NAMRL - 1283

MICROWAVE-INDUCED DEVELOPMENTAL DEFECTS IN THE COMMON MEALWORM (<u>Tenebrio molitor</u>) - A DECADE OF RESEARCH

Richard G. Olsen

December 1981

NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY
PENSACOLA, FLORIDA

MICROWAVE-INDUCED DEVELOPMENTAL DEFECTS IN THE COMMON MEALWORM (*Tenebrio molitor*) - A DECADE OF RESEARCH

Richard G. Olsen

Naval Medical Research and Development Command
MR041.08-01-0002

Reviewed by

Ashton Graybiel, M.D.
Chief Scientific Advisor

Approved and Released by

Captain W. M. Houk, MC, USN
Commanding Officer

9 December 1981

NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY
NAVAL AIR STATION
PENSACOLA, FLORIDA 32508

SUMMARY PAGE

THE PROBLEM

More than a decade ago, Carpenter and Livstone conducted the first experiments that demonstrated microwave-induced effects in Tenebrio molitor, the common mealworm. Results of those experiments were interpreted to show a "nonthermal" microwave effect. Even though subsequent experiments in other laboratories have produced substantial evidence against any "nonthermal" mechanism per se, many researchers in the area of electromagnetic bioeffects still consider the "nonthermal" effects in insects, as first published in 1971, to be valid. It was important that additional experiments be conducted and the cumulative results be rigorously analyzed to resolve this issue. The existence of a nonthermal biological effect would be of significant value to the theoretical study of microwave interaction mechanisms and would probably have an impact on the process of setting microwave exposure standards.

FINDINGS

Statistical analysis of the insect irradiation data shows no microwave-induced effects for exposures of up to 4 hours at dose rates of 63 watts/kilogram. Microwave irradiation, however, at higher intensities (102-126 W/kg) did produce statistically significant effects when applied over a 2-4 hour period. These results are compatible with known microwave interaction mechanisms.

RECOMMENDATIONS

As outlined in this report, experiments in the area of microwave-induced developmental effects in insects have used acute irradiation parameters where the microwave insult is produced over a period of several hours. So far only a thermal effect has been reliably demonstrated. To more fully explore this area, long-term microwave exposure at subthermal levels is needed. The microwave energy could be applied at a dose rate of approximately one W/kg over the entire 8-day pupation period. The results of such experiments would be significant because the entire period of pupation would be irradiated and no strictly thermal effect would occur.

INTRODUCTION AND BACKGROUND

Initial experiments in this area were conducted more than a decade ago at Tufts University (2). Those experiments subjected the insect pupae to 10-GHz irradiation inside a standard WR-90 waveguide transmission system. It was found that 120-min irradiations at a power level of 20 mW and above caused a significant level of defects to be observed in the adult beetles when they emerged approximately 12 days later. The defects occurred mostly in the abdominal region of the insects and were manifested as shriveled abdomen, wings, or elytra or perforations in the elytra. Deaths of the exposed pupae were also observed. In the initial study, it was estimated that microwave irradiation produced a 3 °C temperature rise in the pupae; so, a thermal control was devised to subject pupae to an infra-red heat source to produce an overall temperature rise commensurate with the results of microwave exposure. The thermal controls exhibited no developmental effects; therefore, Carpenter and Livstone (2) concluded that the observed microwave-induced anomalies were nonthermal effects.

Later, at Washington University in the laboratory of F. J. Rosenbaum, more experiments were conducted (8) using a system similar to Carpenter's. Closed-system radiometric measurements in those 9.0 GHz experiments showed that the pupae absorbed about one-third of the total forward waveguide power, resulting in a specific absorption rate (SAR) of 41 W/kg at a power level of 20 mW. Although the control population showed a 36 percent defect rate in Lindauer's experiments compared to a 10 percent value in Carpenter's study, statistically significant microwave effects were observed at the power level used by Carpenter. Also in those experiments, pulsed microwave energy at an average value of 20 mW appeared to produce the same effects as continuous-wave (CW) irradiation.

A year later Liu, Rosenbaum, and Pickard published a report showing a constant-dosage effect in the irradiated pupae (9). A product of waveguide power times duration of irradiation of 4 mW-h produced an equivalent total number of developmental anomalies over the range of 0.25 h (at 16 mW) to 8 h (at 0.5 mW). Duration of pupal state was shown to be longer for irradiated insects in agreement with Carpenter and Livstone's earlier observations. It was further reported that significant increases in teratogenesis were observed at waveguide power levels down to 200 μW for 2-h exposures. Implicit in these results was the possibility that microwave irradiation was a cumulative teratogen with a low intensity threshold.

In 1975 a research project was initiated at the Naval Aerospace Medical Research Laboratory (NAMRL) to study bioeffects of the isolated individual microwave fields (electric and magnetic), using small insects as subjects in a standing wave irradiation system (11). A frequency of 4.0 GHz was originally selected because the electric-to- magnetic field spacing would be about 2 cm for the reflected free-space plane-wave and because a power level of between 100 and 200 watts was available at 4.0 GHz. Since the earlier waveguide experiments were conducted with a nominal 415 volts per meter electric field intensity, the 200 watts of power at 4.0 GHz were deemed to be sufficient to produce any desired level of effects.

A culture of <u>Tenebrio molitor</u> larvae was procured and nurtured into a relatively large culture consisting of approximately one thousand larvae at any given time. The insects were nourished with a high-protein dairy meal and sliced raw potatoes. Early in the development of the insect culture, it was observed that newly emergent, dormant pupae were quickly attacked by the hungry (and mobile) larvae. The larvae tended to eat the tender legs and wing buds of the helpless pupae such that a significant fraction of them would be damaged if left in the main culture more than a few hours after pupation. The traumatized portions of the unfortunate pupae would turn black in time and would typically result in defective adult insects.

The selection protocol for pupae to be used in experiments was to gather freshly emergent specimens each morning and inspect them under 10-power magnification. Those pupae without visible defect were then used in experiments on the following day. After use in an irradiation or a control experiment, the insects were carefully placed in small ventilated serum vials for the balance of the pupal period. Several days after the imago had evolved, the insects were examined for morphological defects.

It is believed that the careful handling and close inspection of pupae in the NAMRL experiments constituted a significant development, for the percent defective controls was very low (1 percent) in contrast to the greater than 30 percent damaged control group presented in the Washington University data (8).

Results of the initial 4-GHz insect irradiations at NAMRL showed very few defects even though the E-plane insects were subjected to a SAR of 29.1 W/kg for as long as 6 hours (11). The irradiations were continued, using a frequency of 5.95 GHz at which 1000 watts of power were available. The initial 5.95-GHz experiments produced many developmental defects but only for very high, thermal dose rates. Few defects were seen for SAR's less than 53 W/kg; therefore, it was concluded that only thermal effects existed for the standing wave irradiation system at 5.95 GHz.

Presentation of the initial NAMRL results at the Annual USNC/URSI Meeting in October, 1976, in Amherst, MA, aroused interest in the Washington University workers in this area. Within a year, W. F. Pickard visited NAMRL and conducted irradiations of Tenebrio pupae; he used several protocols in an effort to resolve whether or not truly nonthermal effects existed (14). In separate experiments, Pickard used insects from the NAMRL culture and insects obtained and handled as those used in previous Washington University experiments. Pickard found that the NAMRL insects exhibited far fewer irradiation-induced defects than the other group which was nourished only with Kellogg's Special "K" and potatoes and was sifted daily to separate the new pupae. Pickard found, moreover, that the smooth plastic cups used in Rosenbaum's laboratory that held each pupa during the transition to adulthood were a source of developmental anomalies. In such cups, the newly formed adults could not right themselves, and they would often become "stuck" to the bottom of the cup during metamorphosis.

During this time, Liu theoretically modeled X-band absorption in a waveguide-mounted Tenebrio pupa (10), and Green initiated further insect irradiation experiments at Washington University to study other factors that might cause developmental anomalies in insects (4). Liu's theoretical analysis used a homogeneous pupa model and showed that maximum microwave absorption should occur at the surface facing the incident irradiation for longitudinally oriented (K-polarized) pupae. Green's experiments used two types of pupal subjects: those without visible blemish (ideal) and those with a nominal amount of visible damage (nonideal). It was seen in Green's data that the "nonideal" insects seemed to be much more susceptible to microwave-induced effects than "ideal" pupae. The "nonideal" pupae, moreover, accounted for 60 percent of a typical experimental population, and use of such imperfect subjects produced a relatively large (approximately 30 percent) fraction of dead insects in the controls.

Green's experiments continued to study the effects of relative humidity and very high microwave power levels on the Tenebrio pupa (5). It was shown in that report from Rosenbaum's laboratory on this topic that statistically significant microwave-induced effects were seen only at relative humidities of 35 percent or less. The initially observed "nonthermal" microwave effect described by Lindauer et al. (8) was thus modified to a "nonthermal-nonideal-low humidity" microwave effect as described by Green et al. (5).

The fact that the presence of other environmental factors had to be included in order to show significant microwave effects greatly weakened Green's argument for a nonthermal mechanism. In the case of "nonideal" subjects, the microwave heating could accelerate the damage already initiated in the pupae, and in low relative humidities, microwave heating could remove needed moisture from the pupae and make them more vulnerable to disease or death.

At NAMRL, the final experiments in this area involved three questions: 1) Could the cumulative nature of the microwave insult be verified using our methods? 2) What was the microwave absorption distribution in waveguide-mounted pupae? 3) What was the statistical significance of the standing wave irradiation experiments involving insect pupae? To answer the first question, constant-dose experiments, conducted at 1123 joules/gram in irradiation lasting from 1.5 to 24 h, showed significant microwave effects (at 5.95 GHz) only for conditions where the pupal temperature exceeded about 40 °C (13). Reciprocity, as reported by Liu et al. (9), was definitely not observed in the results. The NAMRL insect experiments, therefore, do not support the notion that microwave photons are a cumulative teratogen.

To answer the second question, a thermographical analysis of waveguide-irradiated insects was conducted to more fully understand the dosimetry of that configuration (12). Three waveguide systems (L-, C-, and X-band) were used in experiments that recorded the ventral surface temperature distribution for pupae as they were being irradiated. At X-band, thermographic analysis of longitudinally oriented pupae showed maximum heating to be located close to the front surface near the head-thorax interface. The overall profile of thermographically determined absorption was similar to the theoretical predictions of Liu et al. (10). Also significant in the thermographical results was the finding that the ratio of peak-to-average X-band absorption for pupae was about 2.0; therefore, an average pupal SAR of 41 W/kg (at 20 mW) would have localized regions of twice that value. This feature of the X-band dosimetry further weakened the argument for nonthermal microwave effects in insects.

To answer the third question, experiments similar to those initially reported (11) were continued at NAMRL in order to statistically determine the significance of the data. This report contains a compilation of those data along with the results of statistical analysis. It was shown that deleterious microwave effects occurred only where significant thermal burdens were placed on the pupae. It is concluded that those studies conducted elsewhere, exhibiting so-called nonthermal microwave-induced developmental defects, were conducted in the absence of careful culturing and handling protocols such that more than one insult was simultaneously imposed on the subjects. After a close examination of the data, one is hard pressed to accept anything but a thermal basis for the reported effects.

PROCEDURE

A complete description of the apparatus and procedure has been presented in an earlier report (11). The pupae were irradiated in the standing wave of a microwave beam such that some of them were positioned in the plane of the maximum electric field while others were positioned in the plane of the maximum magnetic field. The standing wave was produced by reflecting a plane wave at normal incidence from a metal plate that was suspended in the far-zone of a horn-irradiated anechoic chamber.

Of central importance in these experiments was the protection afforded the insect subjects at all stages. The meticulous selection process and appropriate pupation containers have already been mentioned, but other precautions were also taken. During irradiation, for example, the pupae were carefully placed in ventilated gelatin capsules so that no abrasion could arise from the foamed polystyrene holding blocks that were used to precisely position the pupae in the respective planes. The pupae were never touched by fingers or manipulated with pressure-type holding instruments. This care in handling and husbanding of the subjects resulted in the lowest rate of defects in the sham-irradiated controls of any published data in this area to date.

In catagorizing the defects, the scheme of Carpenter and Livstone (2) was used in examining the adult beetles several days after they emerged:

- D Insect died during pupation;
- G1 Insect developed head and thorax of an adult, but retained the abdomen of a pupa, sometimes with pupal case attached;
- G2 Adult insect had rumpled and grossly distorted elytra and/or shredded wings;
- G3 Adult insect was normal except for small discrete holes in elytra;
- N Adult insect was apparently normal.

Statistical analysis of the results calculated the probability of a chance occurrence of defects for each experimental configuration. This calculation was based on the comparison of two binominal populations for those instances in which the expected number of defects was five or greater (1). The calculation was based on the Fisher Test of exact probability for those instances in which the expected number of defects was less than five (15). Comparison of two binomial populations tested the hypothesis that there was a difference in proportions (normal versus non normal) between the two groups (irradiation group versus the pooled controls). The Fisher Test of exact probability was used to compare the proportion of defects found in the pooled sham-irradiation experiments with the proportion of defects of certain insect exposures.

RESULTS

A summary of the sham-irradiation experiments is given in Table I. Each of the experimental orientations and durations was duplicated in a control experiment in which handling and other physical processes were the same as in irradiation experiments. Table I shows a very low incidence of defects compared to previously reported work from other laboratories (2,8).

Table II gives the irradiation results and statistical comparison to the pooled controls for vertically oriented pupae; that is, for those experiments in which the pupal long axis was parallel with the electric field. Very high SAR was observed for pupae vertically located in the E-plane, and the highest percentage of microwave-induced pupal deaths was seen in the vertical orientation. The statistical analyses of the data of Table II compared to the pooled controls showed a significant microwave effect (or the relatively low probability ($p \leq 0.05$) of a chance occurrence of defects) only for those durations and intensities that allowed a strong thermal build-up in the pupae.

Table III gives the results for horizontally oriented pupae. Here again, significant effects of the irradiation are seen only for the higher intensities and/or durations that allow the pupal temperature to exceed a threshold of approximately 40 °C (11).

TABLE I

Summary of Sham-Irradiation Experiments

Group	Time in Chamber	D	G1	G2	G3	Normal	Total N
Horizontal Orientation	2 hours	1	0	0	3	138	142
	4 hours	0	0	0	2	141	143
	6 hours	0	0	1	1	22	24
	8 hours	0	0	0	0	24	24
Vertical	15 minutes	0	0	0	0	47	47
	30 minutes	0	0	0	0	23	23
	2 hours	0	0	0	0	24	24
	4 hours	0	0	0	0	72	72
	6 hours	0	0	0	0	48	48
Combined Controls		1	0	1	7	539	547

Conditions Seen in Adult Insects: D, G1, G2, G3, Normal

Table II

Results of Irradiation at 5.95 GHz and Vertical Orientation of Pupae

Duration and SAR	Location	Average Dosage J/g	Conditions seen in Adult Insects					Total N	Percent Defective	Probability of Chance Occurrance of Defects
			D	G1	G2	G3	Normal			
30 minutes										
806-960 W/kg	E-Plane	1589	30	2	0	0	4	36	89%	< .001*
126-150 W/kg	H-Plane	248	5	1	0	2	28	36	22%	< .003*
15 minutes										
806-960 W/kg	E-Plane	75	17	5	0	1	13	36	64%	< .005*
126-150 W/kg	H-Plane	124	0	1	0	1	33	35	6%	0.11†
5 minutes										
806-960 W/kg	E-Plane	265	2	0	0	0	58	60	3%	0.21†
126-150 W/kg	H-Plane	41	0	0	1	2	57	60	5%	0.08†

* Based on the comparison of two binomial populations

† Based on the Fisher Test of exact probability

TABLE III

Results of Irradiation at 5.95 GHz and Horizontal Orientation of Pupae

Duration and SAR	Location	Average Dosage J/g	Conditions seen in Adult Insects D	G1	G2	G3	Normal	Total N	Percent Defective	Probability of Chance Occurance of Defects
1 hour										
212-252 W/kg	E-Plane	835	0	0	0	4	8	12	33%	.022*
204-243 W/kg	H-Plane	805	0	0	0	5	7	12	42%	.005*
4 hours										
106-126 W/kg	E-Plane	1670	14	3	2	5	47	71	34%	<.001*
102-122 W/kg	H-Plane	1610	13	3	5	6	45	72	35%	<.001*
2 hours										
106-126 W/kg	E-Plane	835	0	2	2	4	50	58	14%	.007*
102-122 W/kg	H-Plane	805	0	2	0	1	56	60	7%	24*
4 hours										
53-63 W/kg	E-Plane	835	0	0	0	2	46	48	4%	0.17†
51-61 W/kg	H-Plane	805	0	0	0	0	0	48	0%	0.46†
2 hours										
53-63 W/kg	E-Plane	418	0	0	0	0	48	48	0%	0.46†
51-61 W/kg	H-Plane	403	0	0	0	0	48	48	0%	0.46†

* Based on the comparision of two binomial populations
† *Based on the Fisher Test of exact probability*

DISCUSSION

In contrast to previous reports on this topic from other laboratories, statistically significant microwave effects were observed only for irradiation intensities and/or durations that raised the pupal temperature significantly. In developing the statistical methods used in these analyses, the pooled population of control subjects was used. This pooling of control insects was done in all our previously reported experiments in this area. It serves to increase the statistical power of the various tests employed in comparing the irradiation and control data.

That environmental relative humidity played a role in observed microwave effects is a valid proposition. This factor was only recently submitted as an explanation of why effects were observed at certain times and not at others. Aggravating environmental factors have long been known to shift thresholds of microwave effects in animals (3,7). For insects, low relative humidity is a particularly critical environmental factor (6).

The use of "nonideal" subjects as reported by Green, et al. (4,5) was unfortunate. To study properly microwave-induced effects is to study controlled populations of exposed and nonexposed subjects. The introduction of insects known to have suffered observable physical damage of subjective extent is seen as an unnecessary complication of an otherwise simple comparison. It appears that little knowledge can be gained from experiments that use "nonideal" subjects when the definition of "nonideal" is very broad and subjective.

The original report of Carpenter and Livstone (2) in terming the observed microwave effect as "nonthermal" was also in error to a certain extent, since a generalized thermal change was observed in irradiated subjects. Even though equivalent effects could not be produced by simple heating of the pupae inside a small oven, it was not rigorously correct to term the microwave effect "nonthermal" because very little was known at that time about the localized distribution of the absorbed energy. It was later shown that the experimental configuration used by Carpenter and Livstone produced a highly nonuniform heating profile in the insect. In such a configuration, an average temperature rise of 3 °C due to irradiation would produce localized regions of twice that temperature rise.

REFERENCES

1. Byrkit, D.R., Element of Statistics. Second Ed. New York: D. Van Nostrand Co., 1975.

2. Carpenter, R.L., and Livstone, E.M., Evidence for non-thermal effects of microwave radiation: abnormal development of irradiated insect pupae. Trans. IEEE Microwave Theory Tech. MTT-19: 173-178, 1971.

3. Diechmann, W.B., Bernal, E., and Keplinger, M., Effect of environmental temperature and air volume exchange on survival of rats exposed to microwave radiation of 24,000 megacycles. In: Susskind, C. (Ed.), Proceedings of the Third Annual Tri-Service Conference on the Biological Effects of Microwave Radiating Equipments. Berkeley: University of California Printing Dept., 1959. Pp 62-67.

4. Green, D.R., Rosenbaum, F.J., and Pickard, W.F., Biological effects of microwaves on the pupae of Tenebrio molitor, In: Symposium on Biological Effects and Measurement of Radio Frequency, Microwaves. Proceedings of a conference held in Rockville, Maryland, February 16-18, 1977. HEW Publication (FDA) 77-8026. Rockville, MD: US Dept Health, Education and Welfare, Food and Drug Administration, 1977. Pp 253-263.

5. Green, D.R., Rosenbaum, F.J., and Pickard, W.F., Intensity of microwave irradiation and the teratogenic response of Tenebrio molitor. Radio Sci. 14(6S): 165-171, 1979.

6. Green, D.R., The relation of teratogenesis and survival in Tenebrio molitor to microwave irradiation during metamorphosis. M.S. thesis. Washington University, St. Louis, MO, 1978.

7. Houk, W.M., Michaelson, S.M., and Beischer, D.E., The effects of environment on thermoregulatory, serum lipid, carbohydrate, and growth hormone responses of rats exposed to microwaves (Abstract). In: USNC/URSI Program and Abstracts, 1975. P 309.

8. Lindauer, G.A., Liu, L.M., Skewes, G.W., and Rosenbaum, F.J., Futher experiments seeking evidence of nonthermal biological effects of microwave radiation. IEEE Trans. Microwave Theory Tech. MTT-22: 790-793, 1974.

9. Liu, L.M., Rosenbaum, F.J., and Pickard, W.F., The relation of teratogenesis in Tenebrio molitor to the incidence of low-level microwave. IEEE Trans. Microwave Theory Tech. MTT-23: 929-931, 1975.

10. Liu, L.M., Rosenbaum, F.J., and Pickard, W.F., Electric-field distribution along finite length lossy dielectric slabs in waveguide. IEEE Trans. Microwave Theory Tech. MTT-24: 217-219, 1976.

11. Olsen, R.G., Insect teratogenesis in a standing-wave irradiation system. Radio Sci. 12(6S): 199-207, 1977.

12. Olsen, R.G., and Hammer, W.C., Thermographic analysis of waveguide-irradiated insect pupae. Radio Sci. In Press.

13. Olsen, R.G. Constant-dose microwave irradiation of insect pupae, Radio Sci. In Press.

14. Pickard, W.L. and Olsen, R.G., Development effects of microwaves on <u>Tenebrio</u>: Influences of culturing protocol and of carrier frequency. <u>Radio Sci.</u> 14(6S): 181-185, 1979.

15. Siegel, S., <u>Nonparametric Statistics for the Behavioral Sciences</u>. New York: McGraw-Hill Book Co., Inc. 1956.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: NAMRL - 1283	2. GOVT ACCESSION NO.: AD-A11 8822
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): Microwave-Induced Developmental Defects in the Common Mealworm (*Tenebrio molitor*) - A Decade of Research	5. TYPE OF REPORT & PERIOD COVERED: Final
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Richard G. Olsen	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Naval Aerospace Medical Research Laboratory, Naval Air Station, Pensacola, Florida 32508	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: MR041.08-01-0002
11. CONTROLLING OFFICE NAME AND ADDRESS: Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, Maryland 20014	12. REPORT DATE: 9 December 1981
	13. NUMBER OF PAGES: 12
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microwave Effects, Insect Teratogenesis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Microwave-induced developmental effects in insects have been studied at several laboratories during the past decade. Results of the initial experiments were interpreted to show a "nonthermal" microwave effect, but as more studies were conducted by various investigators, a predominantly thermal effect appeared to be the best explanation. This report presents the results of a comprehensive series of insect irradiation experiments including a rigorous statistical analysis of the data. Statistical analysis shows no microwave-induced effects for exposure of up to 4 hours at dose

DD FORM 1473, 1 JAN 73 — EDITION OF 1 NOV 65 IS OBSOLETE

S/N 0102-LF-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) rates of 63 watts/kilogram. Irradiation at higher intensities (102-126 W/kg) did produce statistically significant effects when applied over a 2-4 hour period.