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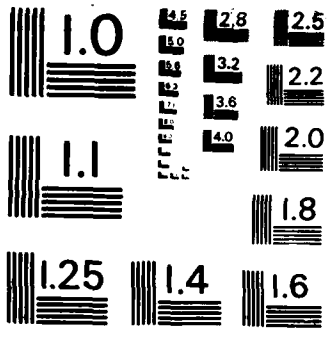

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MICROWAVES AND HUMAN LEUKOCYTE FUNCTION: EXPOSURE OF HUMAN MONONUCLEAR LEUKOCYTES TO MICROWAVE ENERGY PULSE-MODULATED AT 16 Hz OR AT 60 Hz

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NOTICES

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Human mononuclear leukocytes were exposed to 2450 MHz microwaves pulse-modulated at 16 Hz or at 60 Hz, at specific absorption rates up to 4 mW/ml. Such exposures produced no detectable effects on leukocyte viability, or on unstimulated or mitogen-stimulated DNA synthesis or total protein synthesis. The data provided no evidence that exposure to pulse-modulated microwaves is more likely to alter human leukocyte function than is exposure to continuous waves at equivalent energy levels.

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INTRODUCTION

All individuals are exposed to microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves (1-3). These studies were admitted by some of the authors to be poorly reproducible and nonquantitative. Many animal systems have been studied; but the species, microwave power intensities, environmental conditions, and other factors have varied so widely that extrapolation to humans would be exceedingly difficult, even if appropriate (4,5).

In a previous report, we provided data regarding exposure of human leukocytes to microwave energies (continuous wave) at specific absorption rates (SARs) up to 4 mW/ml (6). Exposures resulted in no detectable effects on viability or on unstimulated or stimulated deoxyribonucleic acid (DNA), ribonucleic acid (RNA), total protein, or interferon synthesis by human mononuclear leukocytes. In contrast to the studies cited, our results were highly reproducible.

More recently, investigators have reported that exposure to pulse-modulated microwaves, but not to the unmodulated carrier wave at an equal intensity, alters function of a murine cytotoxic leukocyte line (7). The modulation frequencies implicated included 16 Hz and especially 60 Hz. Some of the earlier studies regarding effects on human leukocytes used pulse-modulated microwaves (1,3).

The studies reported here have been performed to determine whether human leukocytes are affected by exposure to microwave energies pulse-modulated at 16 Hz or at 60 Hz. Exposures to microwave energy at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability, or on unstimulated or stimulated DNA or total protein synthesis by human mononuclear leukocytes. Our results provided no evidence that exposure to pulse-modulated microwaves is more likely to alter human leukocyte function than is exposure to continuous waves at equivalent energy levels.

MATERIALS AND METHODS

Cell Source and Collection of Blood

Peripheral venous blood was obtained by venipuncture from healthy young adult donors (5 male and 7 female, age range 24-35 years) who were taking no medication at the time of the study. Mononuclear leukocytes were obtained from the heparinized whole blood by Ficoll-Hypaque sedimentation (8). Mononuclear leukocytes obtained by this method consisted of 70% to 80% lymphocytes and 20% to 30% monocytes (9); both cell types are required for optimal responses to mitogens.

Except as noted in the following, leukocyte cultures were maintained in medium 199 (Gibco, Grand Island, N.Y.) with modified Earle's salts with glutamine, aqueous penicillin G (100 units/ml), and streptomycin (50 µg/ml). The medium was further supplemented with 10% autologous serum. For studies of total protein synthesis, leukocyte cultures were maintained in leucine-free minimum essential medium (MEM) (Gibco, Grand Island, N.Y.).

Exposure and Sham-Exposure to Microwaves

The mononuclear leukocytes were exposed for two hours in a waveguide system to 2450 MHz microwaves at specific absorption rates (SARs) from 0.29 to 4 mW/ml. Microwaves were pulse-modulated at 16 Hz or at 60 Hz (duty cycle = 0.5).

The waveguide system used in these studies has previously been described in detail (10). Exposure and sham-exposure waveguides are located within a water-jacketed, 37°C CO_2 incubator. Temperature inhomogeneity within the cultures is prevented by continuous shaking of the shelf upon which the waveguides rest. Exposures and sham-exposures were monitored continuously by use of Vitek Electrothermia nonperturbing probes (Vitek, Inc., Boulder, Colo.). No attempt was made to counteract microwave-induced heating of the leukocyte cultures, since we wished to observe any potential microwave-induced effects, thermal or otherwise.

The SARs were determined by analysis of steady-state temperature increments, ΔTss (11). The SAR was the product of: the specific heat (0.97 cal/°C/g); the steady-state temperature increment (°C); and the cooling constant (0.0838/min). The SARs (in milliwatts per milliliter) in this exposure system could be estimated by the product: 5.67 x ΔTss. During prolonged exposures, changes in the thermal environment were expected. The relation between the SAR and the steady-state temperature increment was best represented by a constant (4.63), determined empirically by use of culture medium exposed at absorbed doses between 5 and 45 mW/ml.

The changes in steady-state temperature (mean $\pm$ S.E.) of cultures exposed to microwaves at SARs of 0.29 and 4 mW/ml were 0.03°C $\pm$ 0.03 and 1.04°C $\pm$ 0.06, respectively.

Assays of Leukocyte Viability

Leukocyte viability was determined, from 1 to 7 days after exposure or sham-exposure to microwaves, by use of total cell counts and assays for percent of cells able to exclude trypan blue dye or ethidium bromide (9). The assays of leukocyte function (described in the following) provided additional and even more substantial evidence of leukocyte viability. Mitogen-stimulated lymphocyte transformation responses of human mononuclear leukocytes require participation of both viable monocytes and viable lymphocytes (9).

Assays of DNA and Total Protein Synthesis

Unstimulated and mitogen-stimulated DNA and total protein synthesis by the mononuclear leukocytes were assayed, using established methods,

by cellular incorporation of the tritiated precursors thymidine and leucine, respectively (6,9,12). In brief, mononuclear leukocytes were added to quadruplicate wells of sterile microtiter plates (Costar, Cambridge, Mass.) at a concentration of 5×10^5 cells/ml (1×10^5 cells per well). To the cell cultures were added medium alone, or medium containing phytohemagglutinin (PHA)-M (Difco, Detroit, Mich.). The final volume of the cultures was 0.2 ml per well. PHA was added at an optimal concentration of 160 µg/ml, shown to yield maximum lymphocyte transformation responses with control mononuclear leukocytes (9,13), and at a representative suboptimal concentration (20 µg/ml). Microtiter plates were then incubated at 37°C in 5% CO_2 and air. Cultures were pulsed with the tritiated precursor for the terminal 5 hr of incubation, and were harvested with a semiautomatic cell harvester (Brandel, Inc., Gaithersburg, Md.). Samples were counted with a liquid scintillation counter. Cells were pulsed and harvested from immediately ("zero" days) to 5 days after exposure or sham-exposure to microwaves. For each individual experiment, arithmetic counts per minute (cpm) of quadruplicate cultures were determined.

The absolute counts per minute of tritiated precursor incorporated by the normal (control) PHA-stimulated cells of different individuals varied, as previously established (13). Within each individual experiment, however, the relative responses of microwave-exposed and sham-exposed mononuclear leukocytes were consistent.

Statistical Analyses

Analysis of variance and *Student*'s t-test were used for analysis of the data. There were no significant differences between microwave-exposed leukocytes and sham-exposed leukocytes for any of the assays presented in the Results section.

RESULTS

Leukocyte Viability

In initial studies, mononuclear leukocytes were exposed to 16 Hz pulse-modulated microwaves at an SAR of 0.29 mW/ml, an energy level reported to be associated with pulse-modulated microwave-induced alterations in other functions (14). Exposure of the leukocytes at such an SAR produced no significant changes in cell viability for up to one week after exposure (data not shown). Equivalent viability of microwave-exposed and sham-exposed leukocytes was also noted with exposure of the cells to 16 Hz (Table 1) or to 60 Hz (Table 2) pulse-modulated microwaves at an SAR of 4 mW/ml.

DNA and Total Protein Synthesis

Unstimulated and mitogen-stimulated DNA and total protein synthesis were first examined after exposure of the mononuclear leukocytes to microwaves at an SAR of 0.29 mW/ml, pulse-modulated at 16 Hz. Exposures resulted in no alterations in leukocyte function (data not shown).

TABLE 1. TOTAL VIABLE MONONUCLEAR LEUKOCYTES AFTER EXPOSURE TO MICROWAVE ENERGY PULSE-MODULATED AT 16 Hz (SAR = 4 mW/ml)[a]

	Days After Exposure			
Exposure	1	2	4	7
Microwave	112 ± 24[b]	101 ± 21	82 ± 19	84 ± 18
Sham	108 ± 18	114 ± 21	85 ± 17	84 ± 16

[a]Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

[b]Data represent mean total number of viable cells (total cells x percent viable x 10^{-4} ± S.E.).

TABLE 2. TOTAL VIABLE MONONUCLEAR LEUKOCYTES AFTER EXPOSURE TO MICROWAVE ENERGY PULSE-MODULATED AT 60 Hz (SAR = 4 mW/ml)[a]

	Days After Exposure			
Exposure	1	2	4	7
Microwave	93 ± 19[b]	91 ± 12	64 ± 6	59 ± 5
Sham	102 ± 11	86 ± 7	63 ± 3	60 ± 5

[a]Viability was assayed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

[b]Data represent mean total number of viable cells (total cells x percent viable x 10^{-4}, ± S.E.).

In further experiments, mononuclear leukocytes were exposed to microwaves at an SAR of 4 mW/ml, again pulse-modulated at 16 Hz. The microwave-exposed and sham-exposed leukocytes subsequently showed equivalent unstimulated and mitogen-stimulated DNA synthesis (Fig. 1) and total protein synthesis (Fig. 2).

Leukocytes exposed to microwaves at an SAR of 4 mW/ml, pulse-modulated at 60 Hz, were also assayed for DNA and total protein synthesis. There were no significant differences between the microwave-exposed and sham-exposed mononuclear leukocytes in unstimulated or mitogen-stimulated DNA synthesis (Fig. 3) or total protein synthesis (Fig. 4).

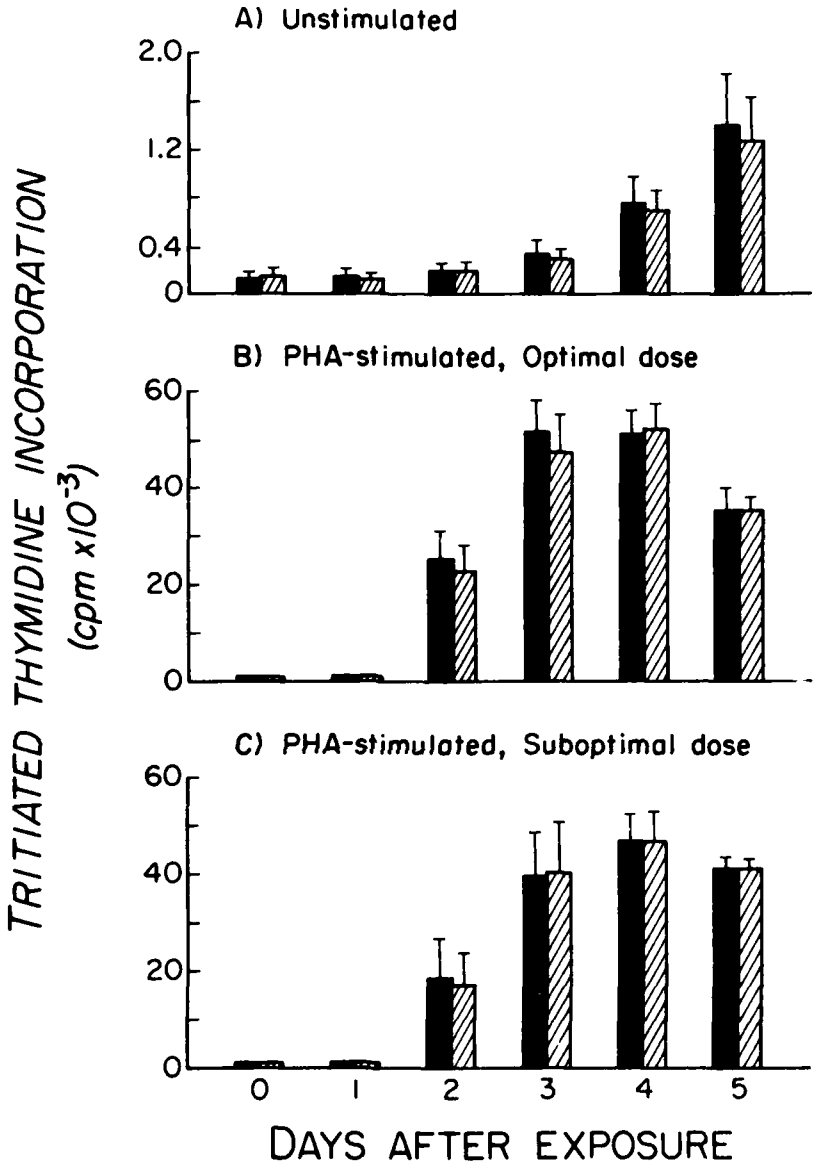

Figure 1. DNA synthesis by microwave-exposed (■) and sham-exposed (▨) human mononuclear leukocytes. Cells were exposed to 2450 MHz microwaves, pulse-modulated at 16 Hz, at an SAR of 4 mW/ml. Unstimulated DNA synthesis and DNA synthesis stimulated with the optimal and suboptimal concentrations of PHA are shown. Columns indicate mean cpm tritiated thymidine incorporation, ± S.E.

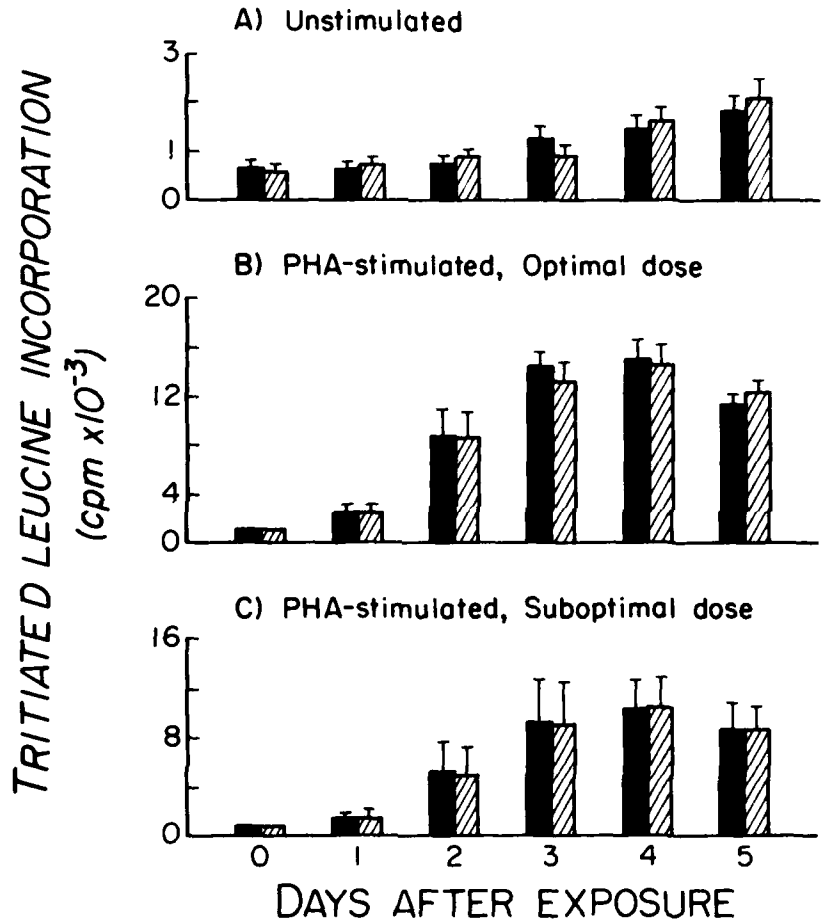

Figure 2. Total protein synthesis by microwave-exposed (■) and sham-exposed (▨) human mononuclear leukocytes. Cells were exposed to 2450 MHz microwaves, pulse-modulated at 16 Hz, at an SAR of 4 mW/ml. Unstimulated total protein synthesis and total protein synthesis stimulated with the optimal and suboptimal concentrations of PHA are shown. Columns indicate mean cpm tritiated leucine incorporation, + S.E.

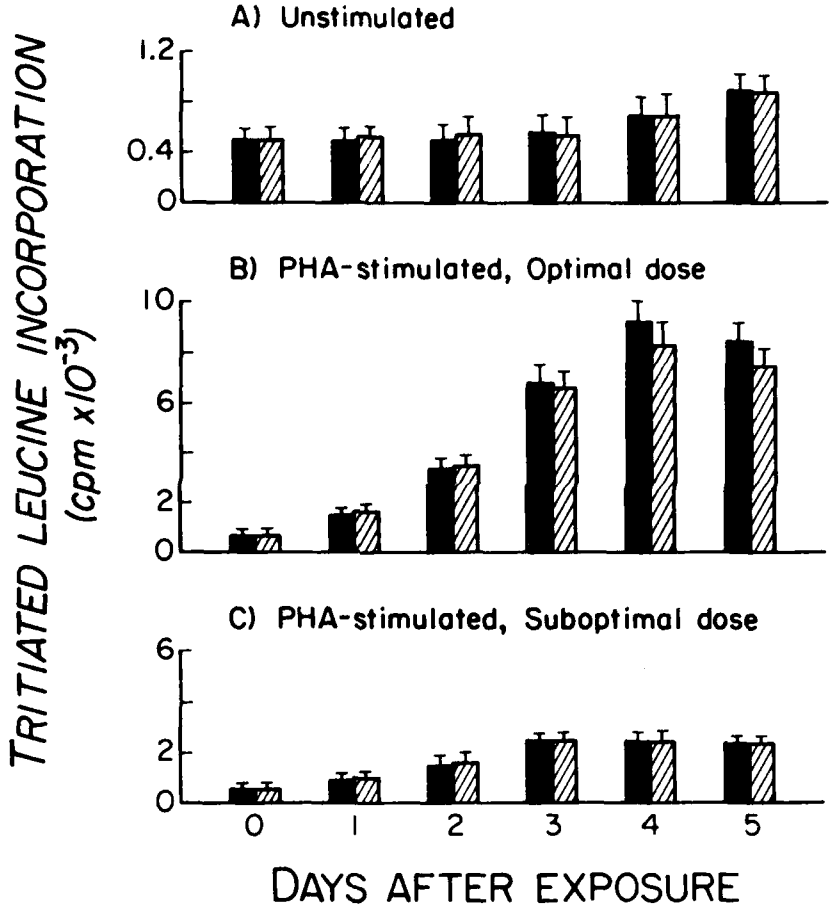

Figure 3. DNA synthesis by microwave-exposed (■) and sham-exposed (▨) human mononuclear leukocytes. Cells were exposed to 2450 MHz microwaves, pulse-modulated at 60 Hz, at an SAR of 4 mW/ml. Unstimulated DNA synthesis and DNA synthesis stimulated with the optimal and suboptimal concentrations of PHA are shown. Columns indicated mean cpm tritiated thymidine incorporation, ± S.E.

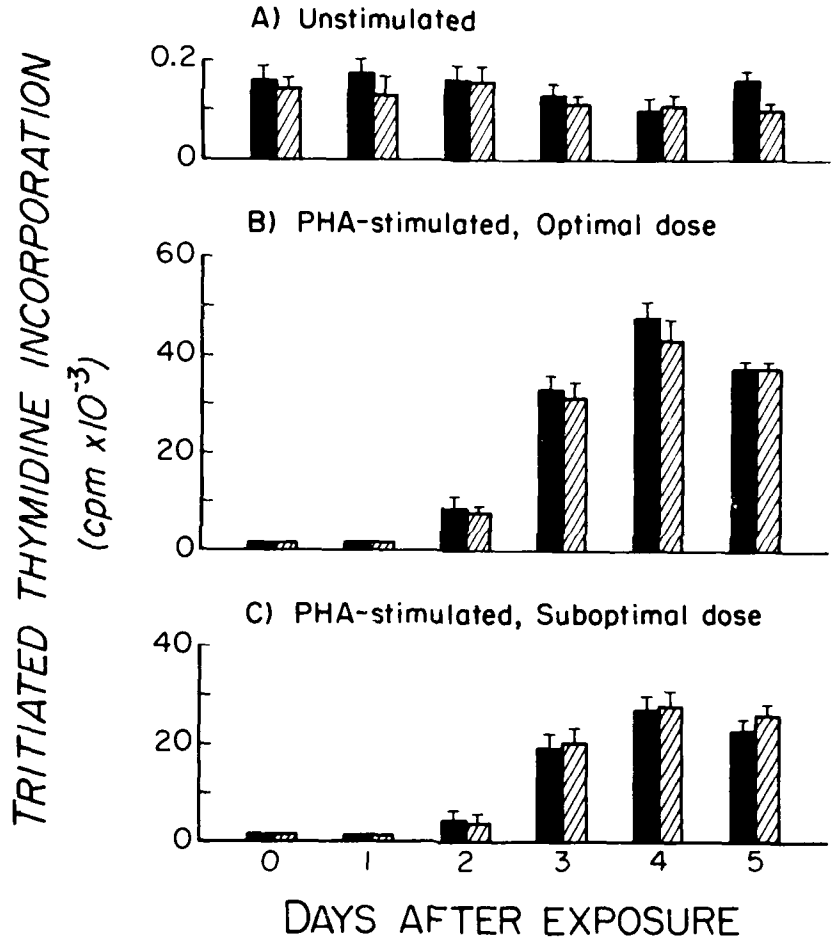

Figure 4. Total protein synthesis by microwave-exposed (■) and sham-exposed (▨) human mononuclear leukocytes. Cells were exposed to 2450 MHz microwaves, pulse-modulated at 60 Hz, at an SAR of 4 mW/ml. Unstimulated total protein synthesis and total protein synthesis stimulated with the optimal and suboptimal concentrations of PHA are shown. Columns indicate mean cpm tritiated leucine incorporation, + S.E.

DISCUSSION

The data from these studies, combined with our earlier observations (6), provide no evidence for a differential susceptibility of human mononuclear leukocytes to microwave energy that is pulse-modulated at 16 Hz or at 60 Hz. Microwave-exposed and sham-exposed leukocytes showed equivalent viability, and equivalent unstimulated or mitogen-stimulated DNA and total protein synthesis.

Effects of sinusoidally amplitude-modulated microwaves on leukocyte cytotoxic function have been reported by others, using a murine lymphoma target cell line and a murine cytotoxic T-lymphocyte line (7). Cytotoxicity was reversibly inhibited, with recovery by 12.5 hours or less after exposure (measured field intensity = 10 mW/cm^2). The detection and magnitude of inhibition varied with the modulation frequencies tested (from 0 to 100 Hz). Suppression of cytotoxicity was maximum at 60 Hz modulation, with smaller and inconsistent effects at 16 Hz. Approximately 20% inhibition was reported with 60 Hz modulation. The effector cell:target cell ratio, or ratios, were not reported, and could not be determined from the description of methods. It is unclear whether suppression of cytotoxicity was observed at all effector:target cell ratios, or was inconsistent. Of note was the significant variability of release of radiolabeled chromium in assays using established cell lines for targets and for effectors: cpm released, from experiments with equivalent microwave exposures, ranged 885 cpm to 5,582 cpm. It is unknown whether human leukocyte cytotoxic functions would be altered by exposure to pulse-modulated microwaves; conclusions derived from murine investigations cannot be ascribed routinely to humans (4).

The current studies do not exclude potential microwave-induced effects on human leukocyte function resulting from exposure at similar SARs, but applied by almost innumerable different possible wave forms (frequencies, modulations, etc.). The literature regarding microwaves includes animal studies reporting beneficial effects attributed to exposure, as well as animal studies reporting deleterious effects, over a broad range of SARs (4). The ubiquitous distribution of microwave energy, and the potential differences between animal models and humans suggest that further investigations with human leukocytes and other cells are warranted.

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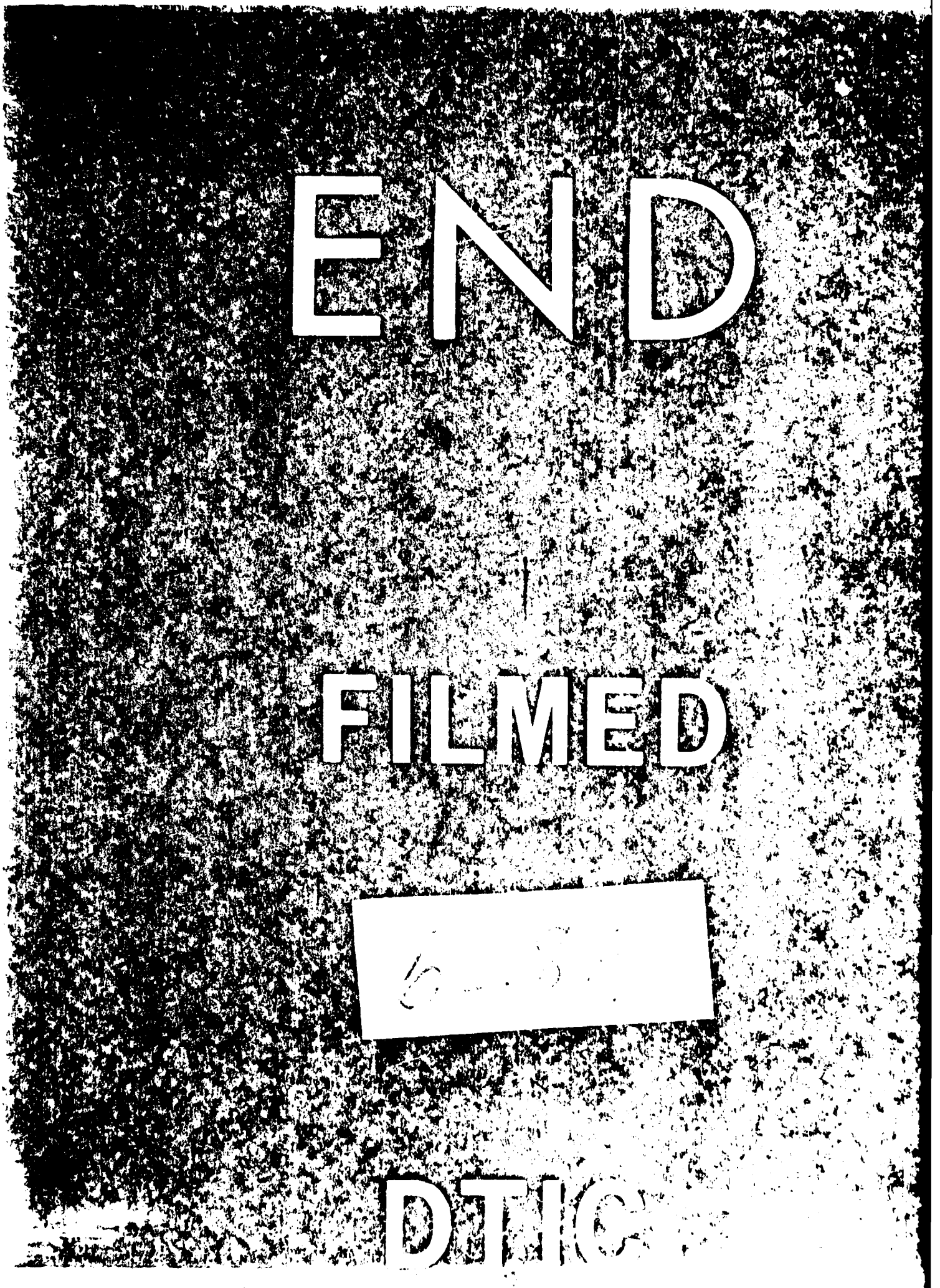
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