Some Results on Low-Level Microwave Treatment of the Mountain Pine Beetle and the Darkling Beetle

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Abstract—Various stages of the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) and pupae of the darkling beetle (*Tenebrio molitor* L.) were treated in waveguide and in "free space" with a wide range of doses of low-level microwave radiation at 10, 35, and 74 GHz. The insects were found to be more resistant to radiation than previously reported in *T. molitor* for similar and weaker doses. It is suggested that this lack of response could be due to different physiological conditions in the *T. molitor* population studied.

I. Introduction

ABNORMAL DEVELOPMENT and reduced survival of the darkling beetle (*Tenebrio molitor* L.) induced by irradiation with low-level microwaves reported by Carpenter and Livestone [1] and later by others [2], [3] prompted us to seek a similar effect on the mountain pine beetle (*Dendroctonus ponderosae*, Hopk.). The latter beetle is the most important destructive insect in merchantable lodgepole pine in western North American forests [4], [5]. Direct control strategies for preventing beetle epidemics call for felling and burning infested trees or treating them with toxic chemicals [6]. Our investigation of the effects of low-level microwave radiation on this beetle was an attempt to provide an environmentally acceptable and, perhaps, a less costly alternative for controlling this forest pest. It is, however, noteworthy that treating various forms of life, including insects other than *T. molitor*, with low-level microwaves has shown results ranging from no effect to various degrees of functional and/or developmental effects that may or may not be permanent [7]–[14].

In this work, initially, various life stages of the mountain pine beetle (MPB) were treated with microwave doses similar to those used by Carpenter and Livestone [1]. As little or no damaging effects were observed, we then repeated their work on *T. molitor* as closely as possible, and also with some modifications such as using free-space radiation and other microwave frequencies. No significant damaging effects were observed. Our results, however, are similar to those of Lessard *et al.* [15], who also did not observe significant developmental damage of *T. molitor* pupae irradiated at comparable low levels of microwaves.

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In the following, we describe our experiments, summarize the results, and suggest possible reasons for the differences between our findings and those of other investigators.

II. The Experiment

Measurements were performed at (nominal) frequencies of 10, 35, and 74 GHz. The experimental arrangement of the microwave apparatus was very similar to that used in [1]–[3], when the insects were irradiated inside the waveguide at 10 GHz. "Free-space" experiments were also performed at 10 GHz to allow the simultaneous irradiation of a large number of insects. In these experiments, the insects were placed on a shallow styrofoam tray which was, in turn, placed on a microwave absorber and they were irradiated by a microwave horn placed a short distance (about 2.5 cm) away. At 35 and 74 GHz, only free-space experiments were conducted; the small sizes of the waveguides at these frequencies did not make it feasible to conduct experiments with the insects inside the waveguide. Several sets of measurements were performed. Most of the experiments were on pupae of both insects but some involved MPB eggs and larvae.

Mountain pine beetle eggs, larvae, prepupae, and pupae of known age were obtained from artificially reared stocks [16]. This rearing system provides highly uniform individuals of all life stages of the insect. Pupae of unknown ages were excavated from field infested bolts. All stages were reared individually to adults and ten days after eclosion of controls, each insect was examined at $7\times$ magnification.

Pupae of *T. molitor* of known ages were obtained from two batches of mature larvae obtained from a local supplier. Approximately 2000 newly received larvae were transferred to wheat bran about 5 cm deep in a $50\times50\times8$ cm stainless steel tray. A 2-mm^2 mesh wire seive was used to separate larvae and pupae from the original diet. Fresh peel from a medium size orange was embedded in the bran and the surface was moistened with atomized distilled water. The insects were incubated in the dark at $24\pm1°$C and 35 to 50-percent relative humidity.

Each day, all the insects were sieved out of their diet. Pupae were reserved in bran in a time-labelled container and held at $24\pm1°$C until used. Dead or moribund pupae and larvae were discarded so that only healthy larvae were returned to the diet. Fresh orange peel was added and the

TABLE I
INITIAL OBSERVATIONS ON MOUNTAIN PINE BEETLE PUPAE EXPOSED IN WAVEGUIDE TO VARIOUS DOSES OF CW MICROWAVE POWER AT 10 GHz

Test[a,b]	Applied Power mW	Duration h	Average Power Density[c] mW/cm²	Dose mW/cm²·h	Treated: Malformed[d]	Treated: Dead[e]	Treated: Normal Adults	Control: Malformed[d]	Control: Dead[e]	Control: Normal Adults
1	20	0.5	8.6	4.3	0(0.0%)	0(0.0%)	10(100.0%)	0(0.0%)	0(0.0%)	10(100.0%)
2	10	1.5	4.3	6.5	0(0.0%)	0(0.0%)	10(100.0%)	0(0.0%)	1(10.0%)	9(90.0%)
3	20	1.0	8.6	8.6	1(10.0%)G3	2(20.0%)	7(70.0%)	0(0.0%)	1(10.0%)	9(90.0%)
4	10	3.0	4.3	12.9	0(0.0%)	0(0.0%)	10(100.0%)	1(10%)G3	0(0.0%)	9(90.0%)
5	20	2.0	8.6	17.2	0(0.0%)	1(10.0%)	9(90.0%)	0(0.0%)	0(0.0%)	10(100.0%)
6	20	2.0	8.6	17.2	4(21.0%)3G3 G1	1(10.0%)	15(79.0%)	0(0.0%)	0(0.0%)	16(100.0%)
7	100	0.5	43.0	21.5	1(10.0%)G1	1(10.0%)	9(90.0%)	0(0.0%)	0(0.0%)	10(100.0%)
8	50	1.5	21.5	32.3	0(0.0%)	0(0.0%)	10(100.0%) }[f]	0(0.0%)	0(0.0%)	10(100.0%)
9	100	1.5	43.0	64.6	0(0.0%)	0(0.0%)	10(100.0%)			
10	50	3.0	21.5	64.6	0(0.0%)	0(0.0%)	10(100.0%) }[f]	0(0.0%)	0(0.0%)	10(100.0%)
11	100	3.0	43.0	129.1	1(10.0%)G1	1(10.0%)	9(90.0%)			
Total for all powers and durations					7(5.8%)	6(5.0%)	109(91.6%)	1(1.0%)	2(2.0%)	93(97%)

[a]Ten pupae per test treatment, except 19 exposed and 16 controls in test 6; tests 1–6 low power, pupae $\frac{1}{2}$–3 days old, tests 7–10 higher power, pupae $\frac{1}{2}$–5 days old; five pupae in a polyethylene container (beem capsule) with a polyurethane plug; two capsules per test, side by side along the waveguide as shown in Fig. 1.
[b]Empty polyethelene containers with polyurethane foam plugs in the waveguide had no significant effect on the wave (power reflection coefficient < 0.00 transmission coefficient 0.999).
[c]Incident power density at the locus of the specimens equals applied power, mW/cross-sectional area of waveguide, cm²; waveguide inside dimensions—2.286 × 1.016 cm.
[d]Teratogenic categories as in [1], except G3 included additional malformations such as knobs on leg joints.
[e]Mortality 10 days after eclosion of adults and included any malformed individuals that had died.
[f]Same control group for each dose.

TABLE II
OBSERVATION OF *Tenebrio molitor* TREATED IN WAVEGUIDE WITH CW MICROWAVE POWER AT 10 GHz[g]

Test	Applied Power mW	Duration h	Average Power Density[c] mW/cm²	Dose mW/cm²·h	Treated: Malformed[d]	Treated: Dead[e]	Treated: Normal Adults	Treated: Total Insects Used	Control: Malformed[d]	Control: Dead[e]	Control: Normal Adults	Control: Total Insects Used	Age Hours
12	20	2.0	8.6	17.2	2(50.0%)G2 G1	3(75.0%)	1(25.0%)	4	0(0.0%)	0(0.0%)	4(100.0%)	4	48
13,14	40	2.0	17.2	34.4	7(46.7%)5G3 2G1	7(46.7%)	4(26.7%)	15	2(25.9%)G2 G1	3(37.5%)	3(37.5%)	8	2
15	10	2.0	4.3	8.6	0(0.0%)	0(0.0%)	4(100.0%)	4					
16	20	2.0	8.6	17.2	3(10.0%)G3	0(0.0%)	27(90.0%)	30 }[f]	1(3.3%)G1	0(0.0%)	29(96.7%)	30	21
17	100	2.0	43.0	86.1	0(0.0%)	0(0.0%)	2(100.0%)	2					
18	20	2.0	8.6	17.2	0(0.0%)	1(12.5%)	7(87.5%)	8	0(0.0%)	1(25.0%)	3(75.0%)	4	24
Total for all powers					12(19.0%)	11(17.5%)	45(71.4%)	63	3(6.5%)	4(8.6%)	41(89.1%)	46	

[c–f]See footnotes of Table I.
[g]In tests 12–17, pupae were placed individually in #00 gelatin capsules, two side by side along the waveguide, head towards the source. In test 18, single pupae were placed in styrofoam blocks almost identical with those used in [1]. Controls were similar but no power applied.

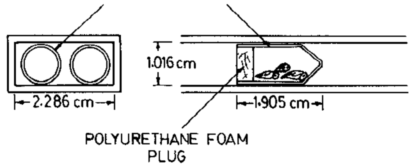

Fig. 1. Arrangement for tests 1–11, Table I.

surface moistened again. After microwave treatment, pupae were individually held in loosely capped, numbered, 25-ml glass vials containing approximately 1 cm of bran, for up to two weeks at 24 ± 1°C. Eclosion of untreated adults occurred nine days after pupation. All individuals were examined at 7× magnification, ten days after treatment.

A. Initial Waveguide Measurements on Mountain Pine Beetle at 10 GHz

In this experiment, mountain pine beetle pupae, 1/2–5 days old, were treated. Five pupae were placed in a polyethylene container with a polyurethane foam plug, two capsules per test, side by side inside an *X*-band waveguide, as shown in Fig. 1. The results are tabulated in Table I. The conditions under which treatment was administered are given as footnotes a–f.

B. Waveguide Measurements on Tenebrio molitor at 10 GHz

The objective of performing these measurements was to reproduce and to confirm the results in [1]–[3]. The results are summarized in Table II. In particular, test 18 was an attempt to replicate exactly the experimental arrangement and procedure in [1], in a case where the most pronounced radiation effects were observed in [1]. Specifically, this involved the application of 20 mW of microwave power for a period of 2 h.

C. Free-Space Irradiation of Tenebrio molitor at 10 GHz

This experiment was performed in order to allow many insects to be exposed to the microwave radiation at the

TABLE III
EXPOSURE OF YOUNG[h] PUPAE OF *Tenebrio molitor* IN FREE SPACE TO VARIOUS DOSES OF CW MICROWAVE POWER AT 10 GHz[i]

Test	Applied Power mW	Duration h	Approx. Average[j] Power Density[c] mW/cm^2	Dose $mW/cm^2 \cdot h$	Treated: Malformed[d]	Treated: Dead[e]	Treated: Normal Adults	Treated: Total	Control[k]: Malformed[d]	Control[k]: Dead[e]	Control[k]: Normal Adults	Control[k]: Total
19	100	2.0	1.8	3.6	3(12.0%)G2	2(8.0%)	20(80.0%)	25				
20	100	3.0	1.8	5.4	2(8.0%)G1	0(0.0%)	23(92.0%)	25}[f]	3(10.7)G3,2G1	0(0.0%)	25(89.3%)	28
21	100	4.0	1.8	7.2	0(0.0%)	1(4.2%)	23(95.8%)	24				
22,23	120	2.0	2.1	4.2	4(9.0%)2G3,2G1	2(4.5%)	38(86.5%)	44	3(13.0%)G3	2(8.7%)	18(78.3%)	23
24	160	2.0	2.9	5.8	7(14.0%)G3,G2,5G1	2(4.0%)	41(82.0%)	50	0(0.0%)	4(16.0%)	21(84.0%)	25
25,26,27	100	2.0	1.8	3.6	2(5.5%)G3,G1	4(11.1%)	30(83.4%)	36	3(6.1%)G1	6(12.2%)	40(81.6%)	49
Total for all powers and durations					18(8.8%)	11(5.3%)	175(85.8%)	204	9(7.0%)	12(9.6%)	104(83.2%)	125

[d–f]See footnotes of Table I.
[h]Tests 19–24, pupae 1 day old; tests 25–27, 2–7 days old.
[i]Specimens placed in a shallow styrofoam tray approximately 6.3×x8.3 cm, placed 2.5 cm from aperture of radiating horn (Fig. 2).
[j]Approximate average power density at the specimen, 2.5 cm from horn aperture = (applied microwave power)/(horn aperture area (cm^2)), aperture area = 56 cm^2.
[k]Controls exposed without microwaves for same periods except 19–21 where control was exposed 4 h.

TABLE IV
EXPOSURE OF MOUNTAIN PINE BEETLE PUPAE TO THREE FREQUENCIES OF MICROWAVES IN FREE SPACE[l]

Test	Frequency GHz	Applied Power mW	Duration h	Approx. Average[m] Power Density mW/cm^2	Dose $mW/cm^2 \cdot h$	Treated: Malformed[d]	Treated: Dead[e]	Treated: Normal Adults	Control: Malformed[d]	Control: Dead[e]	Control: Normal Adults
28	10	120	2.0	2.10	4.2	1(4.0%)G1	0(0.0%)	24(96.0%)			
29	35	30	2.0	0.7	1.4	1(4.0%)G3	1(4.0%)	23(92.0%)][f]	4(16.0%)G2,3G1	1(4.0%)	20(80.0%)
30	74	250	2.0	27.0	54.0	3(12.0%)G3,2G1	2(8.0%)	20(80.0%)			
31	10	60	2.0	1.05	2.1	0(0.0%)	1(4.0%)	24(96.0%)			
32	35	30	2.0	0.7	1.4	3(12.0%)G1,2G3	3(12.0%)	19(76.0%)][f]	2(8.0%)G1	1(4.0%)	22(88.0%)
33	74	12.5	2.0	1.35	2.7	4(16.0%)G1	1(4.0%)	20(80.0%)			
Totals for all frequencies and powers						12(8.0%)	8(5.3%)	130(86.7%)	6(12.0%)	2(4.0%)	42(84.0%)

[d–f]See footnotes of Table I.
[l]25 Pupae per test, various ages in each test excavated from naturally infested lodgepole pine trees.
[m]Approximate average power density at the specimen, 2.5 cm from horn aperture = (applied microwave power)/(horn aperture area (cm^2)); 10 GHz area = 56 cm^2, 35 GHz area = 43.2 cm^2, 74 GHz area = 9.24 cm^2.

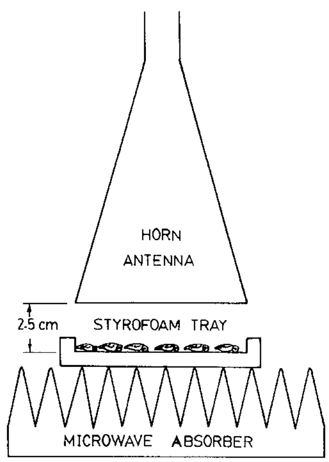

Fig. 2. Free-space irradiation arrangemer

same time and also to ascertain that placing the insects in "containers" in the waveguide did not influence any would-be effect on the irradiated beetles. The irradiating horn arrangement is shown in Fig. 2. The results are given in Table III. The average power densities in this case were small (2.9 mW/cm^2 maximum) relative to those used in [1] and in Section II-A but were much higher than those used in [3] and which were found to be effective in causing significant malformation.

D. Effect of Frequency

Because no significant effect was observed in the previous experiments when the beetles were subjected to 10-GHz microwave radiation, it was decided to look for possible effects at higher frequencies where the (free-space) wavelength λ_0 was comparable with the MPB size (approximately 6 mm). Facilities at two other frequencies were readily available, 35 GHz ($\lambda_0 \simeq 8$ mm) and 74 GHz ($\lambda_0 \simeq 4$ mm), and were used. The results on two samples at 10, 35, and 74 GHz are shown in Table IV.

E. Susceptibility of Other MPB Stages to Microwave Radiation

The possibility that other stages of development of the MPB might be more readily affected by low-level microwave radiation was also investigated. Some measurements were carried out on eggs and prepupal larvae. A summary of these measurements is given in Table V.

TABLE V
DEVELOPMENT OF MOUNTAIN PINE BEETLE EGGS AND LARVAE EXPOSED TO CW 10-GHz MICROWAVE RADIATION IN WAVEGUIDE

Test	Stage	Applied Power	Duration	Average Power Density[c]	Dose	Treated				Control			
		mW	h	mW/cm^2	mW/cm^2·h	Malformed[d]	Dead[e]	Normal Adults	Total Insects Used	Malformed	Dead[e]	Normal Adults	Total Insects Used
	Egg[n]												
	1					0(0.0%)	4(40.0%)	6(60.0%)	10	0(0.0%)	4(80.0%)	1(20.0%)	5
	2					0(0.0%)	4(40.0%)	6(60.0%)	10	0(0.0%)	0(0.0%)	5(100.0%)	5
34	3	20	2.0	8.6	17.2	0(0.0%)	2(20.0%)	8(80.0%)	10	0(0.0%)	3(60.0%)	2(40.0%)	5
	4					0(0.0%)	1(10.0%)	9(90.0%)	10	0(0.0%)	2(40.0%)	3(60.0%)	5
	All stages					0(0.0%)	11(2.75%)	29(72.5%)	40	0(0.0%)	9(45.0%)	11(55.0%)	20
	2					1(3.3%)G3	3(10.0%)	26(86.6%)	30	0(0.0%)	2(20.0%)	8(80.0%)	10
35	3	100	2.0	43.1	86.2	1(3.3%)G3	4(13.3%)	25(83.3%)	30	0(0.0%)	1(10.0%)	9(90.0%)	10
	Both stages					2(3.3%)G3	7(11.7%)	51(85.0%)	60	0(0.0%)	3(15.0%)	17(85.0%)	20
Total for all egg stages, all powers						2(2.0%)	18(18.0%)	80(80.0%)	100	0(0.0%)	12(30.0%)	28(70.0%)	40
Pre-pupal larvae													
36		20	2.0	8.6	17.2	1(6.3%)	0(0.0%)	15(93.7%)	16	0(0.0%)	0(0.0%)	18(100.0%)	18
37		100	2.0	43.1	86.1	0(0.0%)	3(8.6%)	32(91.4%)	35	0(0.0%)	2(5.5%)	34(94.5%)	36
Total for both powers						1(2.0%)	3(5.8%)	47(92.2%)	51	0(0.0%)	2(3.7%)	52(96.3%)	54

[c–e]See footnotes of Table I.
[n]Egg stages after [17].

TABLE VI
POST TREATMENT PERFORMANCE OF FIVE PAIRS OF MOUNTAIN PINE BEETLES[a]

Pair	Treated				Control				Days
	Size[b]		Average Daily Performance[c]		Size[b]		Average Daily Performance		
	Male	Female	Gallery cm	Reproduction	Male	Female	Gallery cm	Reproduction	
1	1.6362	1.9392	1.95	4.9	1.6362	1.9998	1.95	5.1	10
2	1.6968	1.8785	1.70	5.9	1.7574	1.9392	1.85	5.0	10
3	–[d]	1.9998	2.40	7.2	1.6968	1.9998	2.30	8.4	10
4	–[d]	1.9392	2.36	4.9	–[d]	1.9998	1.64	2.9	7
5	1.7574	1.9998	1.76	5.7	–[d]	2.0604	2.40	7.2	10
Average	1.6968	1.9513	2.03	5.72	1.6968	1.9998	2.03	5.72	

[a]Reared from prepupae exposed to 43 mW/cm^2 at 10 GHz for 2 h.
[b]Width of pronotum viewed at 6.3× to the nearest 0.05 mm.
[c]Calculated from gallery length and number of eggs in fresh bolts of lodgepole pine at 24±1°C.
[d]Males escaped after mating.

F. Performance of Treated MPB

In addition to observations on malformations, the beetles' post treatment reproductive performance was also studied. Table VI gives a sample of the performance of beetles which were irradiated at the prepupa stage with a comparatively large dose of microwave radiation. As indicated by this sample, the results generally show no change in the functional behavior of the adult beetles.

III. DISCUSSION

Tests 3 and 6 in Table I suggest reduced survival of MPB as a result of the microwave treatment. However, considering all tests in this series, there was 91.6-percent survival, a reduction of only 5.5 percent from survival in controls. It thus appears that there was no dose-response trend as a result of the treatment. We were also unable to potentiate teratogenesis in this insect.

The tests done to verify microwave-induced teratogenesis and mortality in *T. molitor* given in Tables II and III, with average waveguide doses ranging from 8.6 to 86.1 mWh/cm^2, resulted in an overall reduction of normal adults of approximately 20 percent compared to untreated controls. The results of tests 12 to 18, however, are variable and inconsistent, and there was no apparent overall reduction in production of normal adults. In test 18, Table II (with 20 mW/cm^2), the pupae were placed at the center of the waveguide and thus comparable with Table I in [1]. Calculating percentages in the same manner as used in [1], there was 87.5-percent normal adults produced in our experiment compared to 18.7-percent in [1]. In tests 12 and 16, where the insects were placed side by side in the waveguide, there were, respectively, 25 and 90-percent normal adults. In all cases, including test 12 where the sample was only four insects, the production of normal adults was higher than in [1], as shown in Table II.

In Table III, our data show that the microwave treatment slightly increased survival, with doses in the range 3.6 and 7.2 mWH/cm^2. This is compared to the results of Liu *et al.* [2] who obtained less than 50-percent survival at 2.34 mWh/cm^2. The increase in survival may or may not be significant; the point is that we did not observe similar teratogenic effects to those observed in [2].

Commenting generally on the results of Tables I, II, and III, teratogenesis in *T. molitor* is common and can be genetically and environmentally induced [18]–[20]. It follows that populations raised from various stocks and in different cultural conditions will have various potentials to respond to any factor (e.g., microwaves) which might affect the expression of teratogenesis. Thus, no amount of care or sophistication in the administration of microwave treatments would give reliably reproducible results unless the teratogenic potential was taken into consideration.

From the observations in the MPB given in Table IV, there appears to be, generally, no major effect at the various frequencies and doses used. Tables V and VI also indicate that there is no damaging effect of the microwave treatment on viability of eggs or larvae, nor on the size or reproduction performance of adults.

In all probability the reason for the lack of response of the mountain pine beetle to microwave radiation is the same as that for *T. molitor*. We cannot comment further because, to our knowledge, no other work has been reported on microwave-induced teratogenesis in mountain pine beetle.

In conclusion, our results indicate that the two insects considered were generally more resistant to low-level microwave radiation than reported in most previous investigations on *T. molitor*. However, our results, in conjunction with other carefully conducted studies which gave contrary evidence, suggest that the response to low-level microwave radiation is a strong function of the physiological condition of the cells and tissues being irradiated [21], [22]. This condition could, of course, vary widely and dynamically within a given species. This might also suggest that the setting of microwave safety standards should take into account the dynamic nature of the biological properties of the organisms being irradiated.

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Exposure of Human Mononuclear Leukocytes to Microwave Energy Pulse Modulated at 16 or 60 Hz

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Abstract—**Human mononuclear leukocytes were exposed to 2450-MHz microwaves pulse modulated at 16 or 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.**

I. Introduction

All INDIVIDUALS are exposed to microwave energies to variable degrees. Studies by several investigators 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 (SAR's) up to 4 mW/ml [6]. Such exposures resulted in no detectable effects on viability or on unstimulated or stimulated DNA, RNA, total protein, or interferon synthesis by human mononuclear leukocytes. In contrast to the studies cited above, 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 were performed to determine whether human leukocytes are affected by exposure to microwave energies pulse modulated at 16 or 60 Hz. Such exposures to microwave energy, at specific absorption rates up to 4 mW/ml, resulted in no detectable effects on viability, 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.

II. Materials and Methods

A. Cell Source and Collection of Blood

Peripheral venous blood was obtained by venipuncture from healthy young adult donors (five male and seven female, age range 24–35 years) who were taking no medi-

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