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Interactions of Radiofrequency Radiation on 2-Methoxyethanol Teratogenicity in Rats*

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Concurrent exposures to chemical and physical agents occur in the workplace; exposed workers include those involved with the microelectronics industry, plastic sealers and electrosurgical units. 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 radiofrequency (r.f.; 10 MHz) radiation, which also induces hyperthermia and is teratogenic to exposed animals, and the industrial solvent 2-methoxyethanol (2ME) produces enhanced teratogenicity in rats. A subsequent study replicated and extended that research by investigating the interactive dose-related teratogenicity of r.f. radiation (sham exposure or maintaining colonic temperatures at 42.0°C for 0, 10, 20 or 30 min by r.f. radiation absorption) and 2ME (0, 75, 100, 125 or 150 mg/kg) on gestation days 9 or 13 of rats. The purpose of the present research is to determine the effects of r.f. radiation (sufficient to maintain colonic temperatures at 42.0°C for 10 min) on a range of doses of 2ME (0, 20, 40, 60, 80, 100, 120 and 140 mg kg^{-1}) 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. Dose-related developmental toxicity was observed for 2ME both in the presence and absence of r.f. radiation. However, concurrent RF radiation exposure changed the shape of the dose–effect curve of 2ME. These data indicate that combined exposure effects should be considered when developing exposure guidelines and intervention strategies.

INTRODUCTION

Antagonistic, potentiative and synergistic interactions are frequently observed among various chemical and physical agents.[1] Such interactions may be important in occupational health, because workplace exposures ordinarily involve more than one agent. For example, workers in several industries are potentially exposed to both industrial chemicals and radiofrequency (r.f.) radiation.[2,3] Concurrent exposures to various industrial chemicals and r.f. radiation occur in the electronics industry,[4] and several reports suggest an elevated risk of spontaneous abortions in this industry.[5–9] A second industry with simultaneous exposure to r.f. radiation and putative chemical teratogens is the dielectric heating industry. Glycol ethers, toluene, xylene and other chemicals are frequently found in dielectric heating areas in which r.f. radiation is used to weld or seal vinyl materials.[10–12] A third occupational setting in which combined r.f. radiation and chemical exposures occur is operating rooms, where electrosurgical units (ESUs) are used extensively to cut and coagulate tissues (in about one-half of all surgeries). High r.f. radiation exposures have been documented in these units.[13,14] Several anesthetic gases also found in operating rooms, including nitrous oxide and halothane, are reported to be teratogenic when administered at higher concentrations by inhalation to animals, and are possible human teratogens.[15] Elevated rates of congenital malformations and spontaneous abortions have been reported for female operating-room personnel or wives of male anesthetists.[16,17]

Several reports suggest that r.f. radiation may be teratogenic in humans.[18–20] Other reports indicate that r.f. radiation may produce spontaneous abortions in women given diathermy treatments just prior to, or during, early pregnancy.[21–23] In experimental animals, the teratogenicity of r.f. radiation is associated with the degree and duration of hyperthermia it produces.[24–26] Hyperthermia produces congenital malformations in experimental animals, and it has been hypothesized to produce malformations in humans.[27–30] The threshold for producing developmental toxicity appears to be a 2.5°C elevation of core temperature.[31,32]

Several experimental studies suggest that temperature can affect both the adult[33,34] and the developmental[35–39] toxicity of chemicals. Microwave radiation enhances the teratogenicity of cytosine arabinoside in mice.[40] We initially reported[41] enhanced teratogenicity when rats were exposed to r.f. radiation (maintaining colonic temperature at 42.0°C for 30 min) along with the

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industrial solvent 2-methoxyethanol (2ME; 150 mg kg^{-1} by gavage) on gestation day 13. This glycol ether was chosen as a model compound because of its well-characterized teratogenicity in experimental animals (in every species, and by any exposure route, tested)[42–52] and speculated teratogenicity in humans.[53] Furthermore, as discussed above, adverse reproductive effects have been reported after potential exposures to this or certain other glycol ethers in the semiconductor industry. We anticipated that the results from this model compound (2ME) would be exemplary of potential effects with other industrial chemical teratogens.

Subsequent research was undertaken to characterize the extent of synergism between r.f. radiation and 2ME.[54] The interactive dose-related teratogenicity of r.f. 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^{-1}) was investigated by administering various combinations of r.f. radiation and 2ME to groups of rats on gestation days 9 or 13. Gestation day 20 fetuses were examined for external, skeletal and visceral malformations. The results were consistent with and extended the initial research findings, demonstrating dose-dependent developmental toxicity and interactions. The results also suggested the experimental conditions most needing additional research in the present study. Day 9 exposures generally evidenced little effect by 2ME, either by itself or in combination with r.f. radiation. In contrast, day 13 exposures resulted in highly significant effects from 2ME and r.f. radiation. The structures showing strong evidence of effects from both 2ME and r.f. 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^{-1} 2ME and at a colonic temperature of 42.0°C maintained for 10 min. Previous research suggests that one cannot always predict the nature of interactive dose–effect relationships based on data at limited portions of the dose–effect curve.[1,55] The present research focused on the effects of a single r.f. radiation condition (the lowest condition seen to produce an interaction in the previous study, 42.0°C for 10 min; a subsequent study will investigate lower temperatures and longer durations of exposure to r.f. radiation) on the developmental toxicity of 2ME (to include low doses), with the primary observations on the forepaws after day 13 exposures.

Although this research is undertaken in rats, we would like, ultimately, to estimate the likelihood that the interactive effects of r.f. radiation and 2ME would be observed in humans at occupationally relevant exposures. Because human data are not available either for the extent of possible core temperature increases produced by occupational exposure to r.f. radiation or for the blood levels resulting from occupational exposure to 2ME, the present project (including additional ongoing research investigating lower colonic temperatures for longer durations, which will be published separately) focused on determining the lowest effect level of teratogenicity for combined r.f. radiation (single condition) and 2ME exposures (range of doses). A determination of this lowest effect level facilitates a more accurate risk assessment of human exposures to these two agents.

MATERIALS AND METHODS

Our experimental animals, exposure system and procedures were identical to those described previously.[54] Briefly, virgin female and breeder male CD Sprague-Dawley rats (VAF/plus, 175–200 g; Charles River Breeding Laboratories, Wilmington, MA) were maintained at 24 ± 2°C and 50 ± 10% humidity. Feed was Ziegler® certified laboratory rat chow, with tapwater available *ad libitum*, and room lighting from 7 a.m. to 7 p.m. During the 2-week quarantine period, quality control tests were conducted on rats to ensure that specific pathogens were not introduced into the AAALAC-certified animal facility. The study was conducted in accordance with existing NIH and CDC guidelines for the care and handling of experimental animals. 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 copulatory 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 copulatory plugs or sperm marked day 0 of gestation.

Rats were irradiated in r.f. 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 electric field strength (in V m^{-1}) was measured with a Holaday Instruments Model 3003 field survey monitor. The magnetic field strength (in A m^{-1}) was measured by a Model MFM-10 magnetic field-strength monitor.[56] The near-field synthesizers were enclosed within copper screen wire chambers (Ark Electronics Corp., Models A273 and PSS7S10) to reduce interference from outside r.f. radiation signals and to shield personnel against r.f. 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 ca. 0.4 m^3 min^{-1}, with no detectable air velocity at the location of the animal. Sham exposures were conducted outside the actual near-field synthesizer facilities but inside the environmental chambers for the same duration as the r.f.-exposed animals. Thus, lighting, ventilation, temperature, humidity and handling procedures were the same for sham exposures as for r.f. radiation exposures, but no r.f. field was present during the sham exposure.

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 r.f. 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 to obtain maximum r.f.-induced heating. An r.f.-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. Computer-controlled systems monitored the colonic temperature of the irradiated rats and controlled the r.f. output power such that the colonic temperature was maintained to within ±0.2°C of the target temperature. The output power of the r.f. radiation source initially was set to provide a specific absorption rate (SAR) of 6.6 W kg^{-1} to raise the animal's colonic temperature from its normal baseline of ca. 38°C to 42.0°C (requiring ca. 30 min). Once this colonic temperature was reached, the r.f. radiation output power was adjusted to maintain the colonic temperature at 42.0°C for 10 min, with the SAR varying from 0.8 to 6.6 W kg^{-1}. We previously found that time-averaged SARs required to achieve or maintain colonic temperatures at 42.0°C were not affected by 2ME exposure.[54]

Specific concentrations of 2ME were prepared in distilled water (10 ml kg^{-1}). The solutions were verified by flame ionization gas chromatography to be within 10% of the target concentrations by the NIOSH Division of Physical Sciences and Engineering. Rats were gavaged with the specified dose of 2ME or distilled water immediately prior to 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 ('external' observations). The fetuses were preserved in ethanol. Subsequently, the fetuses were examined by independent observers for malformations of the forelimbs, hindlimbs and tail ('skeletal' observations). They were eviscerated, 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 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.[57] Day 20 fetuses commonly have one or two metacarpals ossified in the forepaws and three or four metatarsals ossified in the hindpaws. Because the phalanges are not normally ossified by day 20, the paw malformations were best observed by a thorough external examination.

A two-by-eight factor design was utilized, with r.f. radiation applied at two levels (sham or 10 min at a colonic temperature of 42.0°C) and 2ME at eight levels (0, 20, 40, 60, 80, 100, 120 and 140 mg kg^{-1}). Pregnant rats ($n = 10$ per group) were randomly assigned to treatment groups.

STATISTICAL METHODS

The choice of statistical method for analyzing a set of data depends, of course, on the underlying question. The question of interest in this study was whether the effect of 2ME and r.f. radiation was greater than additive (this is the best criterion for ascertaining the presence of interaction between two teratogenic agents[1,58,59]). In particular, we were interested in determining if the fraction of fetuses malformed when exposed to 2ME and r.f. radiation was greater than one would expect from merely summing the fraction malformed due to 2ME alone plus the fraction malformed due to r.f. radiation alone.

In previous research,[41] we have tested for an interactive effect by using a test which compares the results of two groups receiving the two agents alone with a third group receiving both agents combined. We have also tested for the additivity of logits using generalized estimating equations.[54]

In the present study, we were primarily interested in determining whether or not there was interaction between two teratogenic agents. For that reason, we chose to test for interaction through a linear regression model, which clearly tests for non-additivity.

In considering the appropriate form of the model, we also considered non-linear effects of the chemical. We looked for quadratic and cubic effects in 2ME and an interaction between the r.f. and the quadratic chemical effect. With only two levels of the r.f., it was not possible to test for any non-linear behavior with regard to this predictor variable. Thus, our initial basic model was

$$p = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3 + \beta_4 R + \beta_5 CR + \beta_6 C^2 R + \epsilon \quad (1)$$

where C = concentration of 2ME (in mg kg^{-1}), R = experimental exposure time (in h) to r.f. radiation at 42°C, ϵ = error term, which is normally distributed with mean 0 and variance σ^2, p = fraction of litter with at least one malformation and β_i = coefficient for predictor variable.

RESULTS

No maternal toxicity was apparent, although no specific tests were run to detect maternal toxicity. There was no increase in the frequency of resorptions. Reductions in fetal weights and virtually all of the malformations that we observed are not reported in detail, as they have been described previously by ourselves[54] and others[47,50] (malformations primarily of the left forepaw, especially microdactyly and ectrodactyly). Table 1 and Fig. 1 present the results of the various exposure combinations.

Testing for effects

Initial examination of the data suggested collinearity among predictor variables, as well as a lack of normality in the residuals. Use of a reduced model with just two terms, for 2ME and the r.f. radiation, showed these predictor variables to be highly significant, as would be expected. When the non-linear and interaction terms of the complete model were included, the coefficients for 2ME and r.f. radiation were not signifi-

Table 1. Mean proportion of fetuses malformed (external exams) in each treatment group, along with the 95% confidence limits ($n = 10$ litters per group)

2-Methoxyethanol (mg kg^{-1}, p.o.)	R.f. radiation: sham exposure	R.f. radiation: 10 min at 42.0°C
	0.033	0.269
0	(−)0.002–0.068	0.103–0.436
	0.057	0.038
20	(−)0.017–0.131	(−)0.011–0.087
	0.058	0.211
40	0.016–0.101	0.018–0.405
	0.051	0.305
60	0.000–0.102	0.085–0.525
	0.076	0.386
80	(−)0.031–0.182	0.167–0.605
	0.041	0.337
100	0.001–0.081	0.118–0.556
	0.248	0.650
120	0.095–0.401	0.426–0.875
	0.194	0.650
140	0.037–0.351	0.377–0.922

cant—a common indication of collinearity among predictor variables.[60] Centering the predictor variables (i.e. subtracting the mean of a predictor variable from each of the original values) in a polynomial model is a useful way of dealing with this problem.[61] Use of the arcsin-square-root transformation, $\sin^{-1}(p^{0.5})$, is commonly recommended to stabilize the variance for proportions.[62] We found that this transformation normalized the residuals for the 'external' data.

Thus our modified regression model was

$$p' = \beta_0' + \beta_1' C' + \beta_2' (C')^2 + \beta_3' (C')^3 + \beta_4' R' + \beta_5' C' R' + \beta_6' (C')^2 \cdot R' + \epsilon \qquad (2)$$

where $p' = \arcsin(\sqrt{p})$, $C' = C -$ (mean of C), i.e. C is centered, $R' = R -$ (mean of R), i.e. R is centered and $\beta_i' =$ coefficient for centered predictor variable.

Although both the 'external' data and 'skeletal' data were taken on the same fetuses, the data were analyzed separately because individual fetuses were not numbered. The correlation between the fractions malformed per litter for the two sets of data was 0.694. The mean fractions malformed for the 'external' and 'skeletal' data sets were 0.2208 and 0.1956, respectively.

The results of linear regression with the complete model (2) with predictor variables centered and the

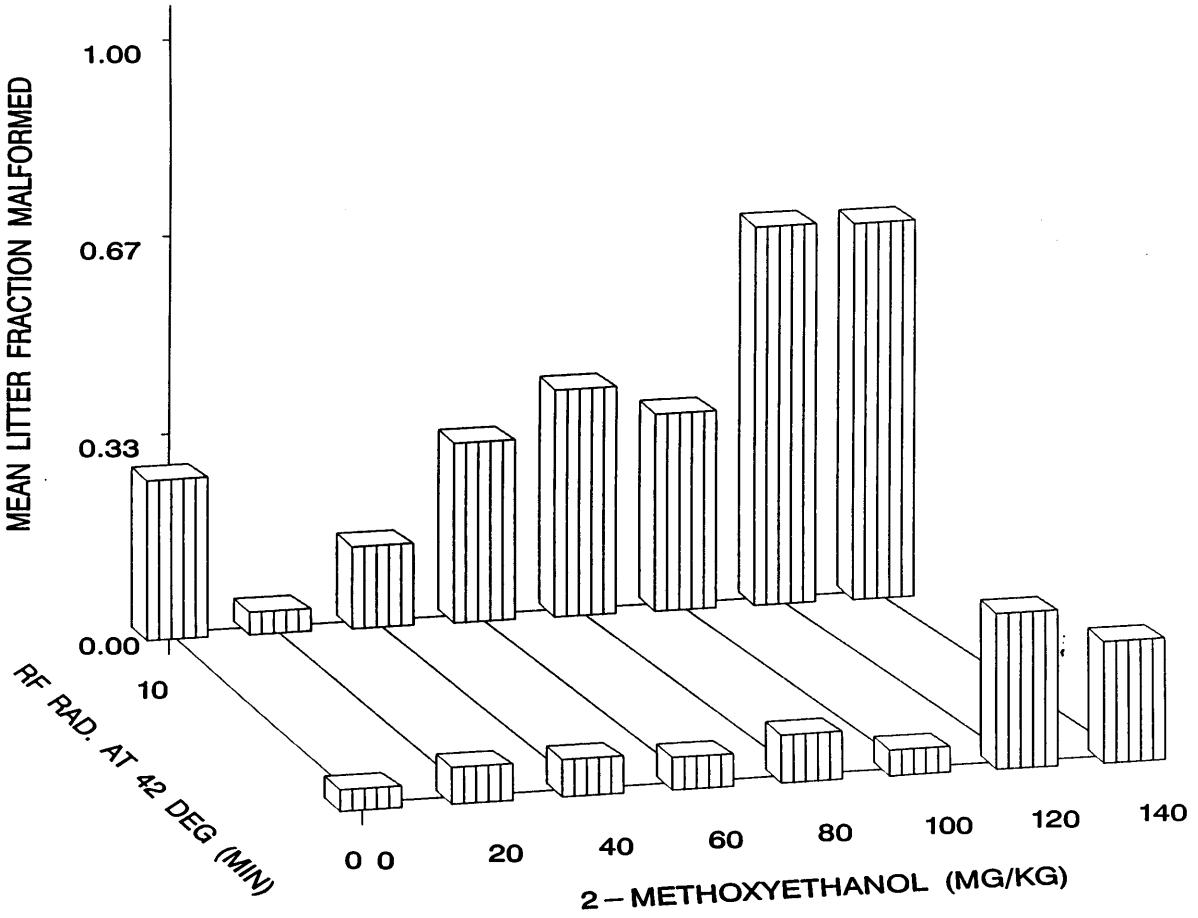

Figure 1. Incidence of external malformations (means of litter means) for the various doses of 2ME in the presence or absence of r.f. radiation (10 min at 42°C).

Table 2. Results of linear regression with the complete model (2)[a]

Parameter	Predictor variable	Parameter estimate	T for H_O: parameter = 0	P value	Standard error of estimate
β_0'	[Intercept]	18.831754	8.38	0.0001	2.247994
β_1'	C'	0.284891	3.30	0.0012	0.086342
β_2'	$(C')^2$	0.002087	2.59	0.0106	0.000806
β_3'	$(C')^3$	−0.000018	−0.81	0.4182	0.000022
β_4'	R'	123.124368	4.56	0.0001	27.007365
β_5'	$C'R'$	1.209716	3.07	0.0025	0.393962
β_6'	$(C')^2R'$	0.005612	0.57	0.5663	0.009762

[a] $R^2 = 0.433$.

dependent variable transformed with the arcsin-square-root transformation, are shown in Table 2.

We performed diagnostic tests on the residuals. The Shapiro–Wilk statistic ($W = 0.987$, $P = 0.823$) supported the assumption of normality for the error terms. Plots of the residuals did not reveal major violations of the assumptions of homogeneity of variance or of independence. In viewing these plots, we recognized that the nature of the dependent variable placed bounds on the possible minima and maxima over certain ranges. For example, if p is the proportion malformed, then $0 \leqslant \arcsin(\sqrt{p}) \leqslant 90$, and so for $p' = \arcsin(\sqrt{p})$ and at the minimum possible residual $\hat{p}' = 0$ ($\hat{p}'$ is the predicted value of p') is $p' - \hat{p}' = 0 - 0 = 0$.

The results for the 'external' data show a significant positive interaction between 2ME and the r.f. radiation ($P = 0.0025$). The impact of 2ME alone ($P = 0.0012$) and the r.f. radiation ($P = 0.0001$) alone was also judged to be significant. There was also evidence for a quadratic effect for 2ME, but no evidence for a cubic effect or an interaction between the quadratic term for 2ME and the r.f. radiation.

The results of the regression for the 'skeletal' data, using model (2) with centered predictor variables and the arcsin-square-root transformation, are shown in Table 3.

In the case of the 'skeletal' data, the diagnostics did suggest violations of certain underlying assumptions in the model. The value of the Shapiro–Wilk statistic ($W = 0.9021$, $P = 0.0001$) suggested that the residuals and, implicitly, the error terms were not normally distributed. A plot of residuals against the predicted values, $\hat{p}'$, clearly indicated that the variance was not homogeneous over the range of $\hat{p}'$.

In general, the results from the 'skeletal' data were similar to those of the 'external' data, except for the non-significant interaction ($P = 0.1274$).

Predictive model

In addition to testing for certain effects in the model, we were also interested in developing a predictive model to describe the effects of 2ME and r.f. radiation on malformations in rat fetuses. For this purpose, we developed a regression model using the original values of the predictor variables and the fraction of the litter malformed (external exams) as the dependent variable. In choosing predictor variables, we used the variables which were shown to be significant in testing for effects with the centered data. Thus our model was

$$p = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_4 R + \beta_5 C{\cdot}R + \epsilon \quad (3)$$

[We used the same subscripts for the coefficients as in model (1).] The results are shown in Table 4.

The fact that the parameter estimate for 2ME was negative and that the 2ME and r.f. radiation appear to have non-significant effects almost certainly reflects collinearity among the predictor variables, as noted earlier. Collinearity is not a problem if one is only interested in using the model as a predictive tool over the appropriate range, as we are here.[60]

One way of examining this predictive model is graphically. A response surface generated from the model

$$\hat{p} = 0.078575 - 0.002384{*}C + 0.000027{*}C^2 + 0.450944{*}R + 0.016654{*}C{*}R$$

Table 3. Results of regression for the 'skeletal' data using model (2)[a]

Parameter	Predictor variable	Parameter estimate	T for H_O: parameter = 0	P value	Standard error of estimate
β_0'	[Intercept]	9.514544	3.05	0.0027	3.122689
β_1'	C'	0.239380	2.01	0.0465	0.119214
β_2'	$(C')^2$	0.003725	3.31	0.0012	0.001126
β_3'	$(C')^3$	0.000008	0.27	0.7868	0.000030
β_4'	R'	129.282487	3.45	0.0007	37.505880
β_5'	$C'R'$	0.842236	1.53	0.1274	0.549368
β_6'	$(C')^2R'$	0.001034	0.08	0.9398	0.013654

[a] $R^2 = 0.325$.

Table 4. Results of regression with predicting model (3)

Parameter	Predictor variable	Parameter estimate	T for H_O: parameter = 0	P value	Standard error of estimate
β_0	[Intercept]	0.078575	1.54	0.1264	0.051123
β_1	C	−0.002384	−1.68	0.0948	0.001418
β_2	C^2	0.000027	2.86	0.0049	0.000009
β_4	R	0.450944	1.20	0.2329	0.376494
β_5	CR	0.016654	3.64	0.0004	0.004580

is shown in Fig. 2a. The surface shows the impact of various model components. We may assess the importance of the interaction component of the model by generating another surface omitting this component. We did so using only the external data (Table 1) with the model

$$\hat{p} = 0.078575 - 0.002384*C + 0.000027*C^2 + 0.450944*R$$

and obtained the surface shown in Fig. 2b, where we note a rather sharp drop in the response for high values of chemical exposure received in combination with exposure to r.f. radiation.

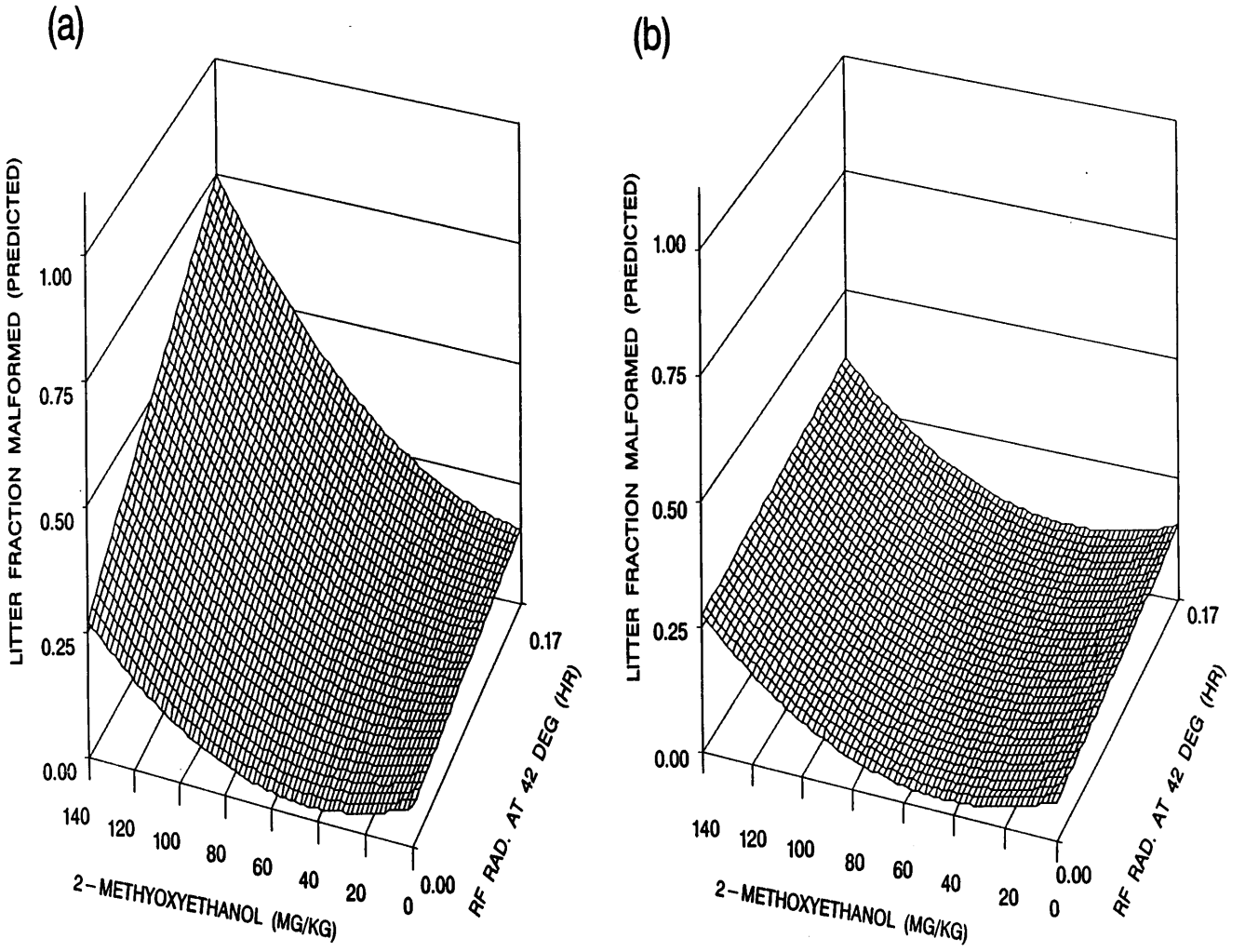

Figure 2. (a) Response surface generated from predictive model (3) [$p = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_4 R + \beta_5 CR + \epsilon$], with 0–140 mg kg^{-1} 2ME and r.f. radiation at 0 or 0.17 h (10 min), which includes the interaction term. (b) The response surface using the same model, but with the interaction term excluded. Note the sharp drop in the predicted proportion of malformations for high levels of 2ME combined with r.f. radiation exposure as compared with (a).

DISCUSSION

The incidence of malformations observed in the present study is consistent with that observed in our previous research.[54] For example, the highest concentration of 2ME (140 mg kg^{-1}) included in the present study produced over twice the incidence of malformations in the presence of r.f. radiation than it did by itself, with about 65% of the fetuses malformed in the presence of r.f. radiation. The range of doses of 2ME included in this study demonstrates interactive effects with r.f. radiation even at low doses of 2ME (see Figs 1 and 2). The higher incidence of malformations in the group given distilled water plus r.f. radiation (Table 1 and Fig. 1) than the groups given low concentrations of 2ME (in the presence of r.f. radiation) is puzzling, and we cannot explain this unexpected result. However, non-linear dose–effect relationships are not unusual at extreme ends of interactions.[1,55,63,64] The data suggest possible antagonism at the lowest concentrations of 2ME. Several possible factors may contribute to the results. It is possible that high levels of r.f. radiation may produce highly reactive free-radicals of oxygen. Ethanol is known to scavenge hydroxyl radicals, and it is possible that 2ME may have a similar effect. Although we have not detected any effect of 2ME on maternal body temperature, it is also possible that low concentrations of 2ME may induce some type of thermotolerance in embryonic tissues, and that this tolerance is not a linear phenomenon. If thermotolerance is involved, the relationship between intracellular pH and thermotolerance[65] is intriguing, as 2ME may affect embryonic intracellular pH.[45] Interestingly, recent research found good fit to a physiologically based pharmacokinetic (PBK) model of the primary metabolite of 2ME (methoxyacetic acid, MAA) at low concentrations, but significant deviations from that model at higher concentrations of MAA in pregnant mice.[66] More details of the dose–effect relationship between 2ME/MAA, thermotolerance, intracellular pH and developmental toxicity will require additional research. Alternatively, it should be noted that 'ramp time' (the time required to bring the colonic temperature to 42°C) in the '0' 2ME group was longer than the other groups (31, 25, 29, 25, 29, 30, 29 and 28 min, in order of ascending concentrations of 2ME). Our previous research indicated the importance of ramp time,[54] but whether or not the time differences contributed significantly to the incidence of malformations is unknown. It appears that r.f. radiation does not affect the biotransformation profile of 2ME,[67] so a proposed mechanism of interaction is yet to be defined. Further research will be necessary to resolve these issues.

The results from the 'external' data indicated a response in excess of additivity at the higher concentrations of 2ME. We see evidence for this in the linear regression analysis in testing the regression coefficient of the interaction term, and graphically from a comparison of response surfaces with (Fig. 2a) and without (Fig. 2b) the interaction term. The significant interaction component that emerges is interesting from a design as well as a biological point of view. Our experimental conditions were based upon our previous research. If the ranges of exposure for either 2ME or the r.f. radiation had been too high or too low, we would have missed the evidence for interaction entirely, obtaining either practically no malformed fetuses or practically all malformed fetuses. In other words, interactive effects are likely to be evident over only certain exposure ranges.

The lack of significance for the interaction coefficient in the 'skeletal' data could be viewed as evidence against a positive interaction, but other possible explanations exist. The lack of significance could also have been due to a lower overall rate of malformed fetuses in the 'skeletal' data, suggesting perhaps the importance of examining fresh specimens in looking for interactions of the nature included in the present study. The lack of evidence could also have been due to the fact that the 'skeletal' data did not meet the underlying assumptions for the linear model, as noted above.

In summary, the present research demonstrates the interactive effects of r.f. radiation on the developmental toxicity of low doses of 2ME in experimental animals. The 'dose' of r.f. radiation used in this study is quite high—approaching a level that may be lethal in a very low number of animals. Ongoing research is focusing on the interaction pattern and lowest combined effect level for the effect of 2ME on r.f. radiation-induced developmental toxicity, both at lower levels of hyperthermia and at longer durations of exposure. Data on such patterns of interaction and lowest combined effects level will be useful in developing workplace exposure guidelines and intervention strategies for combined exposures. No r.f. 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.

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