.0001
LPOST	0.867000	0.937974	1.000000
	0.0005	0.0001	0.0

E = Error SS&CP Matrix

TIME.N represents the contrast between the nth level of TIME and the 1st

	TIME.2	TIME.3

TIME.2	0.0314182693	0.0316244358
TIME.3	0.0316244358	0.0610655066

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Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance

Partial Correlation Coefficients from the Error SS&CP Matrix
of the Variables Defined by the Specified Transformation / Prob > |r|

DF = 9	TIME.2	TIME.3
TIME.2	1.000000	0.721994
	0.0	0.0121
TIME.3	0.721994	1.000000
	0.0121	0.0

Test for Sphericity: Mauchly's Criterion = 0.4295292
Chisquare Approximation = 7.6055893 with 2 df Prob > Chisquare = 0.0223

Applied to Orthogonal Components:
Test for Sphericity: Mauchly's Criterion = 0.7439275
Chisquare Approximation = 2.6623052 with 2 df Prob > Chisquare = 0.2642

Manova Test Criteria and Exact F Statistics for
the Hypothesis of no TIME Effect
H = Type III SS&CP Matrix for TIME E = Error SS&CP Matrix

S=1 M=0 N=3.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.23533234	14.6219	2	9	0.0015
Pillai's Trace	0.76466766	14.6219	2	9	0.0015
Hotelling-Lawley Trace	3.24930980	14.6219	2	9	0.0015
Roy's Greatest Root	3.24930980	14.6219	2	9	0.0015

Manova Test Criteria and Exact F Statistics for
the Hypothesis of no TIME*TRIAL Effect
H = Type III SS&CP Matrix for TIME*TRIAL E = Error SS&CP Matrix

S=1 M=0 N=3.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.72595251	1.6988	2	9	0.2366
Pillai's Trace	0.27404749	1.6988	2	9	0.2366
Hotelling-Lawley Trace	0.37750058	1.6988	2	9	0.2366
Roy's Greatest Root	0.37750058	1.6988	2	9	0.2366

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	0.66638298	0.66638298	10.30	0.0093
Error	10	0.64706806	0.06470681		

Ca (CSF) Analysis

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source: TIME

DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
2	0.05348303	0.02674151	13.18	0.0002	0.0008	0.0002

Source: TIME*TRIAL

DF	Type III SS	Mean Square	F Value	Pr > F	Adj Pr > F G - G	Adj Pr > F H - F
2	0.00972964	0.00486482	2.40	0.1165	0.1307	0.1165

Source: Error(TIME)

DF	Type III SS	Mean Square
20	0.04057289	0.00202864

Greenhouse-Geisser Epsilon = 0.7961
Huynh-Feldt Epsilon = 1.0173

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

TIME.N represents the contrast between the nth level of TIME and the 1st

Contrast Variable: TIME.2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	0.09678775	0.09678775	30.81	0.0002
TRIAL	1	0.00117970	0.00117970	0.38	0.5537
Error	10	0.03141827	0.00314183		

Contrast Variable: TIME.3

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	0.05901249	0.05901249	9.66	0.0111
TRIAL	1	0.01802621	0.01802621	2.95	0.1165
Error	10	0.06106551	0.00610655		

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRIAL	2	A B
TEMPS	3	1 2 3
VACHE	12	096 167 287 346 679 684 691 730 A B C F

Number of observations in data set = 36

General Linear Models Procedure

Dependent Variable: LMESURE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	1.39368744	0.09291250	45.80	0.0001
Error	20	0.04057289	0.00202864		
Corrected Total	35	1.43426033			

R-Square	C.V.	Root MSE	LMESURE Mean
0.971712	1.078126	0.045040	4.17766354

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	0.66638298	0.66638298	328.49	0.0001
VACHE(TRIAL)	10	0.64706806	0.06470681	31.90	0.0001
TEMPS	2	0.05348303	0.02674151	13.18	0.0002
TRIAL*TEMPS	2	0.00972964	0.00486482	2.40	0.1165

Tests of Hypotheses using the Type III MS for VACHE(TRIAL) as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRIAL	1	0.66638298	0.66638298	10.30	0.0093

General Linear Models Procedure
Least Squares Means

TEMPS	LMESURE LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	LSMEAN Number
1	4.16922015	0.01379077	0.0001	1
2	4.26447692	0.01379077	0.0001	2
3	4.24360040	0.01379077	0.0001	3

Pr > |T| HO: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3
1	.	0.0001	0.0011
2	0.0001	.	0.2972
3	0.0011	0.2972	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

TRIAL	TEMPS	LMESURE LSMEAN	Std Err LSMEAN	Pr > |T| HO:LSMEAN=0	LSMEAN Number
A	1	4.00770476	0.01592421	0.0001	1
A	2	4.11347803	0.01592421	0.0001	2
A	3	4.12319410	0.01592421	0.0001	3
B	1	4.33073553	0.02252024	0.0001	4
B	2	4.41547580	0.02252024	0.0001	5
B	3	4.36400669	0.02252024	0.0001	6

Pr > |T| HO: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6
1	.	0.0001	0.0001	0.0001	0.0001	0.0001
2	0.0001	.	0.6708	0.0001	0.0001	0.0001
3	0.0001	0.6708	.	0.0001	0.0001	0.0001
4	0.0001	0.0001	0.0001	.	0.0150	0.3086
5	0.0001	0.0001	0.0001	0.0150	.	0.1217
6	0.0001	0.0001	0.0001	0.3086	0.1217	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

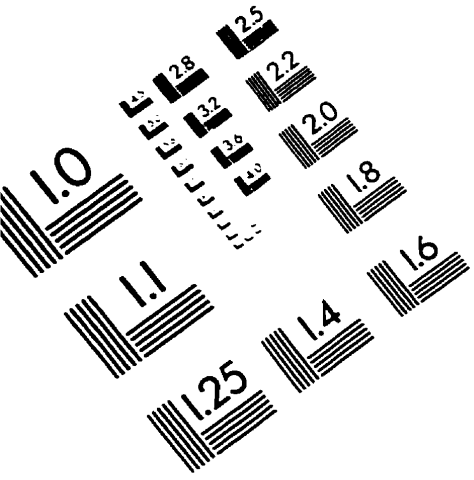
Appendix 5: Milk production data from the Dairy Herd Analysis Service of Québec.

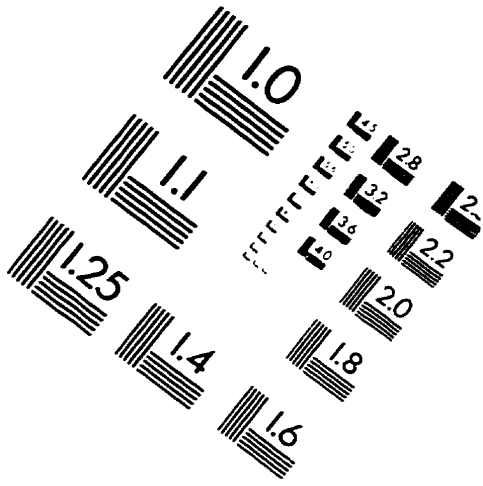
Milk production data from the Dairy Herd Analysis Service of Québec.

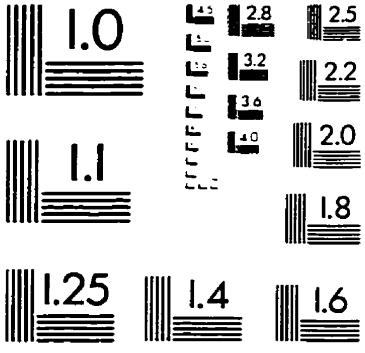
The data presented here correspond to a total of 929,054 lactation records collected form September 1979 to December 1989 on 383,097 cows. From these data 90,560 cows with lifetime performance records were processed to obtain the following information about first lactations. Data are actual means ± standard deviation. (Jairath **et al.** 1995)

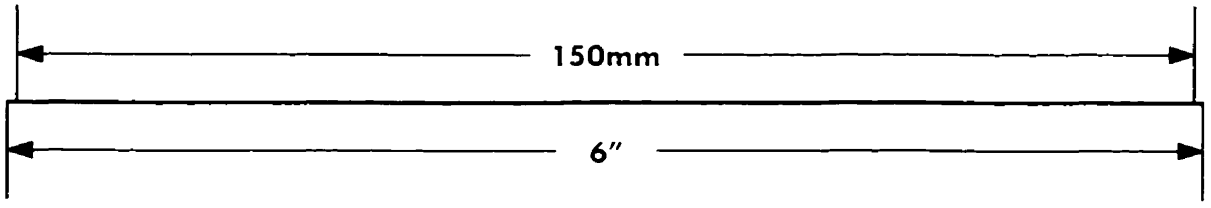
*Variable	Mean	SD
Milk, kg/305 days	5155.67 ±	1944.99
Fat, kg/305 days	183.28 ±	70.99
Protein, kg/305 days	162.62 ±	62.56
Milk yield, kg/day	17.91 ±	3.46
Fat yield, kg/day	0.63 ±	0.13
Protein yield, kg/day	0.56 ±	0.11
Fat %	3.56 ±	0.40
Protein %	3.15 ±	0.22

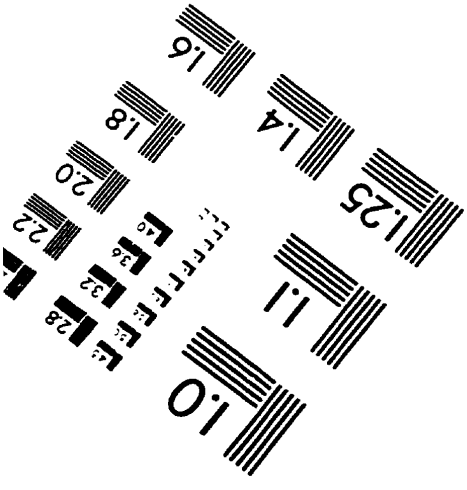
IMAGE EVALUATION
TEST TARGET (QA-3)

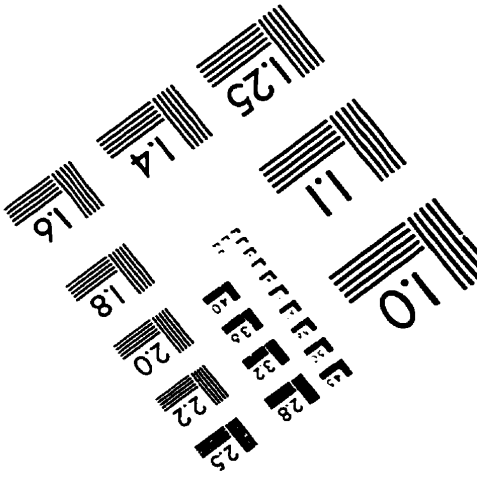












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