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# EVIDENCE OF IMMUNE STIMULATION FOLLOWING SHORT-TERM EXPOSURE TO SPECIFIC EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELDS

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#### ABSTRACT

There is increasing evidence that extremely low frequency (ELF) electromagnetic fields (EMFs) interact with immune cells. Even more evident is that immune cells are activated when exposed to these fields for a short period. Signal specificity and dosimetry appear to play a role. In this study, four groups of laboratory mice received daily exposure to a specific electromagnetic field with an intensity of 5µT for one hour, four hours and twenty-four hours (continuously) respectively for a period of seven days. The control group received no exposure and was used as standard for comparison. Following exposure, whole blood was analysed for leukocyte count, CD3, CD4, CD8 and CD19 analysis. The results for the twenty-four hour exposure group indicated increased total leukocyte, lymphocyte, CD3 and CD4 values and a decreased neutrophil values. These findings provide evidence that the immune system is indeed stimulated by exposure to EMFs.

#### **KEYWORDS**

Immunent, extremely low frequency electromagnetic fields, immune stimulation, leukocytes

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### **INTRODUCTION**

Life without electricity in the 21<sup>st</sup> century is unthinkable, since almost our entire existence has become dependent on electricity and wireless telecommunication.<sup>[1]</sup> Where electricity is generated, electromagnetic fields (EMFs) are created.<sup>[2]</sup> Advanced electrical technology has created an electromagnetic environment in which people live and work, resulting in constant concern about the environment and human health.

EMFs comprise a wide spectrum of frequency, which ranges from extremely high at the highest end of the ionising spectrum to extremely low at the lowest end of the non-ionising spectrum. The field of interest for this study fell within the extremely low frequency (ELF) spectrum between 50 and 60 Hz. These are the fields to which people are exposed on a daily basis, through household appliances, computers and telecommunication.<sup>[2-4]</sup> Low frequency (LF) fields (which extend from the higher end of the ELF band up to 300GHz) have been suggested to be harmful to human health.<sup>[5,6]</sup> Some research efforts have indicated that there is no conclusive evidence that exposure to low fieldstrength EMFs poses a health risk.<sup>[2,3,7,8]</sup> It is this uncertainty that has elicited the on-going research in this area for the last three decades.<sup>[2]</sup> The evidence and claims of scientists in this regard have been found to be somewhat controversial.<sup>[2,3,7,8]</sup> It seems that short-term exposure to ELF-EMF does not have a detrimental effect on human biological systems,<sup>[9]</sup> while long-term exposure can cause chronic stress, resulting in tissue damage.<sup>[10,11,8]</sup>

There has been growing interest in studies that indicate the possibility of therapeutic and health benefits from short-term exposure to ELF-EMFs.<sup>[12-16]</sup> Recently, the immune system has been targeted for research in this field. It was found that shortterm exposure to fields in the ELF range affects the blood cell levels and results in lymphocyte proliferation.<sup>[4]</sup> This same research group indicated an increased phagocytic activity in macrophages; hence, indicating an effect on the innate immune response. Several authors suggested that short-term exposure to ELF-EMF could stimulate the immune response, specifically on a cellular level, leading to the production of cytokines.<sup>[17,18,4,16]</sup> The research of Cuppen et al.<sup>[17]</sup> led to a hypothesis that ELF-EMF could alert or stimulate the immune system through mild stress induction on cells due to cytokine production. Simkó and Mattson assumed an effect at cellular level, where cells respond to EMF by reacting as they would to an unspecific stressor.<sup>[13]</sup> However, sufficient supporting evidence to validate this postulation is lacking.

Recent research studies indicated that exposure to a specific EMF signal (the Immunent BV signal with multiple waveforms [20-5000Hz]) led to decreased mortality in fish<sup>[17]</sup> and also improved the feed conversion in chickens.<sup>[19]</sup> In addition, this same signal proved to enhance the immune system when a study on chickens, infected with coccidiosis, indicated reduced intestinal lesions after exposure to this specific signal.<sup>[19]</sup> The patented Immunent signal<sup>[20]</sup> is created by a device which emits a specific ELF-EMF signal with a different waveform and intensity from a

standard therapeutic signal.<sup>[16]</sup> With this specific signal, several research studies have shown that short-term exposure to the signal enhances the immune system.<sup>[4,13,17,19]</sup>

The hypothesis by Cuppen et al.<sup>[20]</sup> suggests cytokine involvement needs to be investigated further, specifically in mammals. Once validated, this hypothesis could mean a breakthrough in the medical field with respect to boosting an early immune response.<sup>[4]</sup> However, there is limited research in this field on mammals and there is a need for replication of existing evidence that short-term exposure to ELF-EMF could promote innate immunity and find new evidence to support this theory. If there is evidence that short-term exposure to the ELF-EMF Immunent BV signal can stimulate the immune system of fish and chickens,<sup>[21]</sup> then it could be possible that the same findings could be reproduced in mammals, specifically mice (Mus. musculus), thus endorsing the findings of previous research in lower animals. The aim of the current study was to investigate whether short-term exposure to ELF-EMF from the specific Immunent BV signal could affect the immune system in mice and if the duration of exposure to the signal has any significant effect on the results.

## METHODOLOGY

The research study was based on a longitudinal study design and was conducted at the Central University of Technology (CUT). The experimental design involved the following four processes: (a) exposure of the mice to the EMF signal; (b) anaesthetisation, blood collection and euthanasia of the animals; (c) biological analysis; and (d) data capturing and processing for statistical analysis. Approval for the study was granted by the animal ethics committee of the UFS (No. 20/08). Rules and regulations of the Experimental Animal Act (Review of animal care legislation in South Africa, 1962) were strictly adhered to with the help of University of the Free State (UFS) Animal Unit where the exposure period was set up and conducted.

The sample comprised one hundred healthy young adult (approximately six weeks old) male mice of the BALB/c N1H strain with a mean weight of 23±1.5 grams. The weight was kept within this range to avoid dominant behaviour by larger mice. Male mice were chosen in order to exclude hormonal influences. Well-ventilated, plastic cylinders were designed and built to house the cages containing the mice during the exposure period. Copper coils were spun around each cylinder to create the electromagnetic field controlled from the Immunent BV exposure control system. The Immunent generator, supplied by Immunent BV,<sup>[22]</sup> was used for the exposure procedure. The signal produced by the generator was unique. It consisted of multiple waveforms, which created complex, continuously changing EMFs with steep rise times and exponential decays. The experimental group of mice were uniformly exposed to the EMFs (multiple frequencies 20-5000Hz) with an intensity of 5µT. Mice were housed in Perspex cages, which were lined with sawdust for bedding. The design of the cages allowed free access to food and water. The diet consisted of a balanced diet of Epol mouse pellets. Cages were visited daily to check on food, water, temperature and power supply. Controlled environmental conditions, such as room temperature of 21°C and light switches controlling a twelve-hour day and twelve-hour night routine were maintained. Human handling of the mice was kept to a minimum, to prevent stress in the animals.

The experiment was set up in three experimental groups of twenty-five mice each and a control group of twenty-five mice. The cages housing the mice were placed inside the cylinders where they were exposed to the EMF signal. The duration of daily exposure for each experimental group (referred to as Groups A, B and C) were as follows: (1) twenty-four hours (continuous) for Group A; (2) four hours for Group B; and (3) one hour for Group C. The control group (K) was housed in a separate room and totally removed from any exposure to EMFs created by the Immunent signal. In all other aspects (housing, room temperature, lighting, diet and daily visits), the control group was treated the same way as the experimental group. The mice were subjected to this exposure for 7 days. After completion of exposure, the mice were anaesthetised in a chamber containing Allantoin gas. This process was performed by qualified personnel from the Animal Unit. Approximately 1ml whole blood was collected (through orbital bleeding) for the biological analysis. The anticoagulant used was ethylenediaminetetraacetic acid (EDTA) powder. While anesthetised, mice were euthanized by cervical dislocation.

The white blood cell counts (leukocyte count) were analysed on an ABX Pentra 60 blood cell analyser (reagents from Scientific Group). The total leukocyte count was determined as well as the differential lymphocyte, monocyte, neutrophil, eosinophil and basophil values (expressed as percentage of the total leukocytes). High, low and medium controls were included for quality control. The immunophenotyping of lymphocyte subsets was performed on a Fluorescence-Activated Cell Sorting (FACS) Calibur Analyser, using rat anti-mouse markers (from Beckman Coulter). Isotype controls were included for all the markers. Whole blood was analysed for the total T-lymphocyte (CD3), helper T-lymphocyte (CD4), cytotoxic T-lymphocyte (CD8) and B-lymphocyte (CD19) values.

Raw data was compiled in Excel and sent for statistical analysis by a statistics consultant. Descriptive statistics, namely means and standard deviations (or medians and percentiles), were calculated for the different groups. For data producing a Gaussian distribution, means and standard deviations were calculated, whereas the data producing a non-Gaussian curve, median and percentiles were calculated. The mean (or median) values for each of the exposure groups were compared individually with the mean (or median) value of the control group using the t-test (or Kruskal-Wallis test) for independent samples. The ANOVA test (or Kruskal-Wallis test) was used for comparison between the exposure groups (intergroup comparison). A significance level of 0.05 was used.

# RESULTS

The leukocyte values and immunophenotyping from 100 mice were analysed. Results from clotted samples were disregarded for analysis. A summary of the results from the leukocyte values and immunophenotyping is captured in Tables 1-4. It should be noted that the differential leukocyte values and immunophenotyping values were expressed as percentage values. The histograms in Figures 1-3 illustrate only the results showing significant differences. The mean (median) for the exposure groups versus the control group are presented (Table 1 and 3), in an attempt to determine whether the exposure had an effect on the cell values. This is followed by the intergroup comparison (Table 2 and 4) to determine whether the duration of exposure had an effect on cell values.

### Leukocyte values

The descriptive statistics and statistical significance for mean or median differences between the various experimental groups and the control group for the leukocyte parameters are depicted in the Table 1 and figures 1a-1c.

The data revealed a significant mean or median difference in total leukocyte (p=0.0391), lymphocyte (p=0.0149) and neutrophil (p=0.0014) values between group A (twenty four hour) and the control group. For the monocytes, eosinophils and ba-

sophils, there were no significant median differences between the experimental groups and the control group (p>0.05). In figures 1a-1c the total leukocyte and lymphocyte values for group A was significantly higher than that of the control group, whereas the neutrophil values of group A was significantly lower.

From these histograms it is also evident that the difference between the exposure groups and the control group seemed to increase as the duration of exposure time increased. In spite of the differences not all being statistically significant, many parameters were found to be significant in group A, which received the maximum exposure duration. A similar observation was made for the decreased neutrophil values.

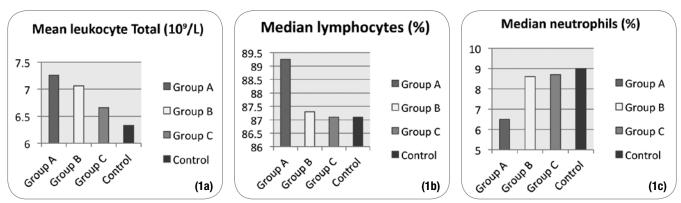
Table 2 and Figures 2a-2c depict the differences for leukocyte parameters between all of the groups (ANOVA test) and between the individual groups (intergroup comparison).

The results in Table 2 indicate no significant intergroup varia-

Parameter		A (24hr)	B (4hr)	C (1hr)	K (Control)
Total leukocytes	Mean (10 <sup>9</sup> /L)	7.26	7.06	6.66	6.33
	SD	1.66	1.44	1.42	1.39
	p-value t-test	0.0391*	0.0773	0.4032	
	Median (%)	89.25	87.30	87.10	87.10
Lymphocytes	Inter-quartile range	87.3-92.55	85.60-90.90	85.50-89.20	84.25-89.05
-	p-value Kruskal-Wallis test	0.0149*	0.4715	0.5555	
Monocytes	Median (%)	2.60	3.00	3.10	3.25
	Inter-quartile range	1.70-3.60	2.30-3.80	2.20-4.10	2.50-4.90
-	p-value Kruskal-Wallis test	0.0833	0.5549	0.6309	
	Median (%)	6.50	8.60	8.70	9.00
Neutrophils	Inter-quartile range	5.60-8.70	6.90-10.10	7.20-10.20	7.80-9.85
	p-value Kruskal-Wallis test	0.0014*	0.2712	0.6169	
Eosinophils	Median (%)	0.10	0.20	0.20	0.20
	Inter-quartile range	0.10-0.20	0.10-0.30	0.10-0.40	0.10-0.40
	p-value Kruskal-Wallis test	0.1360	0.9030	0.8469	
Basophils	Median (%)	0.40	0.40	0.40	0.40
	Inter-quartile range	0.30-0.50	0.30-0.50	0.30-0.40	0.30-0.50
	p-value Kruskal-Wallis test	0.6656	0.8356	0.2562	

Table 1: Descriptive statistics and p-values for mean and median leukocyte parameters of the experimental and control groups.

\*(p<0.05 indicates significant difference)

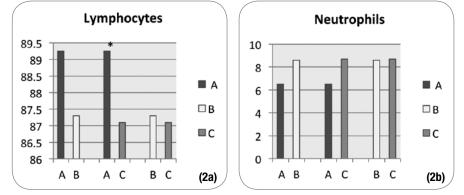


Figures 1a-c: Mean or median values for the total leukocytes (a), lymphocytes (b), and neutrophils (c) of all the groups (\*significant difference p<0.05)

Parameter	A vs. B vs. C (ANOVA/ Kruskal- Wallis test) p-value	A vs. B (t-test/ Kruskal-Wallis test) p-value	A vs. C (t-test/ Kruskal-Wallis test) p-value	B vs. C (t-test/ Kruskal-Wallis test) p-value
Total leukocytes	0.3772	0.6520	0.1830	0.3385
Lymphocytes	0.0338*	0.0423*	0.0151*	0.7195
Monocytes	0.1563	0.1272	0.0771	0.7709
Neutrophils	0.0190*	0.0435*	0.0072*	0.4786
Eosinophils	0.2636	0.2379	0.1219	0.6228
Basophils	0.1475	0.7251	0.0945	0.0879

Table 2: Intergroup comparison for leukocyte parameters of the experimental and control groups.

\*(p<0.05 indicates significant difference)



Figures 2a & b: Intergroup comparison of median, lymphocyte and neutrophils values (\*significant difference p<0.05)

tion for the total leukocyte, monocyte, eosinophil and basophil results (p>0.05). There was, however, a significant variation shown by the ANOVA test between the three groups for the lymphocyte (p=0.0338) and neutrophil (p=0.0190) paramaters (Figures 2a and 2b).

The results for the lymphocyte percentage indicated a significant median difference between groups A and B (p=0.0423) as well as between groups A and C (p=0.0151), but none was found between groups B and C (p>0.05). For the neutrophils, there was a significant median difference between groups A and B (p=0.0435) as well as between groups A and C (p=0.0072), and, as with the lymphocytes, none was found between groups B and C (p>0.05). For both the parameters, the most significant median differences were found between groups A and C. Once again, it is evident that the longer the exposure duration, the more significant the difference became.

#### Immunophenotyping

The descriptive statistics and statistical significance for mean differences between the experimental groups and the control group for the immunophenotyping results are summarised in

Parameter		A (24hr)	B (4hr)	C (1hr)	K (Control)
CD3	Median (%)	34.13	34.17	35.77	25.70
	SD	8.86	11.64	11.01	13.73
	p-value t-test	0.0218*	0.0372*	0.0137*	
CD4	Median (%)	26.52	25.72	26.87	19.20
	SD	7.01	8.65	8.81	11.11
	p-value t-test	0.0138*	0.0400*	0.0193*	
CD8	Median (%)	8.37	8.43	9.94	6.7
	SD	2.96	3.35	2.85	3.19
	p-value t-test	0.0762	0.0934	0.0015*	
CD19	Median (%)	23.08	23.22	17.32	17.88
	SD	12.10	12.03	12.78	13.77
	p-value t-test	0.1849	0.1885	0.8933	

\*(p<0.05 indicates significant difference)

Table 3. The immunophenotyping parameters are expressed as percentage values.

There was a significant increase in the CD3 percentage of groups A (p=0.0218), B (p=0.0372) and C (p=0.0137) compared to the control group. In addition, a significant mean difference in CD4 between groups A (p=0.0138), B (p=0.0400), C (p=0.0193) and the control group was found. For CD8, only group C showed a significant mean difference (p=0.0015) from that of the control group. No significant mean differences were found between any of the exposure groups and the control group for CD19 percentages. A remarkable finding in this part of the study was that in spite of the fact that the total lymphocyte values were higher than the control group in only the continuously exposed group (Group A), the CD3 and CD4 (T lymphocyte) values were found to be higher in all of the exposure groups.

Table 4 indicates the differences between all of the groups (ANOVA test) and between the individual groups (intergroup comparison). In this case no significant differences (p>0.05) were found between any of the exposure groups for the immunophenotyping parameters. The ANOVA test (A vs. B vs. C) in-

dicated no significant differences (p>0.05) between the groups for the immunophenotyping parameters.

#### DISCUSSION

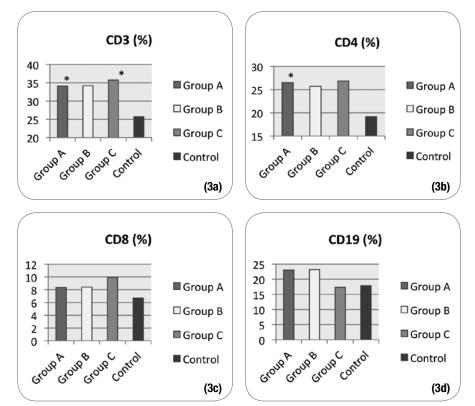
The comparative data between the various exposure groups and the control group revealed that there was a significant difference in lymphocyte and neutrophil results between the exposure groups and the control groups; indicating a strong possibility that short-term exposure to ELF-EMF could stimulate the immune response. The intergroup comparison revealed that there was a significant variation between the different groups for the lymphocyte and neutrophil results; suggesting that the duration of exposure had an effect on the cell values.

Most of the statistically significant differences were evident in the lymphocyte parameters. The immunophenotyping analysis for specific lymphocyte sub-populations revealed that these significant differences were limited to T-lymphocytes. The increased lymphocyte value most likely accounted for the increased total leukocyte count, which was confirmed by the immunophenotyping results in Table 3. It can also be deduced

Parameter	A vs. B vs. CA vs. B (t-test)A vs. C (t-test)(ANOVA) p-valuep-valuep-value			B vs. C (t-test) p-value	
CD3	0.3772	0.6520	0.1830	0.3385	
CD4	0.0338	0.0423	0.0151	0.7195	
CD8	0.1563	0.1272	0.0771	0.7709	
CD19	0.0190	0.0435	0.0072	0.4786	

Table 4: Intergroup comparison for immunophenotyping parameters of the experimental and control groups

\*(p<0.05 indicates significant difference)



Figures 3a-d: Mean values for CD3 (3a), CD4 (3b), CD8 (3c) and CD19 (3d) percentages of all the groups (\*significant difference p<0.05)

from the immunophenotyping that T-lymphocytes represented the specific group of lymphocytes that was increased. The significant differences for the leukocyte parameters in Table 1 suggest that exposure to the ELF-EMF signal did have an effect on the leukocyte values. There was also an apparent variation between the groups for the leukocyte values, where the tendency increased with an increase in the duration of exposure. The most significant difference was found in group A (twenty four hour exposure), the experimental group which received the maximum daily exposure. Hence, the deduction that the longer the exposure, the more significant the difference became. This provides evidence that the duration of exposure plays an important role or has an effect on the immune cell values. This effect, as a result of duration of exposure, was also reported by Goraca et al.<sup>[15]</sup> when they suggested that the oxidative stress parameters depended on "working time" (duration of exposure) of the ELF-EMF field.

In the human body, immune stimulation is characterised by an increased lymphocyte count, due to activation of lymphocytes when stimulated by a "foreign agent or stimulus".<sup>[23]</sup> Hence, the increased leukocyte count in this study could possibly indicate an immune stimulation. Markov et al.[14] indicated that ELF-EMFs interact with lymphocytes and Selmaoui et al.<sup>[24]</sup> found an increased IL-6 in healthy young men exposed to a 9-hour intermittent field (10µT). An increased leukocyte count could, therefore, be due to increased cytokine production.<sup>[17,21,22]</sup> This could explain the association of the increased lymphocyte concentration in this study. The reason for this is that activation of the innate immune system is accompanied by cytokine production.<sup>[4]</sup> Cytokines play an important role in T-lymphocyte differentiation in the immune response.<sup>[23]</sup> Once stimulated, the immune system is no longer in a state of homeostasis. In a study by Markov et al.<sup>[14]</sup> they found that ELF-EMF affected homeostatically unstable cells. This concurs with the hypothesis of Cuppen et al.<sup>[17]</sup> that ELF-EMF exposure can "put the immune system into a state of alert".

The reason for the decreased neutrophil values in this current study is unclear. In the study by Elmusharaf et al.<sup>[19]</sup> the authors suggested that EMF treatment could possibly result in increased blood flow, just like it would in an inflammatory reaction, hence leading to relocalization or colonization of phagocytic cells to potentially damaged tissues.<sup>[20]</sup> The author is of opinion that this suggestion could possibly explain the reduced neutrophil count in our study. In other words, the neutrophil value was decreased in the peripheral blood due to relocation of these cells to the tissues when being stimulated by ELF-EMF. De Kleijn et al.<sup>[4]</sup> mention that inflammatory cytokines play a role in regulation of other immune cells such as neutrophils. Coico, Sunshine and Benjamini<sup>[22,23]</sup> explain neutrophil migration (also known as transendothelial migration) under the influence of cytokines. One of the cytokines involved in this process is IL-8. A possible explanation for the decreased neutrophil count could then be due to IL-8 release following activation of lymphocytes with consequent relocation of neutrophils to the tissues. The finding of increased phagocytic activity following short-term exposure to ELF-EMF has been described by several authors.  $^{\scriptscriptstyle [4,13,15,26,27]}$  In yet another study, increased macrophage activity was found to be associated with increased superoxide anion (O2/free radical) production.<sup>[25]</sup> However, these analyses were not performed in this study, but could direct toward consideration for future research. Varani et al.<sup>[28]</sup> found that ELF-EMF exposure resulted in an increase of A2A adenosine receptor density in neutrophils. Adenosine receptors interact on the neutrophil surface as an anti-inflammatory agent.<sup>[29]</sup> This A2A adenosine receptor could be considered as yet another possibility of a biological mechanism explaining the involvement of neutrophils in the immune response following exposure to ELF-EMF.

Taking a critical look at the results obtained for the immunophenotyping, it was found that short-term exposure to ELF-EMF resulted in increased CD3 and CD4 lymphocyte values for all the exposure groups (twenty-four, four and one hour exposure). The most significant increase in values was seen in group C (one hour exposure). There was also a significant increase in CD8 counts for group C. No significant differences were found for the CD19 values. Therefore, short-term exposure to the specific Immunent signal resulted in increased T-lymphocyte values, but had no effect on B-cell values. Since no significant differences were found in the intergroup comparison, it can be deduced that the duration of exposure did not affect the results of the immunophenotyping. A possible explanation for the increased CD3 and CD4 lymphocyte percentages for all the exposure groups could be that the T-lymphocytes were signalled by the increased IL-6 levels. This fits in well with the findings by Selmaoui et al.<sup>[24]</sup> who reported raised IL-6 levels in young men when exposed to 50Hz EMFs. The increase, and possible activation, of CD4 cells can then result in activation and increased number of CD8 cells.<sup>[25]</sup> Aldinucci & Pessina<sup>[18]</sup> demonstrated increased proliferative response and cytokine (INFy and IL-6) release when cells were expose to ELF-EMF. IL-6 activates Tcells and IL-2 production CD4 cells also produce IL-2 which, in turn, induces proliferation and differentiation of CD8 cells.<sup>[25]</sup> This could possibly be the outcome of the underlying biological process resulting in the increased CD4 values and also the increased CD8 values in group C. These results agree well with the results for the leukocyte parameters of the current study, confirming that the increased lymphocyte values were limited to the T-lymphocytes and that cytokine involvement in this process cannot be excluded.

The results from the current study revealed evidence of immune stimulation in mice after short-term exposure to the Immunent signal. Since the biological analysis involved cell counts, it is evident that the effect on the immune system is at cellular level. Specific cells that indicated this effect were the T-lymphocyte population. This was confirmed in both the full blood count and the immunophenotyping. The findings of the current study also suggest that the longer the duration of exposure, the greater the effect on the cell values. It should be kept in mind, however, that the exposure duration for the current study was limited to seven days. The researcher is of the opinion that the critical issue in this study was the dosimetry and the physical characteristics of the signal. Future investigations are advised which target the understanding of the biological mechanisms, using the Immunent exposure system and repeating all the experiments from the current study on higher animals, such as swine or primates. Further research on the mechanism of neutrophil involvement, specifically on cytokine level, is advised. Specific recommendations include a repeat of the current experiment and determining interleukin-2, interleukin-6 and interleukin-8 levels.

# CONCLUSION

From this study, it can be concluded that short-term (seven days) exposure to the Immunent signal can stimulate the immune response on a cellular level and that the duration of exposure plays a role on this effect. The subsequent health effects and potential therapeutic application could be beneficial, not only in health departments, but also in the farming industry where a significant amount of research has already produced evidence proving the benefits of the Immunent signal.

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