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Interactions of Radiofrequency Radiation-Induced Hyperthermia and 2-Methoxyethanol Teratogenicity in Rats

B.K. Nelson,* David L. Conover, Edward F. Krieg, Jr, Diana L. Snyder, and Richard M. Edwards

Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, Ohio

Radiofrequency (RF) radiation is used in a variety of workplaces. In addition to RF radiation, many workers are concurrently exposed to numerous chemicals; exposed workers include those involved with the microelectronics industry, plastic sealers, and electrosurgical units. The developmental toxicity of RF radiation is associated with the degree and duration of hyperthermia induced by the exposure. Previous animal research indicates that hyperthermia induced by an elevation in ambient temperature can potentiate the toxicity and teratogenicity of some chemical agents. We previously demonstrated that combined exposure to RF radiation (10 MHz) and the industrial solvent, 2-methoxyethanol (2ME), produces enhanced teratogenicity in rats. The purpose of the present research is to determine the effects of varying the degree and duration of hyperthermia induced by RF radiation (sufficient to maintain colonic temperatures at control [38.5], 39.0, 40.0, or 41.0 °C for up to 6 h) and 2ME (100 mg/kg) administered on gestation day 13 of rats. Focusing on characterizing the dose-response pattern of interactions, this research seeks to determine the lowest interactive effect level. Day 20 fetuses were examined for external and skeletal malformations. The results are consistent with previous observations. Significant interactions were observed between 2ME and RF radiation sufficient to maintain colonic temperatures at 41 °C for 1 h, but no consistent interactions were seen at lower temperatures even with longer durations. These data indicate that combined exposure effects should be considered when developing both RF radiation and chemical exposure guidelines and intervention strategies. *Bioelectromagnetics 18:349–359, 1997.* © 1997 Wiley-Liss, Inc.

Key words: RF radiation; industrial solvents; glycol ethers; developmental toxicity; synergism

INTRODUCTION

Radiofrequency (RF) radiation may be developmentally toxic in humans [Marchese, 1953; Rubin and Erdman, 1959; Cocozza et al., 1960; Hoffman and Dietzel, 1966; Imrie, 1971; Brown-Woodman et al., 1988]. In experimental animals, the teratogenicity of RF radiation is associated with the degree and duration of hyperthermia it produces [Lary et al., 1982; 1983; Lary and Conover, 1987]. Hyperthermia produces congenital malformations in experimental animals, and it has been hypothesized to produce malformations in humans [Edwards, 1986; Lary, 1986; Warkany, 1986; Kimmel et al., 1993; Edwards et al., 1995]. The threshold for producing developmental toxicity appears to be a 2.5 °C elevation of core temperature for short term or acute exposures [Germain et al., 1985; Lary et al., 1986]. Lary et al. [1983] found that maintaining colonic temperature at 42 °C for 15 min or 41 °C for 2 h significantly increased developmental toxicity in rats.

Temperature can affect both adult [Keplinger et al. 1959; Weihe, 1973; Goldstein et al., 1990] and developmental [Ferm and Kilham, 1977; Fraser, 1977; Ferm and Ferm, 1979; Hanlon and Ferm, 1986; Shiota et al., 1988] toxicity of chemicals. Several kinds of radiation have been observed to affect the developmental toxicity of drugs and chemicals [Nelson, 1994]. For example, Marcickiewicz et al. [1986] reported that microwave radiation (2450 MHz; at 1 and 10 mW/cm^2 [Specific Absorption Rate (SAR)= 0.4–0.5 and 4–5 W/kg, respectively; no detectable colonic temperature rise] or 40 mW/cm^2 [SAR = 16–18 W/kg; producing a 1.5–2 °C rise in colonic temperature] for 2 h daily throughout gestation) enhances the teratogenicity

*Correspondence to: B.K. Nelson, Division of Biomedical and Behavioral Science, NIOSH C-24, Cincinnati, OH 45226.

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of cytosine arabinoside in mice. The report by Chiang et al. [1995] suggests that even low levels of radiation (pulsed 15.6 kHz; 40 μT 4 h day on gestation days 7–16; no detectable rise in colonic temperature) may enhance the teratogenicity of this same drug in mice.

Nelson et al. [1997] briefly reviewed three industries in which workers are potentially exposed to both RF radiation and industrial chemicals [Stewart and Elkington, 1985; Cohen, 1986]. 1) The electronics industry [Cone, 1986], where several reports suggest an elevated risk of reduced fertility and spontaneous abortions [Rudolf and Swan, 1986; Paustenbach, 1988; Pastides et al., 1988; Schenker, 1996; Correa et al., 1996; Gray et al., 1996; Hammond et al., 1996; Lumm et al., 1996; Pinney and Lemasters, 1996; Swan and Forest, 1996]). 2) The dielectric heating industry (where some glycol ethers, toluene, xylene, and other chemicals are frequently found in areas in which RF radiation is used to weld or seal vinyl materials [Conover et al., 1981; Cox and Conover, 1981; Cox et al., 1982]. 3) Operating rooms where electrosurgical units (ESUs) are used extensively to cut and coagulate tissues (high RF radiation exposures have been documented in these units [Fox et al., 1976; Ruggera, 1977] and several potentially teratogenic anesthetic gases are found [Schardein, 1993]; elevated rates of congenital malformations and spontaneous abortions have been reported for female operating room personnel or wives of male anesthetists [ASA, 1974; Goldstein et al., 1976].

The large numbers of people exposed to RF radiation along with various chemicals, coupled with literature reports of toxic interactions between RF radiation and several chemicals, suggested the need to investigate combined exposures. Given the background information cited above, it is curious that neither the ''physical agent'' community nor the ''chemical agent'' community has addressed combined effects when developing exposure guidelines and intervention strategies. We initially reported enhanced teratogenicity when rats received short-term, high level RF radiation exposure (maintaining colonic temperature at 42.0 °C for 30 min) along with the industrial solvent 2-methoxyethanol (2ME; 150 mg/kg by gavage) on gestation day 13 [Nelson et al., 1991]. This glycol ether was chosen as a model compound because of its well-characterized teratogenicity in experimental animals [NIOSH, 1983, 1991; Nelson et al., 1984, 1989; Hanley et al., 1984; Horton et al., 1985; Hardin and Eisenmann, 1987; Sleet et al., 1987, 1988] and speculated teratogenicity in humans [Bolt and Golka, 1990]. Furthermore, as discussed above, adverse reproductive effects have been reported after potential exposures to this or certain other glycol ethers in the semiconductor industry.

Subsequent research was undertaken to characterize the extent of synergism between RF radiation and 2ME [Nelson et al., 1994]. The interactive dose-related teratogenicity of RF radiation (sham exposure or maintaining colonic temperatures at 42.0 °C for 0, 10, 20, or 30 min) and 2ME (0, 75, 100, 125, or 150 mg/kg) was investigated by administering various combinations of RF radiation and 2ME to groups of rats on gestation days 9 or 13. Day 9 exposures generally evidenced little effect by 2ME, either by itself or in combination with RF radiation. In contrast, day 13 exposures resulted in highly significant effects from 2ME and RF radiation. The structures showing strong evidence of effects from both 2ME and RF radiation after exposure on gestation day 13 were the forepaw digits, forepaw phalanges, hindpaw digits, hindpaw phalanges, hind limbs, metacarpals, and metatarsals. A lowest interactive effect level was not determined in this study, as significant interactions were observed even at 75 mg/kg 2ME.

Consequently, a third study focused on the effects of a short-term RF radiation exposure (42 °C for 10 min) on the developmental toxicity of various doses of 2ME, with the primary observations on the forepaws after day 13 exposures. Interactive effects were observed even at the lowest levels of 2ME [Nelson et al., 1997]. We were unable to detect differences in 2ME pharmacokinetics due to RF radiation exposure [Cheever et al., 1997], which would have been the most straight-forward mechanism of interaction. In contrast with the previous studies, the present research focused on the interactive effects of 2ME on the developmental toxicity of longer-term (up to 6 h), lower-level (39, 40, or 41 °C) RF radiation exposure conditions. This made the RF exposure conditions more occupationally relevant.

Although this research is undertaken in rats, we would like, ultimately, to estimate the likelihood that the interactive effects of RF radiation and 2ME would be observed in humans at occupationally-relevant exposures. Since human data are not available either for the extent of possible core temperature increases produced by occupational exposure to RF radiation or for the blood levels resulting from occupational exposure to 2ME, this research has focused on determining the lowest effect level of teratogenicity for various combinations of RF radiation and 2ME exposures. A determination of this lowest effect level facilitates a more accurate risk assessment and/or intervention strategies for human exposures to these two agents.

MATERIALS AND METHODS

Our experimental animals, exposure system, and procedures were identical to those described previously

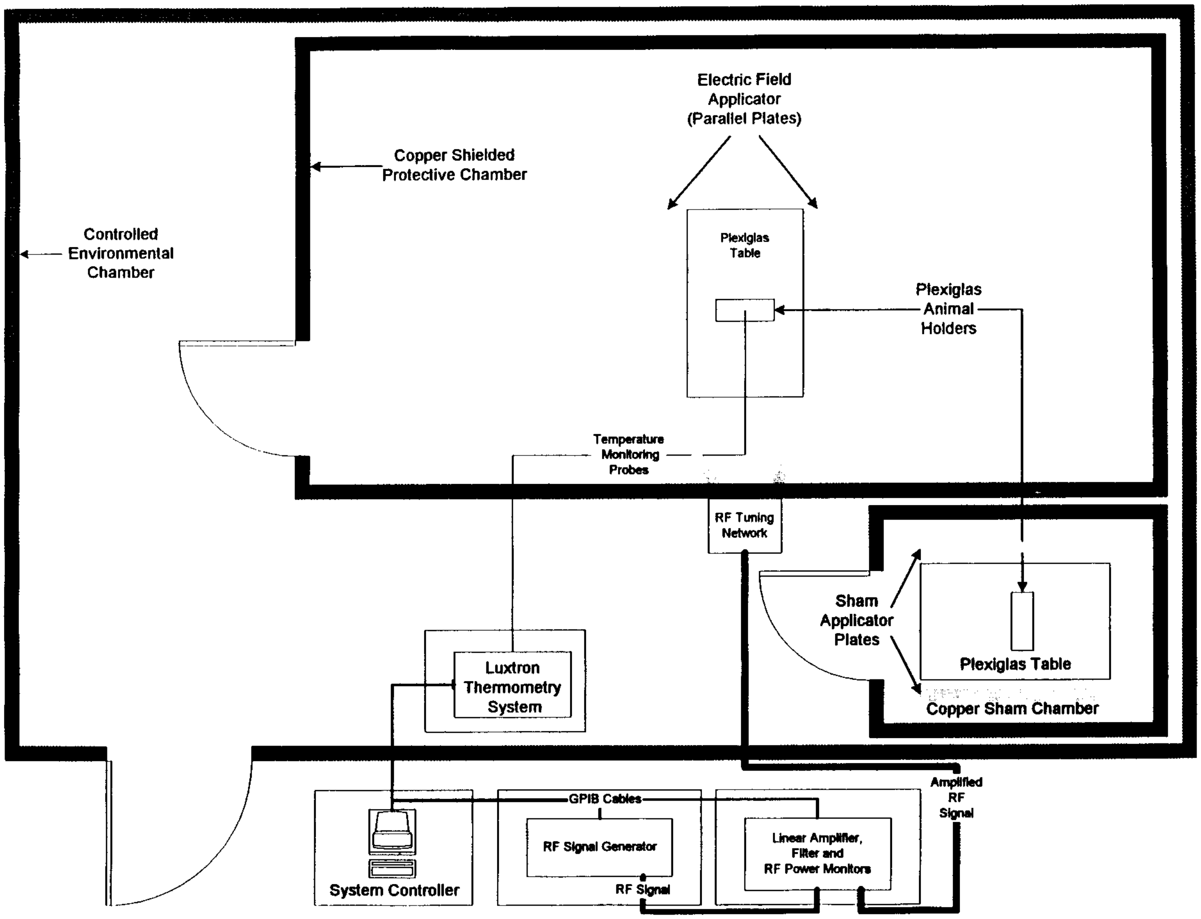

Fig. 1. Schematic diagram of the entire RF radiation exposure system.

[Nelson et al., 1994, 1996]. Briefly, virgin female (175–200g) and breeder male (275–300g) CD Sprague-Dawley rats (VAF/plus; Charles River Breeding Laboratories, Wilmington, MA) were maintained at 24 ± 2 °C and 50 ± 10% humidity. Feed was Ziegler® certified laboratory rat chow, with tap water available ad lib, and room lighting was on from 7:00 am to 7:00 pm During the 2-week quarantine period, quality control tests were conducted to ensure that rats used in the study were healthy and that specific pathogens were not introduced into the AAALAC-certified animal facility. For breeding, females were placed individually with males in the afternoon, and the paper under each male's cage was examined the following morning for vaginal plugs. Vaginal smears were taken from females having no vaginal plugs to evaluate the presence of sperm or estrus cycle of the female. Presence of vaginal plugs or sperm marked day 0 of gestation.

Figure 1 presents a a schematic diagram of the entire exposure system, and Figure 2 a drawing of the parallel-plate exposure setup. Rats were irradiated in RF near-field synthesizer facilities operated in the dominant electric field mode under continuous wave conditions at a frequency of 10 MHz. The frequency was controlled accurately (frequency resolution to 1 Hz at 10 MHz) by a Hewlett-Packard Model 8660C or 8660D synthesized signal generator. The signal from the generator was amplified by an Amplifier Research Model 1000L or 200L linear amplifier to provide power to the near-field synthesizer. The near-field synthesizers were enclosed within copper screen wire chambers (Ark Electronics Corp., Model A273 and PSS7S10) to reduce interference from outside RF radiation signals and to shield personnel against RF radiation exposure outside of the system. These copper wire chambers were housed in Forma Scientific Model 7010 or 74668 environmental chambers. All exposures, including sham exposures of control animals, were conducted at an ambient temperature of 24(±1.0)°C and a relative humidity of 50(±10)%. The air exchange rate in the 33 m^3 environmental chambers was approximately 0.4 m^3/minute, with non-detectable air velocity at the loca-

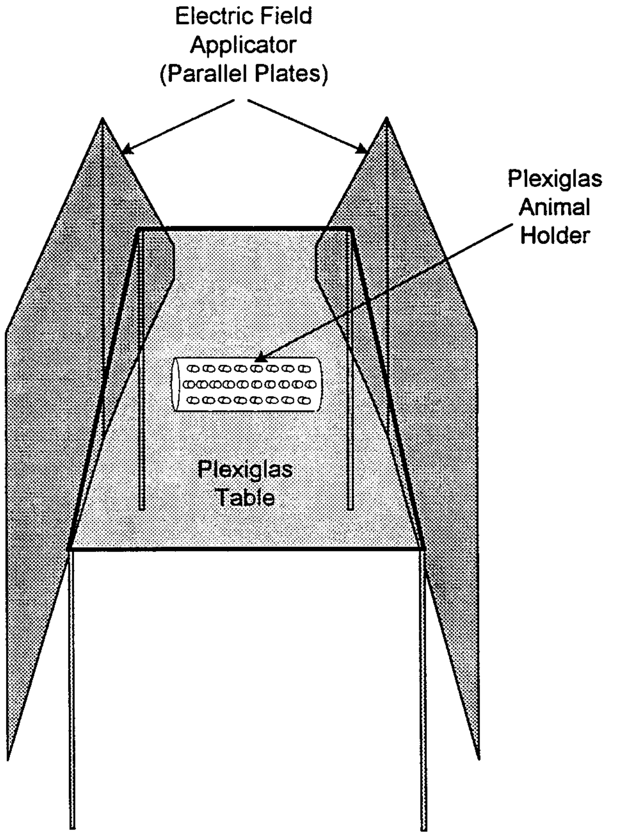

Fig. 2. Drawing of the parallel-plate RF radiation exposure setup.

tion of the animal. In the RF exposure chamber, the 60 Hz electric field strength ranged from 0.17 to 0.20 volts/meter and the magnetic flux density ranged from 0.02 to 0.06 μT. In the sham exposure chamber, the 60 Hz electric field strength ranged from 0.17 to 0.20 volts/meter and the magnetic flux density ranged from 0.05 to 0.18 μT.

Each rat was irradiated once, without anesthesia, in a cylindrical Plexiglas holder perforated with 12-mm holes. The holders were designed to prevent the rat from changing its orientation relative to the RF field and to allow circulation of air about the rat. Each rat was oriented so that its long axis (length) was parallel to the incident electric field so that maximum RF-induced heating was obtained. An RF-insensitive temperature probe (Luxtron Corp., model MPM) covered by a sterile closed-end catheter was inserted 5 cm into the animal's colon, and secured with elastic adhesive tape. The tail was taped under the abdomen, and the animal was placed in a mesh bag to prevent the tail and paws from protruding outside the animal holder. Computer-controlled systems monitored the colonic temperature of the irradiated rats and controlled the RF output power such that the colonic temperature was maintained to within ±0.2 °C of the target temperature. The output power of the RF radiation source initially was set to provide a SAR of 7.9 W/kg to raise the animal's colonic temperature from its normal baseline of approximately 38.5 to 39.0 °C, 40.0, or 41.0 °C (requiring approximately 20 min). Once the target colonic temperature was reached, the RF radiation output power was adjusted to maintain the target colonic temperature for the desired duration, with the SAR varying from 0.8 W/kg to 7.9 W/kg. We previously found that time-averaged SARs required to achieve or maintain colonic temperatures at 42.0 °C were not affected by 2ME exposure [Nelson et al., 1994].

Average whole-body SAR calibrations were conducted using numerical modeling [Durney et al., 1978], calorimetry [IEEE, 1991], and reflection coefficient techniques [Greene, 1977]. First, numerical modeling data were used to determine the SAR for rats. The electric field strength for exposed rats was determined at a constant RF output power of 250 W. An SAR was calculated using numerical modeling [medium rat (320 g), E-polarization at 10 MHz] and electric field strength data. Second, calorimetry (saline solution phantom model) was used to determine the SAR at 250 W. Third, reflection coefficient measurement techniques [Greene, 1977] developed at the National Bureau of Standards (NBS) were used to determine the SAR at 250 W. Due to the unique design of the exposure system developed at NBS [Greene, 1974], the average whole-body SAR for an exposed rat (or any exposed specimen) can be determined by measuring only the forward and reflected powers on the transmission line feeding the electric field applicator. SAR calibrations conducted with all three techniques were in good agreement.

For rats exposed in this study, average whole-body SAR was determined for each rat using numerical modeling techniques [Durney et al., 1978]. During exposure system calibration, the electric field strength for exposed rats was determined as a function of the net RF power (forward minus reflected power) delivered to the electric-field applicator. SAR was calculated using numerical modeling [medium rat (320 g), E-polarization at 10 MHz] and field strength data. A computer-automated system was used to determine average whole-body SAR.

A concentration of 100 mg/kg 2ME was prepared in distilled water based on administration of 10 ml liquid/kg, and was verified by flame ionization gas chromatography to be within 10% of the target concentration by the NIOSH Division of Physical Sciences and Engineering. Rats were gavaged with the specified dose of 2ME or distilled water immediately prior to

TABLE 1. Numbers of Animals in Exposure Design (Includes Groups of Rats Exposed to RF Radiation Sufficient to Maintain Colonic Temperatures at the Specified Level and Duration and Administered Distilled Water [dw] or 2ME [100 mg/kg] on Gestation Day (13)*

RF Radiation	RF Radiation Duration (Hours)					
Sham	0	1	2	3	4	6
Sham						
−*dw*	10[†]		10		10	10
−2*ME*	10[†]		10		10	10
39.0 °C						
−*dw*	10		10		10	10
−2*ME*	10		10		10	10
40.0 °C						
−*dw*	10	10	10	10	10	
−2*ME*	10	10	10	10	10	
41.0 °C						
−*dw*	10	10				
−2*ME*	10	10				

*Potential maternal overheating at colonic temperatures of 41.0 °C for longer than 1 h and at 40.0 °C for longer than 4 h prohibited including longer exposure durations at those temperatures.

[†]Maintained in sham condition to correspond with RF radiation at 41.0 °C for 0 h (based on the ''ramp time'' for approximately 25 animals during pilot studies, this was determined to be 20 min). The rats in the other cells in this column were maintained for 0 time after the specified colonic temperature was achieved.

preparing the animals for irradiation or sham irradiation (preparation time of 5–10 min).

Dams were sacrificed on day 20, and fetuses were removed serially. Fetuses were blotted dry, weighed, and examined for malformations by trained observers who were blind to the treatment conditions of the fetuses at the time of observation (''fresh tissue'' observations). The fetuses were preserved in ethanol. Subsequently, the fetuses were examined by independent observers for malformations of the forelimbs, hindlimbs and tail, including examination for internal and skeletal malformations (''fixed-tissue'' observations). They were eviscerated, singly stained for 24 h with alizarin red-S in 5% potassium hydroxide, 8 h in 5% potassium hydroxide, 18 h in 2 parts benzyl alcohol, 2 parts of 70% ethyl alcohol, and 1 part glycerin, and stored in 1 part ethyl alcohol and 1 part glycerin. Stained fetal skeletons were evaluated for the number of caudal ossification sites and bone malformations and variations using a technique modified from Staples and Schnell [1963]. Single-stained day 20 fetuses commonly have 1–2 metacarpals ossified in the forepaws and 3–4 metatarsals ossified in the hindpaws. Since the phalanges are not normally ossified by day 20, the paw malformations were best observed by a thorough external examination.

A factorial design with missing cells was utilized, with 2ME at 0 or 100 mg/kg, and RF radiation applied to maintain colonic temperatures for the durations shown in Table 1. Ten blocks of dams were successively run over a period of about one year. All treatment combinations appeared in each block. Dams were randomly assigned to treatment conditions within a block.

STATISTICAL METHODS

Analysis of Variance (ANOVA) was used to test for effects of Time, Temperature, 2ME, and their interactions. The model that was used is

$$X_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_1 + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\delta_{il} + \beta\gamma_{jk} + \beta\delta_{jl} + \gamma\delta_{kl} + \alpha\beta\gamma_{ijk} + \alpha\beta\delta_{ijl} + \alpha\gamma\delta_{ikl} + \beta\gamma\delta_{jkl} + \alpha\beta\gamma\delta_{ijkl} + \zeta(\alpha\beta\gamma\delta)_{m(ijkl)} + \varepsilon_{ijklm}.$$

where: X_n is the value of the dependent variable for a fetus

μ is the grand mean

α_i represents the random effect of block

β_j represents the effect of time (duration of exposure to RF radiation)

γ_k represents the effect of colonic temperature

σ_l represents the effect of 2ME

ζ_m represents the random effect of dam

and, ε_n is a random, independent residual effect of pups. Combinations of Greek letters indicate interactions, and parentheses indicate nesting. The main effects and interactions of interest were tested using the interaction

TABLE 2. Specific Absorption Rates (SAR; Mean W/kg ± standard deviation) of Groups of Rats Exposed to RF Radiation Sufficient to Maintain Colonic Temperatures at the Specified Level and Duration and Administered Distilled Water (*dw*) or 2*ME* (100 mg/kg) on Gestation Day 13

	RF Radiation Duration (Hours)					
RF Radiation	0	1	2	3	4	6
Sham						
−*dw*	—		—		—	—
−2*ME*	—		—		—	—
39.0 °C						
−*dw*	7.38 ± .49		1.20 ± .34		1.24 ± .69	1.55 ± .41
−2*ME*	7.90 ± .69		1.18 ± .26		1.37 ± .63	1.24 ± .41
40.0 °C						
−*dw*	7.67 ± .68	2.31 ± .54	1.80 ± .63	1.79 ± .46	1.88 ± .64	
−2*ME*	7.36 ± .51	2.10 ± .34	2.12 ± .42	2.08 ± .38	1.93 ± .44	
41.0 °C						
−*dw*	7.64 ± .63	2.65 ± .51				
−2*ME*	7.36 ± .39	2.75 ± .34				

with block that corresponded to it, e.g., the main effect of Time was tested with the Block × Time interaction. Contrasts were done if a main effect or interaction was statistically significant.

RESULTS

A separate publication reports that acute maternal weight loss during the RF radiation exposure increased with increasing duration and colonic temperature, but the 24-h weight loss was inconsistent, and no significant effects on overall pregnancy weight gain were observed [Snyder et al., 1997]. As we have observed before, 2ME did not affect SARs (Table 2).

Virtually all of the malformations we observed involved the forepaws as described previously by our laboratory [e.g., Nelson et al., 1994, 1996] and by others [e.g., Horton et al., 1985; Hardin and Eisenmann, 1987]. Routinely, the left forepaw has a higher incidence of malformation than does the right forepaw; hence these are separated. A few hindpaw malformations were observed.

Fresh Tissue Observations

The main effect of 2ME was significant for fetal weight [$F(1, 9) = 51.5$, $P = .0001$] (i.e., across all treatment conditions, 2ME consistently reduced fetal weights). The mean fetal weight (± Standard Deviation) of the combined groups receiving 100 mg/kg 2ME (3.12 ± 0.38 g; female = 3.04 g, male = 3.21 g) was less than the mean of those receiving distilled water (dw; 3.46 ± 0.39 g; female = 3.36 g, male = 3.55 g) (Figure 3).

The main effect of 2ME was significant for curvature in the left forepaw digits [$F(1, 9) = 5.5$, $P = .04$] (i.e., digit curvature can be primarily attributed to 2ME). The proportion of fetuses with curvature in the group receiving 100 mg/kg 2ME (0.02 ± 0.09) was greater than those receiving dw (0.003 ± 0.01).

The main effect of 2ME was also significant for curvature of the right forepaw digits [$F(1, 9) = 7.4$, $P = .02$]. The proportion of fetuses with curvature in

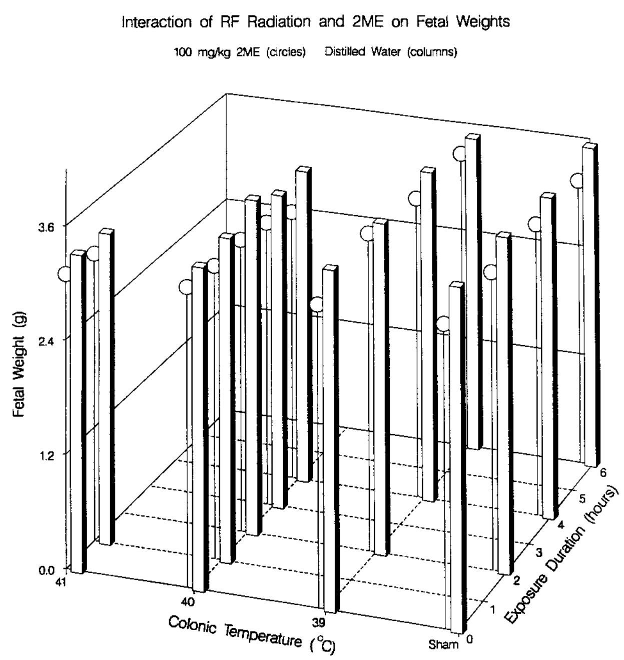

Fig. 3. Fetal weights after combined exposure to RF radiation sufficient to elevate colonic temperatures from (sham) to 39, 40, or 41 °C for up to 6 h, along with exposure to vehicle (distilled water, dw) or 100 mg/kg 2-methoxyethanol (2ME). Main effect of 2ME is significant.

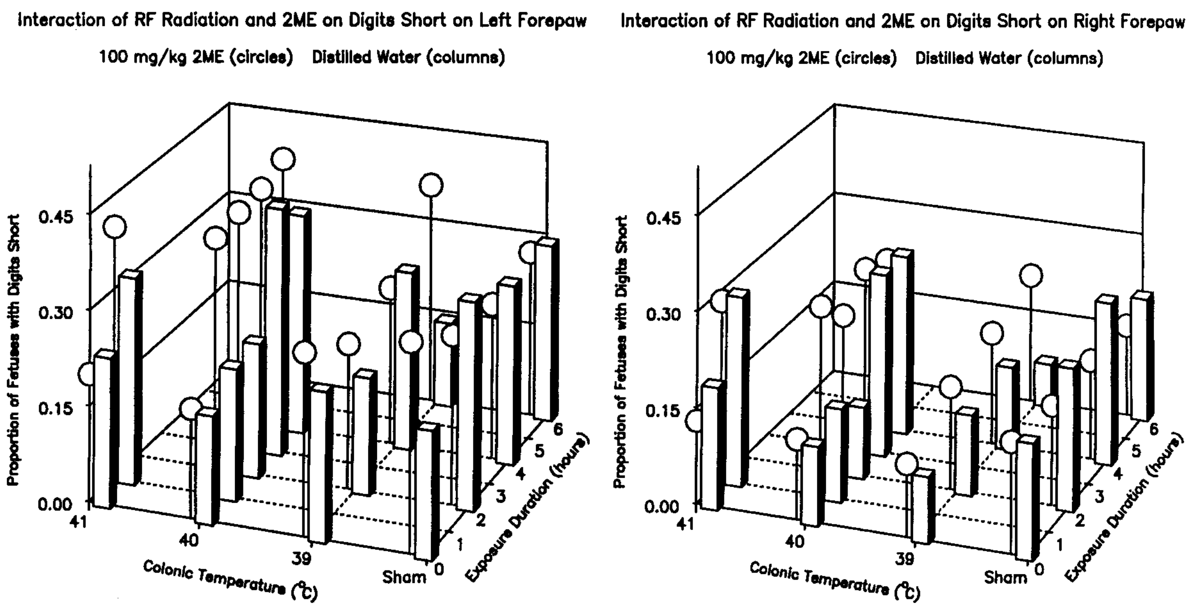

Fig. 4. Effect of RF radiation (colonic temperature at sham, 39, 40, or 41 °C) for up to 6 h, along with exposure to dw or 100 mg/kg 2ME to produce short digits. Panel A depicts left forepaw (main effect of exposure time is significant). Panel B depicts right forepaw (Temperature × 2ME interaction is significant).

the group receiving 100 mg/kg 2ME (0.02 ± 0.08) was greater than those receiving dw (0.002 ± 0.01).

The main effect of RF radiation-induced temperature rise was also significant for curvature of the right forepaw digits [$F(3, 27) = 3.3$, $P = .04$]. Contrasts were done to compare the proportion of fetuses with curvature at each temperature to every other temperature. The proportion at 40 °C (0.02 ± 0.1) was significantly greater than in the sham condition (0.01 ± 0.02) [$P = .02$] and at 41 °C (0.002 ± 0.01) [$P = .03$].

The main effect of Time was significant for digits short on the left forepaw [Figure 4(a); $F(5, 45) = 3.1$, $P = .02$] (i.e., generally as exposure time increased, the incidence of short digits increased), although the Time × Temperature interaction was not significant. Contrasts were done to compare the proportion of fetuses with short digits at each time to every other time within a given temperature. The only temperature at which significant differences were obtained was at 40 °C, where the average of each proportion (since dw and 2ME means did not differ significantly at these points) differed from the 0 time point (each $P < .05$). The proportions for dw and 2ME, respectively, at 0 h were 0.17 ± 0.26 and 0.17 ± 0.20; at 1 h were 0.21 ± 0.23 and 0.40 ± 0.27; at 2 h were 0.21 ± 0.21 and 0.41 ± 0.31; at 3 h were 0.40 ± 0.25 and 0.42 ± 0.17; and at 4 h were 0.35 ± 0.29 and 0.44 ± 0.32.

The Temperature × 2ME interaction was significant for digits short on the right forepaw [Figure 4,(b); $F(3, 27) = 3.8$, $P = .02$]. Contrasts were done to test for a 2ME effect at each temperature, but none of the contrasts was significant.

The Time × Temperature × 2ME interaction was significant for digits missing on the left forepaw [Figure 5(a); $F(6, 54) = 2.6$, $P = .03$]. Contrasts were done at each temperature to compare the effect of 2ME at times greater than zero h to the effect at zero h. The only contrast that was significant was at 41 °C. The difference between the proportion of fetuses having missing digits in the group receiving 100 mg/kg 2ME (0.19 ± 0.34) and the group receiving dw (0.02 ± 0.04) at 1 h was significantly greater than the difference at 0 hrs (both 0.00 ± 0.00) [$P < .0001$].

The same pattern of statistical significance was found for the contrasts for digits missing on the right forepaw, although the Time × Temperature × 2ME interaction was not statistically significant [$P = .10$].

The main effect of 2ME was significant for the right forepaw appearing thicker than normal [$F(1, 9) = 9.0$, $P = .02$]. That is, the proportion of fetuses with thicker forepaws in the group receiving 100 mg/kg 2ME (0.006 ± 0.02) was greater than those receiving dw (0.001 ± 0.1).

Fixed Tissue Observations

The Time × Temperature × 2ME interaction was significant for forepaw ectrodactyly (i.e., short or miss-

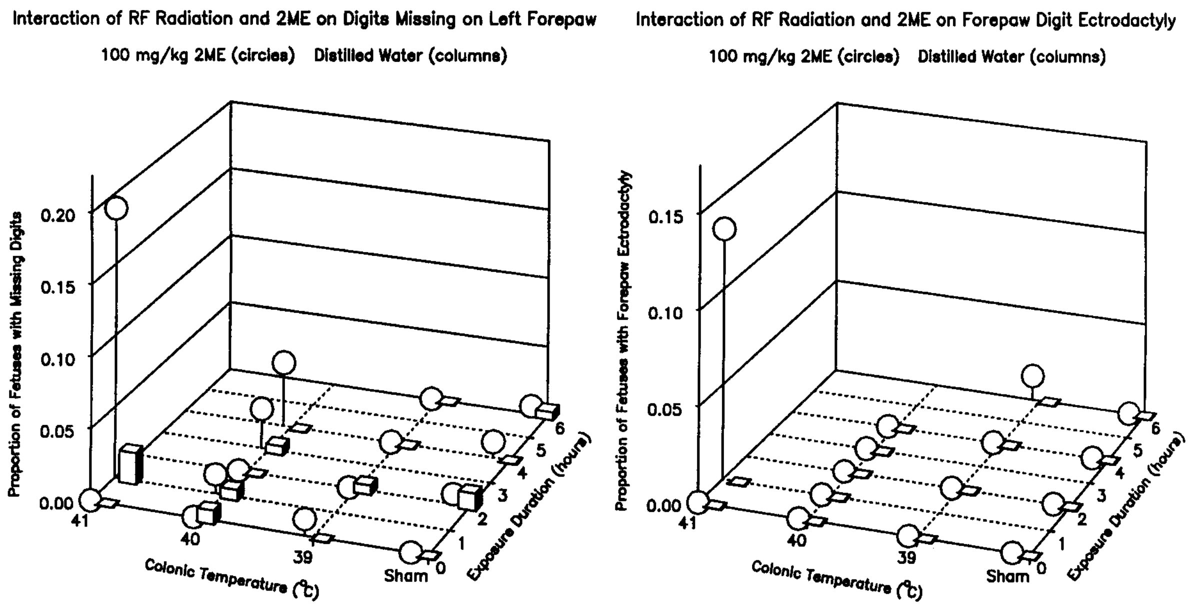

Fig. 5. Effect of RF radiation (colonic temperature at sham, 39, 40, or 41 °C) for up to 6 h, along with exposure to dw or 100 mg/kg 2ME to reduce number of digits on right forepaw (Panel A; Time × Temperature × 2ME interaction is significant) or produce forepaw ectrodactyly (Panel B; Time × Temperature × 2ME interaction is significant).

ing digits) [Figure 5(b); $F(6, 54) = 3.2$, $P = .01$]. Contrasts were done as described above. The only contrast that was significant was at 41 °C. The difference between the proportion of fetuses having Ectrodactyly in the group receiving 100 mg/kg 2ME (0.13 ± 0.22) and the group receiving dw (0.00 ± 0.00) at 1 h was significantly greater than the difference at 0 h (both 0.00 ± 0.00) [$P < .0001$].

The Time × Temperature × 2ME interaction was significant for hindpaw ectrodactyly [$F(6, 54) = 3.9$, $P = .003$]. Contrasts were done as described above. The only contrast that was significant was at 41 °C. The difference between the proportion of fetuses having hindpaw ectrodactyly in the group receiving 100 mg/kg 2ME (0.06 ± 0.09) and the group receiving dw (0.00 ± 0.00) at 1 h was significantly greater than the difference at 0 h (both 0.00 ± 0.00) [$P < .0001$].

The main effect of 2ME was nearly significant for forelimb microdactyly [$P = .05$]. The proportion of fetuses with microdactyly in the group receiving 100 mg/kg 2ME (0.06 ± 0.17) was greater than the proportion of those receiving dw (0.014 ± 0.05).

The Time × Temperature interaction was significant for forelimb microdactyly [$F(6, 54) = 2.3$, $P = .05$]. Contrasts were done at each temperature to compare the proportions malformed at times greater than zero hrs to the proportion at zero h. The only significant contrast was at 41 °C, where the proportion at 1 h (0.11 ± 0.24) was greater than that at 0 h (0.05 ± 0.19) [$P = .0441$].

As might be expected from the fetal weight differences, the main effect of 2ME was significant for the number of caudal centra (i.e., ossification sites) [$F(1, 9) = 227.8$, $P = .0001$]. The mean number of caudal centra of the group receiving 100 mg/kg 2ME (2.42 ± 0.9311; female = 2.41, male = 2.43) was less than the mean of those receiving dw (3.64 ± 0.7930; female = 3.65, male = 3.64).

DISCUSSION

The incidence of malformations observed in the present study is consistent with that observed in our previous research [Nelson et al., 1994, 1997]. As expected, there were general trends toward increasing developmental toxicity with increasing colonic temperature and increasing duration of RF radiation exposure. There were some indications that concurrent exposure to 2ME and RF radiation produced greater developmental toxicity than exposure to either agent alone, although this trend was not observed in every measure of developmental toxicity. Generally, the observations in fresh tissue fetuses were verified in the fixed tissues. Consistent with our previous study [Nelson et al., 1997], however, more malformations were detected in the fresh tissue observations than in the fixed tissue observations. An exception to this statement was the observation of significant malformations of the hindpaws in the fixed tissues, but not in fresh tissues.

Taken together, the present observations suggest that there may be less interaction of RF radiation and 2ME at less severe conditions of RF radiation exposure. This point is interesting, as our previous observations [Nelson et al., 1997] suggested that there were still interactions of 2ME and RF radiation at even the lowest levels of 2ME used—although it appeared that antagonistic effects were observed at these levels in contrast to synergistic effects at higher levels. Since interactions are observed at the lowest levels of 2ME but not at lower ''levels'' of RF radiation, it may suggest that RF radiation exerts more of an effect on 2ME developmental toxicity than 2ME exerts on RF radiation developmental toxicity. Although we have not been able to detect changes in the biotransformation profile of 2ME from concurrent RF radiation exposure [Cheever et al., 1997], it may be profitable to focus future research on the ability of RF radiation to alter 2ME teratogenicity.

Our observations that there were not interactions in the absence of significant hyperthermia also differ from those of Marcickiewicz et al. [1986]; (2450 MHz; SARs = 0.4–0.5 and 4–5 W/kg, 2 h daily throughout gestation) and Chiang et al. [1995]; pulsed 15.6 kHz; 40 μT 4 h day on gestation days 7–16). Both of these investigators reported enhanced teratogenicity of ara-C in mice with no detectable rise in colonic temperature. The different results may relate to different radiation types and/or frequencies, different basal metabolic rates of mice (approximately 9–10 W/kg) used by Marcickiewicz et al. [1986] and Chiang et al. [1995] vs. rats (approximately 6–7 W/kg) used in our study, single or repeated exposures, different exposure durations, and biotransformation profile of ara-C vs 2ME. Predictions as to whether or not interactions would be expected from different chemical/drug exposures may not be as simple as predicting if hyperthermia is expected from radiation exposure or not.

Throughout this study, the lower incidence and less severe nature of the malformations sometimes made it difficult to classify a fetus as ''normal'' or ''malformed.'' It appears that many of our observations in this study clustered in areas near thresholds, both for degrees as well as durations of hyperthermia. Not surprisingly, then, some inconsistencies in dose-effect relationships were seen in the results. For example, we saw a higher proportion of curvature in right forepaw digits at 40 than at 41 °C. Also, we saw significant duration-dependent effects in left forepaw short digits at 40 °C, but not at 41 °C (although the trend was there at 41 °C, and it was evident in left forepaw digits missing). We believe that these ''inconsistencies'' are reflective of our observations at or near the threshold for effects, rather than ''U-shaped'' dose effect curves that have been described in toxicology [Davis and Svendsgaard, 1990, 1994]. Our earlier research [Nelson et al., 1994; 1997] found significant interactions between various doses of 2ME and RF radiation sufficient to elevate colonic temperatures to 42 °C for up to 30 min. Although it appears that there were still interactions between RF radiation and 2ME at 1 h exposure at 41 °C, the present results demonstrated no consistent patterns of interaction at lower levels and longer durations of RF radiation exposure.

It should be noted that it is possible that different patterns may be observed if a different dose of 2ME had been selected. The level used in this study (viz., 100 mg/kg) is approximately the threshold dose for teratogenicity by itself. For example, fetal weights were reduced by 2ME in this study (also reflected by reduced caudal centra), and left and right forepaw digits were curved, but other malformations were not observed in the absence of RF radiation. Had different doses of 2ME been selected (or a host of other exposure parameters changed), it is possible that slightly different malformation incidences and interactions may be seen—but we feel that similar trends would be observed and conclusions reached.

Another issue concerns the effect of environmental temperature and humidity on the interactions reported in this study. We have observed a wide range of temperature and humidity in workplaces where RF radiation is used. Other than the interactions which we observed between 2ME and RF radiation at 41 °C for 1 h in this study, it appears that we were near an effect threshold in several instances (i.e., suggestions that there may be an interaction, but not a clear, consistent pattern). If environmental temperature and/or humidity affect the interaction, however, we might see different thresholds for interactive developmental toxicity as temperature or humidity are altered. There are several reasons to suspect that environmental changes (temperature/humidity) may affect the interactive effects. Environmental changes can alter the rat's ability to control body temperature [Besch, 1990]. In addition, chemical teratogenicity in experimental animals interacts with environmental and body temperature [e.g., Ferm and Kilham, 1977; Ferm and Ferm, 1979; Edwards, 1986; Lary, 1986; Warkany, 1986; Kimmel et al., 1993]. Therefore, the next phase of our research is addressing the effect of environmental temperature and humidity on the interaction between RF radiation and 2ME.

In summary, the present research demonstrates the interactive effects of RF radiation on the developmental toxicity of a relatively low dose of 2ME in rats.

Data on patterns of interaction and lowest combined effects level will be useful in developing workplace exposure guidelines and intervention strategies for combined exposures. No RF radiation or chemical exposure guidelines or intervention strategies address combined physical and chemical agent interaction effects. Clearly, however, the data from the present study indicate that combined exposure effects should be considered when developing these guidelines and strategies.

REFERENCES

ASA (American Society of Anesthesiologists) (1974): Report of an ad hoc committee on the effects of trace anesthetics on the health of operating room personnel. Anesthesiology 41:321.

Besch EL (1990): Environmental variables and animal needs. In Rollin BE, Kesel ML (eds): ''The Experimental Animal in Biomedical Research,'' Vol. 1. Boca Raton: CRC Press, pp 113–152.

Bolt HM, Golka K (1990): Maternal exposure to ethylene glycol monomethyl ether acetate and hypospadia in offspring: A case report. Br J Ind Med 47:352–353.

Brown-Woodman PDC, Hadley JA, Waterhouse J, Webster WS (1988): Teratogenic effects of exposure to radiofrequency radiation (27.12 MHz) from a shortwave diathermy unit. Ind Health 26:1–10.

Cheever KL, Swearingen TF, Edwards RM, Nelson BK, Werren DM, Conover DL, DeBord DG: 2-Methoxyethanol metabolism, embryonic distribution, and macromolecular adduct formation in the rat: The effect of radiofrequency radiation. (Submitted, 1997)

Chiang H, Wu RY, Shao BJ, Fu YD, Yao GD, Lu DJ (1995): Pulsed magnetic field from video display terminals enhances teratogenic effects of cytosine arabinoside in mice. Bioelectromagnetics 16:70–74.

Cocozza G, De Blasio A, Nunziata B (1960): Remarks on short-wave embryopathy. Pediatria (Napoli) 68:7–23.

Cohen R (1986): Radiofrequency and microwave radiation in the microelectronics industry. In: ''Occupational Medicine: State of the Art Reviews,'' Vol. 1 (No. 1: The Microelectronics Industry). Philadelphia: Hanley and Belfus, Inc., pp 145–154.

Cone JE (1986): Health hazards of solvents. In: ''Occupational Medicine: State of the Art Reviews,'' Vol. 1 (No. 1: The Microelectronics Industry). Philadelphia: Hanley and Belfus, Inc., pp 69–87.

Conover DL, Murray WE Jr, Foley ED, Lary JM, Parr WH (1981): Measurement of electric- and magnetic-field strengths from industrial radio-frequency (6–38 MHz) plastic sealers. Proceedings of the IEEE 68(1):17–20.

Correa A, Gray RH, Cohen R, Rothman N, Shah F, Seacat H, Corn M (1996): Ethylene glycol ethers and risks of spontaneous abortion and subfertility. Am J Epidemiol 143:707–717.

Cox C, Conover DL (1981): ''Industrial Hygiene Survey Report in Support of Epidemiologic Study of RF Heat Sealer Operations at National Blank Book Co., Inc.,'' Report # 88.25, Cincinnati, Ohio: National Institute for Occupational Safety and Health.

Cox C, Murray WE, Foley EP (1982): Occupational exposures to radiofrequency radiation (18–31 MHz) from RF dielectric heat sealers. Am Ind Hyg Assoc J 43:149–153.

Davis JM, Svendsgaard DJ (1990): U-shaped dose-response curves: Their occurrence and implications for risk assessment. J Toxicol Environ Health 30:71–83.

Davis JM, Svendsgaard DJ (1994): Nonmonotonic dose-response relationships in toxicological studies. In Calabrese EJ (ed): ''Biological Effects of Low Level Exposures: Dose-Response Relationships.'' Boca Raton: Lewis Publishers, pp 67–85.

Durney CH, Johnson CC, Barber PW, Massoudi H, Iskander MF, Lords JL, Ryser DK, Allen SJ, Mitchell JC (1978): Radiofrequency radiation dosimetry handbook (second edition). Report SAM-TR—78–22, USAF School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, Texas, p 95.

Edwards MJ (1986): Hyperthermia as a teratogen: A review of experimental studies and their clinical significance. Teratog Carcinog Mutagen 6:563–582.

Edwards MJ, Shiota K, Smith MSR, Walsh DA (1995): Hyperthermia and birth defects. Reprod Toxicol 9:411–425.

Ferm VH, Ferm RR (1979): Teratogenic interaction of hyperthermia and vitamin A. Biol Neonate 36:168–172.

Ferm VH, Kilham L (1977): Synergistic teratogenic effects of arsenic and hyperthermia in hamsters. Environ Res 14:483–486.

Fox JWC, Knadle RT, Brook RH (1976): Radiofrequency in the operating-theatre. Lancet 1:962.

Fraser FC (1977): Interactions and multiple causes. In Wilson JG, Fraser FC (eds): ''Handbook of Teratology,'' Vol 1: General Principles and Etiology. New York: Plenum, pp 445–463.

Germain MA, Webster WS, Edwards MJ (1985): Hyperthermia as a teratogen: Parameters determining hyperthermia-induced head defects in the rat. Teratology 31:265–272.

Goldstein BD, Paz J, Giuffrida JG, Palmes ED, Ferrand EF (1976): Atmospheric derivatives of anesthetic gases as a possible hazard to operating-room personnel. Lancet 2:235–237.

Goldstein RS, Hewitt WR, Hook JB (1990): ''Toxic Interactions.'' New York: Academic.

Gray RH, Correa A, Hakim R, Cohen R, Corn M, Shah F, Rothman N, Hou W, Seacat H (1996): Ethylene glycol ethers and reproductive health in semiconductor workers. Occup Hyg 2:331–338.

Greene FM (1974): Development and construction of an electromagnetic near-field synthesizer. NBS Technical Note 652. Boulder, Colorado: National Bureau of Standards.

Greene FM (1977): Measurement of RF power-absorption in biological specimens (10–100 MHz). DHEW (NIOSH) Publication No. 77–146. Cincinnati, Ohio: National Institute for Occupational Safety and Health, pp 9–17.

Hammond SK, Hines CJ, Hallock MF, Woskie, SR, Kenyon EM, Schenker MB (1996): Exposures to glycol ethers in the semiconductor industry. Occup Hyg 2:355–366.

Hanley TR Jr, Yano BL, Nitschke KD, John JA (1984): Comparison of the teratogenic potential of inhaled ethylene glycol monomethyl ether in rats, mice, and rabbits. Toxicol Appl Pharmacol 75:409–422.

Hanlon DP, Ferm VH (1986): Teratogen concentration changes as the basis of the heat stress enhancement of arsenate teratogenesis in hamsters. Teratology 34:189–193.

Hardin BD, Eisenmann CJ (1987): Relative potency of four ethylene glycol ethers for induction of paw malformations in the CD-1 mouse. Teratology 35:321–328.

Hoffman D, Dietzel F (1966): Abortions and malformations following short wave irradiation during pregnancy. Geburtsh Frauenheilkd 26:554–557.

Horton VL, Sleet RB, John-Greene JA, Welsch F (1985): Developmental phase-specific and dose-related teratogenic effects of ethylene glycol monomethyl ether in CD-1 mice. Toxicol Appl Pharmacol 80:108–118.

IEEE (1991): IEEE recommended practice for the measurement of potentially hazardous electromagnetic fields-RF and microwave. IEEE Standard C95.3-1991. New York: Institute of Electrical and Electronics Engineers, pp 72–74.

Imrie AH (1971): Pelvic short wave diathermy given inadvertently in early pregnancy. J Obstet Gynaecol Br Commonw 78:91–92.

Keplinger ML, Lanier GE, Deichmann WB (1959): Effects of environmental temperature on the acute toxicity of a number of compounds in rats. Toxicol Appl Pharmacol 1:156–161.

Kimmel CA, Cuff JM, Kimmel GL, Heredia DJ, Tudor N, Silverman PM, Chen J (1993): Skeletal development following heat exposure in the rat. Teratology 47:229–242.

Lary JM (1986): Hyperthermia and teratogenicity. In Anghileri LJ, Robert J (eds): ''Hyperthermia in Cancer Treatment,'' Vol. I. Boca Raton: CRC Press, pp 107–126.

Lary JM, Conover DL (March 1987): Teratogenic effects of radiofrequency radiation. IEEE Engineer Med Biol Mag, pp 42–46.

Lary JM, Conover DL, Foley ED, Hanser PL (1982): Teratogenic effects of 27.12 MHz radiofrequency radiation in rats. Teratology 26:299–309.

Lary JM, Conover DL, Johnson PH, Burg JR (1983): Teratogenicity of 27.12 MHz radiation in rats is related to duration of hyperthermic exposure. Bioelectromagnetics 4:249–255.

Lary JM, Conover DL, Johnson PH, Hornung RW (1986): Dose-response relationship between body temperature and birth defects in radiofrequency-irradiated rats. Bioelectromagnetics 7:141–149.

Lumm SH, Kutcher JS, Morris CB (1996): Spontaneous abortions and glycol ethers used in the semiconductor industry: An epidemiological review. Occup Hyg 2:339–354.

Marchese GS (1953): Premature birth and multiple congenital malformations due to short waves. Minerva Nipiol 3:100–101.

Marcickiewicz J, Chazan B, Niemiec T, Sokolska G, Troszynski M, Luczak M, Szmigielski S (1986): Microwave radiation enhances teratogenic effect of cytosine arabinoside in mice. Biol Neonate 50:75–82.

Nelson BK (1994): Interactions in developmental toxicology: A literature review and terminology proposal. Teratology 49:33–71.

Nelson BK, Conover DL, Brightwell WS, Shaw PB, Werren D, Edwards RM, Lary JM (1991): Marked increase in the teratogenicity of the combined administration of the industrial solvent 2-methoxyethanol and radiofrequency radiation in rats. Teratology 43:621–634.

Nelson BK, Conover DL, Shaw PB, Werren DM, Edwards RM, Hoberman AM (1994): Interactive developmental toxicity of radiofrequency radiation and 2-methoxyethanol in rats. Teratology 50:275–293.

Nelson BK, Conover DL, Shaw PB, Snyder DL, Edwards RM (1996): Interactions of radiofrequency radiation on 2-methoxyethanol teratogenicity in rats. J Appl Toxicol 17(1):31–39, 1997.

Nelson BK, Setzer JV, Brightwell WS, Mathinos PR, Kuczuk MH, Weaver TE, Goad PT (1984): Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ Health Perspect 57:261–271.

Nelson BK, Vorhees CV, Scott WJ Jr, Hastings L (1989): Effects of 2-methoxyethanol on fetal development, postnatal behavior, and embryonic intracellular pH. Neurotoxicol Teratol 11(3):273–284.

NIOSH (1983): ''Current Intelligence Bulletin # 39: Glycol Ethers—2-Methoxyethanol and 2-ethoxyethanol.'' DHHS (NIOSH) Publication No. 83–112, Cincinnati, Ohio: National Institute for Occupational Safety and Health.

NIOSH (1991): ''Criteria for a Recommended Standard: Occupational Exposure to Ethylene Glycol Monomethyl Ether, Ethylene Glycol Monoethyl Ether, and their Acetates.'' DHHS (NIOSH) Publication No. 91–119, 296 pp, Cincinnati, Ohio: National Institute for Occupational Safety and Health.

Pastides H, Calabrese EJ, Hosmer DW, Harris DR (1988): Spontaneous abortion and general illness symptoms among semiconductor manufacturers. J Occup Med 30:543–551.

Paustenbach DJ (1988): Assessment of the developmental risks resulting from occupational exposure to select glycol ethers within the semiconductor industry. J Toxicol Environ Health 23:29–75.

Pinney SM, Lemasters GK (1996): Spontaneous abortions and stillbirths in semiconductor employees. Occup Hyg 2:387–404.

Rubin A, Erdman WJ (1959): Microwave exposure of the human female pelvis during early pregnancy and prior to conception. Am J Phys Med 38:219–220.

Rudolph L, Swan SH (1986): Reproductive hazards in the microelectronics industry. In: ''Occupational Medicine: State of the Art Reviews.'' Vol. 1 (No. 1: The Microelectronics Industry). Philadelphia: Hanley and Belfus, Inc. pp 135–143.

Ruggera PS (1977): Near-field measurements of RF fields. In: ''Symposium on Biological Effects and Measurement of Radio Frequency/Microwaves, Proceedings of a Conference,'' HEW Pub. (FDA) #77-8026, U.S. Dept. HEW, PHS, FDA, BRH, Rockville MD, pp 104–116.

Schardein JL (1993): ''Chemically Induced Birth Defects,'' 2nd Edition, Revised and Expanded. New York: Marcel Dekker, Inc., 902 pages.

Schenker MB (1996): Reproductive health effects of glycol ether exposure in the semiconductor industry. Occup Hyg 2:367–372.

Shiota K, Shionoya Y, Ide M, Uenobe F, Kuwahara C, Fukui Y (1988): Teratogenic interaction of ethanol and hyperthermia in mice. Proc Soc Exp Biol Med 187:142–148.

Sleet R, Greene JA, Welsch F (1987): Teratogenicity and disposition of the glycol ether 2-methoxyethanol and their relationship in CD-1 mice. In Welsch F (ed): ''Approaches to Elucidate Mechanisms in Teratogenesis,'' Washington: Hemisphere Pub Corp, pp 33–57.

Sleet RB, Greene JA, Welsch F (1988): The relationship of embryotoxicity to disposition of 2-methoxyethanol in mice. Toxicol Appl Pharmacol 93:195–207.

Snyder DL, Edwards RM, Conover DL, Nelson BK (1997): Weight loss in pregnant rats during and 24 hours after radiofrequency radiation-induced hyperthermia: Effects of level and duration, animal restraint, and solvent exposure. (Submitted).

Staples RE, Schnell (1963): Refinement in rapid clearing technique in the KOH-alizarin red S method for fetal bone. Stain Technol 39:61–63.

Stewart JH, Elkington KJ (1985): Electronics: Semiconductor manufacturing. In Cralley LV, Cralley LJ, Knowlton JC (eds.): ''Industrial Hygiene Aspects of Plant Operations, vol. 3, Engineering Considerations in Equipment Selection, Layout, and Building Design.'' New York: MacMillan Publishing Co, pp 453–462.

Swan SH, Forest W (1996): Reproductive risks of glycol ethers and other agents used in semiconductor manufacturing. Occup Hyg 2:373–386.

Warkany J (1986):Teratogen update: Hyperthermia. Teratology 33:365–371.

Weihe WH (1973): The effect of temperature on the action of drugs. Annu Rev Pharmacol 13:409–425.