Synergism Between Sinusoidal-50 Hz Magnetic Field and Formaldehyde in Triggering Carcinogenic Effects in Male Sprague–Dawley Rats

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Background *Experimental rodent bioassays performed up to now have failed to provide conclusive confirmation of the carcinogenicity of extremely low frequency magnetic fields (ELFMF).*
Objectives *To evaluate the potential synergistic carcinogenic effects of concurrent exposure to ELFMF and formaldehyde in four groups of male and female Sprague–Dawley rats.*
Methods *One group was exposed from prenatal life until natural death to S-50 Hz MF and to formaldehyde in drinking water from 6 weeks of age for 104 weeks, two groups were treated only with formaldehyde or only with MF and one group served as untreated control.*
Results *Compared to untreated controls, exposure to MF and formaldehyde causes in males a statistically significant increased incidence of malignant tumors ($P \leq 0.01$), thyroid C-cell carcinomas ($P \leq 0.01$), and hemolymphoreticular neoplasias ($P \leq 0.05$). No statistically significant differences were observed among female groups.*
Conclusions *Life-span exposure to MF and formaldehyde induces statistically significant carcinogenic effects in male rats.* Am. J. Ind. Med.

KEY WORDS: carcinogenic synergism between S-50 Hz MF plus formaldehyde; Sprague–Dawley rats; life-span bioassays; hemolymphoreticular neoplasias; thyroid C-cell carcinomas

INTRODUCTION

Early epidemiological studies have shown that human exposure to extremely low frequency magnetic fields (ELFMF) increases the risk of leukemia in children [Wertheimer and Leeper, 1979], as well as lymphomas/leukemias in adults [Milham, 1982], breast cancer in women aged less than 55 years [Wertheimer and Leeper, 1979] and also in men [Matanoski and Breysse 1989; Demers et al., 1991; Matanoski et al., 1991; Tynes et al., 1992].

Experimental long-term bioassays in which ELFMF were administered alone to rodents, failed to confirm the carcinogenic potential of ELFMF exposure [Margonato et al., 1995; Mandeville et al., 1997; Yasui et al., 1997; Boorman et al., 1999; McCormick et al., 1999].

Moreover, several short-term studies were conducted to evaluate the carcinogenic effects of combined exposure to ELFMF and well-known physical and chemical carcinogenic

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agents. Up to now, the results of these studies have shown either equivocal or weak evidence of the ELFMF capacity to augment the carcinogenic effects of those carcinogens [IARC, 2002]. However, life-span experimental studies designed to evaluate the carcinogenic effects of combined exposure to ELFMF and low-doses of known carcinogens, trying to simulate possible human exposure situations, have never been performed. On the basis of this background, the Ramazzini Institute planned a large project of integrated experiments with a view to evaluating the carcinogenic potential of ELFMF alone or in combination with low doses of other well-known carcinogenic agents. The distinctive common characteristics of this project were (i) the use of a large number of male and female rats per group in order to enhance the sensitivity of the studies; (ii) starting the exposure to ELFMF right from prenatal life and continuing until natural death; (iii) inclusion of all rats in each litter in order to consider family effects; (iv) the availability of almost 20,000 male and female historical controls monitored over the years in a standardized way, including the same diet and same environmental conditions; (v) all rats submitted to complete necropsy and histological evaluation; (vi) the experiments of the project started concurrently in order to compare the effects of the different exposure situations; and (vii) the animals were those born during the simultaneous breeding of 540 breeders.

The design of the project encompasses four experiments in which groups of male and female Sprague–Dawley rats were exposed only to S-50 Hz MF (first experiment including 5,029 rats); in the other three experiments, groups of rats were exposed to S-50 Hz MF plus γ radiation (657 rats), or to formaldehyde (805 rats), or to Aflatoxin B1 (642 rats). Overall the project encompasses 7,133 rats and all the experiments started concurrently.

Carcinogenic Effects on Sprague–Dawley Rats Exposed for the Life-Span to S-50 Hz MF and Formaldehyde in Drinking Water

This paper will report on the results of the study in which a group 200 male and 203 female Sprague–Dawley rats were exposed from prenatal life until natural death to 1,000 μT S-50 Hz MF plus 50 ppm of formaldehyde administered in drinking water from 6 to 104 weeks of age and then observed until natural death. Moreover, one group of 200 males and 202 females was exposed only to formaldehyde, one group of 253 males and 270 females was exposed to 1000 μT and one group of 500 males and 501 females served as negative controls (Table I).

Formaldehyde is a widely produced and used chemical product. Common non-occupational sources of exposure to formaldehyde include combustion processes, release from building materials and tobacco smoke, absorption through the skin from cosmetics. Occupational exposure to formaldehyde occurs in a wide range of occupations and industries. The highest air exposures (2–5 ppm) were measured during varnishing of furniture and wooden floors, in the garment industry, as tissue preservative in embalming and anatomy laboratories, in hospitals for disinfection, in the treatment of fur, and in certain jobs within board mills and foundries, in the production of resins and plastic products [IARC, 2012]. Formaldehyde is considered by IARC to be carcinogenic to humans, causing cancer of the nasopharynx and leukemia [IARC, 2012]. Because of its large-scale industrial use and its environmental occurrence, formaldehyde may be considered a diffuse chemical agent to which large groups of the population may be exposed.

In a life-span bioassay conducted in our laboratory, formaldehyde was administered in drinking water to male and female Sprague–Dawley rats at the intensity levels of 0, 50, 100, 500, 1,000, or 1,500 mg/L. The study showed statistically significant leukemogenic effects in males at the concentration of 100–1,500 ppm; at 50 ppm a statistically non-significant increased incidence of lymphomas/leukemias was observed compared to controls [Soffritti et al., 1989, 2002].

The aim of the present study was to evaluate the potential co-carcinogenic effects of concurrent exposure to 1,000 μT S-50 Hz MF plus formaldehyde administered at 50 ppm in drinking water in Sprague–Dawley rats with particular reference to hematological neoplasias.

MATERIALS AND METHODS

S-50 Hz MF Exposure Conditions and Facilities

In order to give all the experimental groups the same environmental conditions (i.e., a temperature of 22 ± 3°C, a relative humidity of 40–60% and 12 hr/day homogeneous diffusion of light), the rats were located in a room of $60 \times 15 \times 4$ m, in all more than 900 sqm.

The MF exposure system was constructed so as to satisfy a number of technical conditions, namely: (i) the magnetic field was linearly polarized; (ii) the field lines were horizontal and parallel to the ground; (iii) the field uniformity was better than $\pm 10\%$; (iv) the current supply had a maximum harmonic distortion of 3%; (v) the field rise time at power-up was at least 10 periods (for 50 Hz, 200 ms); (vi) the current generator was noiseless; (vii) the joule effect on windings did not alter the environmental temperature, a maximum variation of 2°C being tolerated near coils; (viii) coil noise and vibration were absent; and (ix) the natural field level was no more than 0.1 μT and all mutual interaction of the system was avoided, while in any case the control group stayed in the same room.

TABLE I. Long-Term Syncarcinogenic Study in Sprague–Dawley Rats Exposed to Sinusoidal 50 Hz Magnetic Field (S-50 Hz MF) and Formaldehyde

Plan of the experiment					
	Animals		Treatment		
Group No.	Sex	No.	S-50 Hz MF[a] (μT)	Other[b]	Duration of the study
I	M	500	0	0	Life-span
	F	501			
	M + F	1001			
II	M	253	1,000 C	0	Life-span
	F	270			
	M + F	523			
III	M	200	0	Formaldehyde	Life-span
	F	202		50 mg/L	
	M + F	402			
IV	M	200	1,000 C	Formaldehyde	Life-span
	F	203		50 mg/L	
	M + F	403			
Total		2,329			

[a]The treatment with ELFMF-50 Hz for 19 hr/day, in continuous mode (C), started on the 12th day of pregnancy and lasted until natural death.
[b]Formaldehyde was administered in drinking water, starting at 6 weeks of age for 104 weeks.

The exposure system was constructed by Ansaldo Energia SpA (Genoa, Italy). In order to satisfy the requirements on stray fields, a good solution was obtained using a toroidal shaped device (Fig. 1). The configuration obtained by the optimal design computer program complies both with the test volume field requirements and stray fields outside the magnetic structure. Indeed, about 1 m away from the external torus boundary producing 1 mT, the magnetic field level was approximately 0.1 μT [Montanari, 2003]. The toroid consisted of 24 coils made of three turns of an insulated copper cable, mounted on a superstructure of aluminum composed of two insulated parts in order to avoid a closed loop subject to total field. The total copper cross-section was $11 \times 28\ mm^2$ and the total current used for 1 mT level was 359.6 A. Mounted inside the toroidal magnet was a wooden support structure for rat cages. Each toroidal device was designed for the allocation of 500 rats, 5 per cage, distributed on five levels. The toroidal device has an external diameter of 4.7 m and is 2.1 m high. The 12 devices needed were identical and the different levels of magnetic field were obtained by properly tuning the power supplies which were of current controlled type. A magnetic field probe produced by LAES s.a.s. (Rapallo, Italy) was placed on each device to monitor the field and an IT system continuously stored the exposure data throughout the study. The details of the exposure system have been described by Montanari [2003].

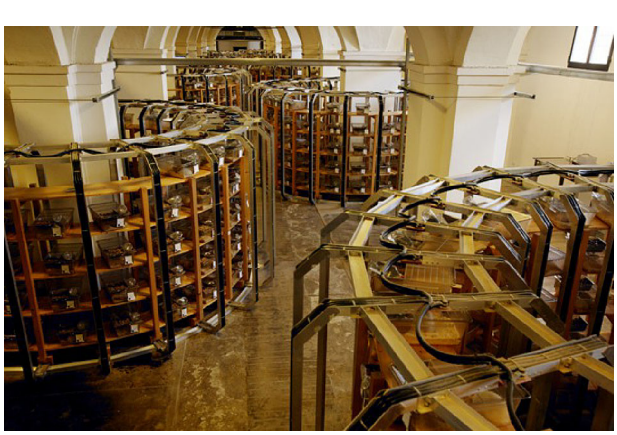

FIGURE 1. System of exposure for 50 Hz MF.

The magnetic exposure conditions were positively evaluated by a representative of the USA National Institute of Standards and Technology in the framework of the scientific collaboration between the US NIEHS/National Toxicology Program and the Ramazzini Institute.

Formaldehyde

Formaldehyde was supplied in aqueous solution by Carlo Erba Reagenti srl (Rodano, Milan, Italy) at a concentration of 40%. The major impurities were the following: formic acid 0.02%; chloride 0.0005%; color (APHA) 10%; heavy metals (Pb) 0.0002%; residue on ignition 0.01%; sulfate 0.002%; iron 0.0001%; lead 0.0001%; and methyl alcohol (stabilizer) 10%.

Diet

All the animals received ordinary feed delivered in pellets ad libitum and provided by "Laboratorio Dottori Piccioni" (Milan, Italy) as has been the practice at the Cesare Maltoni Cancer Research Center of the Ramazzini Institute (CMCRC/RI) for more than 40 years.

Experimental Animals

The animals used for the experiment were Sprague–Dawley rats from the same colony used for more than 40 years at the CMCRC/RI. The basic expected tumorigram and its fluctuations are based upon data derived from more than 20,000 historical controls.

The generation of experimental animals was performed as follows: (i) inbred breeders of the same generation were randomized by body weight in four groups in such a way as to have no more than one brother and sister per group; (ii) the size of breeder groups was dictated by the number of offspring required; (iii) mating of the breeders that generated the offspring for the experiments was strictly outbred (made possible by the pedigree identification number of each animal); it was synchronized among groups and lasted 5 days; (iv) all the offspring of each litter from these breeders were assigned to the respective experimental groups.

All the male and female breeders were euthanized by CO_2 over-exposure, respectively, 3 weeks after birth and 1 week after weaning of the offspring (at 4–5 weeks of age).

The experimental animals were identified by ear punch (Jackson Laboratory method) and distributed by sex, litter by litter, until the planned number for each group was reached. After weaning, animals received ordinary feed and water or formaldehyde solution ad libitum on the basis of the experimental group to which they belonged. Animals were housed 5 per cage, in polycarbonate cages ($41 \times 25 \times 15$) with covers made of non-magnetic material and a shallow layer of white wood shaving as bedding. All the animals were kept in a temperature-controlled environment at 23 ± 2°C and 50–60% of relative humidity, with 12hr/day light/dark alternation.

The experiments were conducted according to the Italian law regulating the use and humane treatment of animals for scientific purposes [Decreto Legislativo, 1992].

Treatments

Treatment with S-50 Hz MF began during fetal life exposing the female breeders from the 12th day of pregnancy and for the offspring it lasted until natural death. Daily duration of the exposure lasted 19 hr/day throughout the study. The exposure system was deactivated (switched off) for 5 hr a day in order to allow animal clinical observation as well as room and animal cleaning.

Formaldehyde was administered to the rats from 6 weeks of age for 104 weeks in drinking water at concentrations of 50 mg/L and supplied ad libitum. Every morning a fresh water solution of formaldehyde was prepared at the required concentration; the residual drinking water from the day before was removed, and the glass drinking bottles were washed and filled with fresh solution. One group of control animals received tap water, without formaldehyde, for the entire duration of the experiment.

Conduct of the Experiment

All the animals were kept under observation until spontaneous death, apart moribunf animals which were sacrificed in order to avoid unnecessary sufferings. From each group, starting from 6 weeks of age, the mean daily drinking water and feed consumptions were measured in a sample of 100 animals per group (50 males and 50 females) every 2 weeks for the first 8 weeks, and then every 4 weeks for 110 weeks. Status and behavior of animals were examined 3 times daily and they were clinically examined for gross changes every 2 weeks for the first 8 weeks and every 4 weeks until the end of the experiment. The animals were weighed every 2 weeks for the first 8 weeks from 6 weeks of age, and then every 4 weeks for 110 weeks, and then every 8 weeks until the end of the experiments.

Upon death, all animals underwent systematic necropsy. Histopathology was routinely performed on the following organs and tissues: skin and subcutanous tissue, mammary glands, the brain, pituitary gland, Zymbal glands, parotid glands, submaxillary glands, Harderian glands, cranium (with bone marrow, oral and nasal cavities, and external and internal ear ducts) (five sections), tongue, thyroid and parathyroid, pharynx, larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes, and any other organs or tissues with pathological lesions.

From each gross lesion (>1 cm in diameter) observed at necropsy, a portion of tissue was taken, flash frozen in liquid nitrogen, and stored at −70°C for molecular biology purposes.

The organs and tissues collected during necropsy were preserved in a 70% solution of Solvanol (a mixture of ethyl and isopropyl alcohol, respectively, approximately 60% and 40%, obtained from Vital srl, Bologna, Italy), and 30% distilled water, apart from bone tissues which were preserved in 10% formalin and then decalcified.

All slides were examined microscopically by the same group of pathologists; a senior pathologist reviewed all

tumors and any other lesion of oncological interest. The histopathological evaluation and classification of benign and malignant tumors were performed following the criteria of the US National Toxicology Program [Boorman et al., 1990].

Statistical evaluation of the various malignant tumors was based on the Cox proportional hazard regression model [Cox, 1972] which was adjusted for possible differential survival. The P-values ($P \leq 0.05$; or $P \leq 0.01$) are reported in the text and in the tables. For those endpoints for which some dose groups had no cases, a simple Mantel–Haenszel model was used since there was no difference in survival between the groups exposed. The statistical software used for the analysis was "EGRET Software Analysis" by the Cytel Software Corporation (Cambridge, MA).

RESULTS

The experiment proceeded smoothly without any noticeable unexpected alteration of the clinical status of the animals in the various groups.

At the beginning of the treatment, we observed a decrease in the mean daily water consumption in the two groups of males drinking water with formaldehyde compared to the negative control group. The decrease was sharper in animals treated with formaldehyde and S-50 Hz MF than in those treated with formaldehyde alone and was evident in males until about 54 weeks of age (Fig. 2). In males, no differences were observed among all groups in body weight (Fig. 3) or in survival (Fig. 4). In females, no changes in water consumption, body weight, or survival were observed among the groups. Statistically significant oncological results relating to the incidence of animals bearing tumors and to site-specific tumors are reported in Tables II and III.

Multiple tumors of differing type and site, of differing type at the same site, of the same type in bilateral organs, of the same type in the skin, in subcutaneous tissue, in mammary glands or at distant sites of diffuse tissue (e.g., bones and skeletal muscles) were counted as simple/independent tumors. Multiple tumors of the same type in the same tissue and organ, apart from those listed below, were counted only once.

Comparison With Untreated Controls

Carcinogenic effects of exposure to 1,000 μT plus formaldehyde compared to untreated controls are reported in Tables II and III.

Benign and malignant tumors

The incidence of benign and malignant tumors is reported in Table II. No statistically significant differences in the incidence of benign tumors were observed among the treated groups compared to controls. A statistically significant increased incidence ($P \leq 0.01$) of malignant tumors was observed in males exposed to S-50 Hz MF plus formaldehyde compared to untreated controls. The evidence of an increased cumulative hazard for total malignant tumor in males exposed to 1,000 μT plus formaldehyde compared to untreated control and the other groups is shown in Table IV and Figure 5.

C-cell thyroid gland tumors and hemolymphoreticular neoplasias

The incidences of C-cell adenomas and C-cell carcinomas of the thyroid gland and hemolymphoreticular

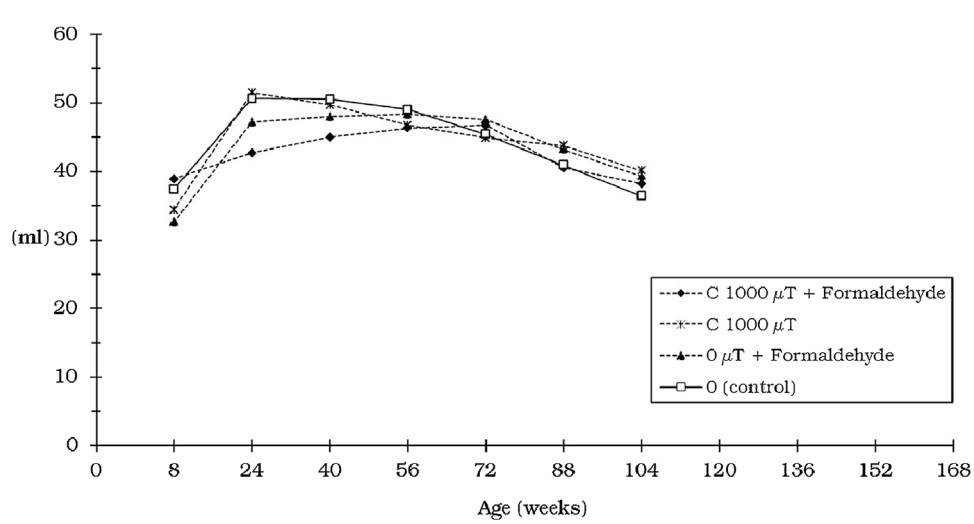

FIGURE 2. Mean daily water consumption in male Sprague–Dawley rats.

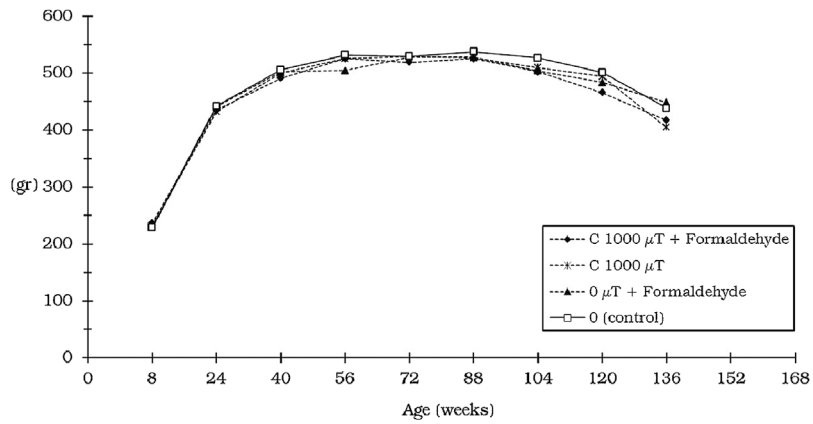

FIGURE 3. Mean body weight in male Sprague–Dawley rats.

neoplasias (HLRN) compared to untreated controls are reported in Table III.

The data show a statistically significant increased incidence of C-cell carcinoma of the thyroid in males after exposure to 1,000 μT plus formaldehyde ($P \leq 0.01$). An increase in the cumulative hazard for C-cell carcinomas in males exposed to 1,000 μT plus formaldehyde compared to the other groups is shown in Table IV and Figure 6. No difference was observed in the incidences of C-cell adenomas among groups. When C-cell adenomas were lumped with C-cell carcinomas, a statistically significant difference ($P \leq 0.01$) was observed in females exposed to formaldehyde alone (Tables III and IV). C-cell thyroid adenomas were large, most of the time occupying the whole lobe, compressing the surrounding tissues, in one case bilaterally, the cells in general being small and showing pale-colored cytoplasm. C-cell carcinomas were nodules of 0.3–0.7 cm macroscopically observed during the necropsies; microscopically the neoplasias involved almost all the lobe of the thyroid (Fig. 7) with infiltration of the adjacent tissue (Fig. 8) and permeation of vessels (Fig. 9). C-cell carcinoma of the thyroid is a very rare tumor in male and female Sprague–Dawley rats from our colony.

A statistically significant increased incidence ($P \leq 0.05$) of HLRN occurred in males treated with S-50 Hz MF plus formaldehyde compared to negative controls (Table III). An increased cumulative hazard for leukemias and lymphomas in males exposed to 1,000 μT and formaldehyde compared to the other groups is shown in Table IV and Figure 10.

The majority of HLRN involved multiple organs and tissues and lumping together lymphomas/leukemias is justified because solid and circulating phases are common in many hematopoietic neoplasms and a distinction would be artificial [Harris et al., 2001]. In our untreated historical

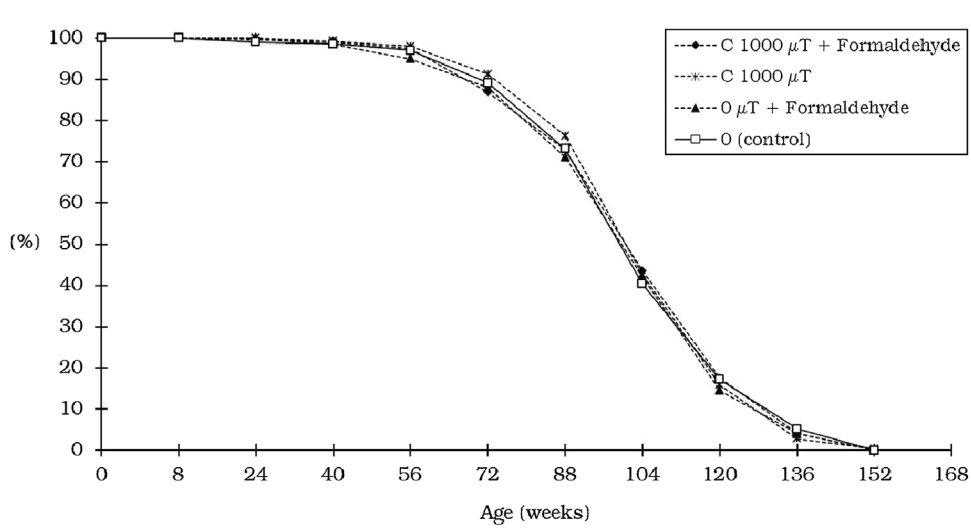

FIGURE 4. Survival in male Sprague–Dawley rats.

TABLE II. Incidence of Benign and Malignant Tumors in Male (M) and Female (F) Sprague–Dawley Rats Exposed to S-50 Hz MF[a] and/or Formaldehyde[b] Compared to Untreated Controls

	Animals		Tumor-incidences			
			Benign tumors		Malignant tumors	
Group No. (μT/mg/L)	Sex	No.	No.	%	No.	%
I	M	500	253	50.6	175	35.0
(0/0)	F	501	341	68.1	213	42.5
II	M	253	132	52.2	86	34.0
(1,000/0)	F	270	196	72.6	118	43.7
III	M	200	101	50.5	83	41.5
(0/50)	F	202	148	73.3	95	47.0
IV	M	200	99	49.5	97	48.5*,**
(1,000/50)	F	203	144	70.9	77	37.9

[a]The treatment with S-50 Hz MF for 19 hr/day, in continuous mode, started on the 12th day of pregnancy and lasted until spontaneous death.
[b]Formaldehyde was administered in drinking water for 104 weeks, starting at 6 weeks of age.
*Statistically significant ($P \leq 0.01$) using Cox Proportional Hazard Model. **Statistically significant ($P \leq 0.05$) when compared to Group II.

controls over the past 10 years, the overall incidence of HLRN was 17.2% (range 9.5–23.4%) out of 1,814 males. Compared to the highest value of the range among historical controls, the incidence of HLRN in males treated with 1,000 μT and formaldehyde was slightly lower, but still significantly higher if compared to the concurrent untreated controls.

Comparison With the 1,000 μT MF-Treated Group

Comparing the oncological results between animals exposed to S-50 Hz MF plus formaldehyde with the group exposed to 1,000 μT (Tables II and III), it may be observed that the exposure to 1,000 μT and formaldehyde induces in males a statistically significant increased incidence of malignant tumors ($P \leq 0.05$), C-cell thyroid carcinomas ($P \leq 0.05$), and HLRN ($P \leq 0.05$). An increased hazard ratio is shown in males for thyroid C-cell carcinomas ($P = 0.04$) and HLRN ($P = 0.02$) (Table V).

Comparison With the 50 mg/L Formaldehyde-Treated Group

Comparing the oncological results in animals exposed to S-50 Hz MF plus formaldehyde with the group exposed only to formaldehyde (Tables II and III), no statistically significant increase was observed in the incidences of the various types of tumors in males; a statistically significant decrease ($P = 0.03$) in the hazard ratio of C-cell adenomas plus C-cell carcinomas was observed in females (Table V).

TABLE III. Incidence of C-cell Thyroid Tumors and Hemolymphoreticular Neoplasias (HLRN) in Male (M) and Female (F) Sprague–Dawley Rats Exposed to S-50 Hz MF[a] and/or Formaldehyde[b] Compared to Untreated Controls

			Tumor incidences							
			C-cell tumor of the thyroid							
	Animals		Adenomas		Carcinomas[c]		Adenomas plus carcinomas		HLRN[d]	
Group No. (μT/mg/L)	Sex	No.	No.	%	No.	%	No	%	No.	%
I (0/0)	M	500	20	4	5	1.0	25	5.0	83	16.6
	F	501	12	2	8	1.6	20	4.0	68	13.6
II (1,000/0)	M	253	11	4.3	3	1.2	14	5.5	36	14.2
	F	270	14	5	3	1.1	17	6.3	44	16.3
III (0/50)	M	200	13	6	3	1.5	16	8.0	44	22.0
	F	202	15	7	5	2.5	20	9.9*	23	11.4
IV (1,000/50)	M	200	8 (9)[e]	4	8	4.0*,***	16	8.0	47	23.5**,***
	F	203	6	3.0	3	1.5	9	4.4	25	12.3

[a]The treatment with S-50 Hz for 19 hr/day, in continuous mode, started on the 12th day of pregnancy and lasted until natural death.
[b]Formaldehyde was administered in drinking water for 104 weeks, starting at 6 weeks of age.
[c]In males, the overall incidence of C-cell carcinomas among 1,814 untreated historical controls of the past 10 years was 1.1% (range: 0.1–2.1%).
[d]In males, the overall incidence of HLRN among 1,814 untreated historical controls of the past 10 years was 17.2% (range: 9.5–23.4%).
[e]Between brackets () is reported the total number of tumors (one animal may bear more than one tumor).
*Statistically significant ($P \leq 0.01$) using Cox Proportional Hazard Model.
**Statistically significant ($P \leq 0.05$) using Cox Proportional Hazard Model.
***Statistically significant ($P \leq 0.05$) when compared to Group II.

TABLE IV. Hazard Ratios (HR) for Male (M) and Female (F) Sprague–Dawley Rats Bearing Total Malignant Tumors (TMT), Thyroid C-Cell Tumors (C-ca, C-cell Adenomas; C-cc, C-Cell Carcinomas), and Haemolymphoreticular Neoplasias (HLRN) Compared to Untreated Controls

					Thyroid C-cell tumors								
		TMT			C-cc			C-cc plus C-ca			HLRN		
Group No. (μT[a]/mg/L[b])	Sex	HR	95%CI	*P*-value	HR	95%CI	*P*-value	HR	95%CI	*P*-value	HR	95%CI	*P*-value
II	M	0.97	0.75–1.26	0.83	1.25	0.30–5.25	0.76	0.98	0.49–1.95	0.95	0.86	0.58–1.27	0.45
(1,000/0)	F	1.13	0.90–1.42	0.28	0.85	0.22–3.21	0.81	1.85	0.97–3.54	0.06	1.30	0.89–1.91	0.17
III	M	1.24	0.95–1.61	0.11	1.66	0.39–6.97	0.49	1.67	0.89–3.14	0.11	1.36	0.94–1.96	0.10
(0/50)	F	1.10	0.86–1.40	0.46	1.47	0.48–4.50	0.50	2.37	1.27–4.40	**0.01**	0.83	0.52–1.34	0.45
IV	M	1.45	1.13–1.86	**0.004**	4.59	1.47–14.35	**0.009**	1.72	0.91–3.24	0.09	1.44	1.01–2.06	**0.05**
(1,000/50)	F	0.92	0.71–1.20	0.53	0.88	0.23–3.34	0.86	0.88	0.37–2.09	0.76	0.96	0.60–1.52	0.85

[a]The treatment with S-50 Hz for 19 hr/day, in continuous mode, started on the 12th day of pregnancy and lasted until natural death.
[b]Formaldehyde was administered in drinking water for 104 weeks, starting at 6 weeks of age.
In bold are reported the statistically significant p-values using Cox proportional hazard model.

DISCUSSION

The present study was planned in the framework of an integrated experimental project encompassing several life-span studies, including combined exposure to ELFMF and carcinogenic agents, designed to evaluate the potential of S-50 Hz MF in the carcinogenic process.

Up to now, apart from some promotional subchronic studies, to our knowledge, no study has been performed to test the potential syncarcinogenic effects of life-span exposure to ELFMF and known carcinogenic agents in rats and mice.

The purpose of this study was to evaluate if concurrent exposure to S-50 Hz MF from prenatal life until natural death and to formaldehyde, a human carcinogen, administered in drinking water from 6 weeks of age for 104 weeks, had the capacity to augment the incidence of malignant tumors in Sprague–Dawley rats compared to controls. The results have

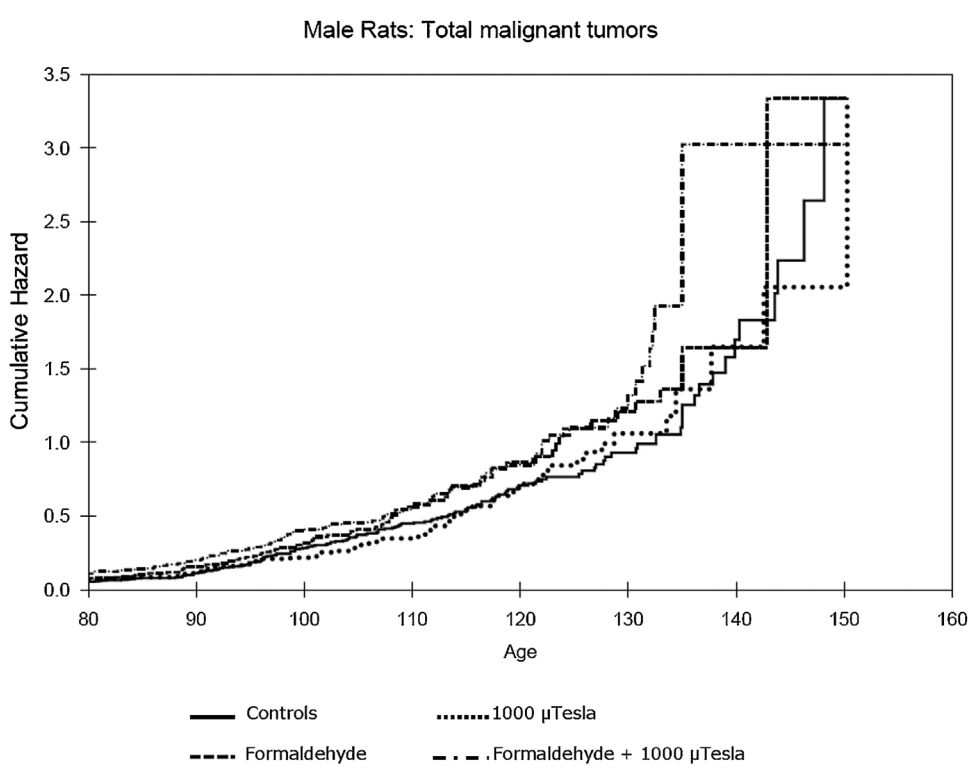

FIGURE 5. Cumulative hazard for Kaplan–Meier estimation for total malignant tumors in male Sprague–Dawley rats.

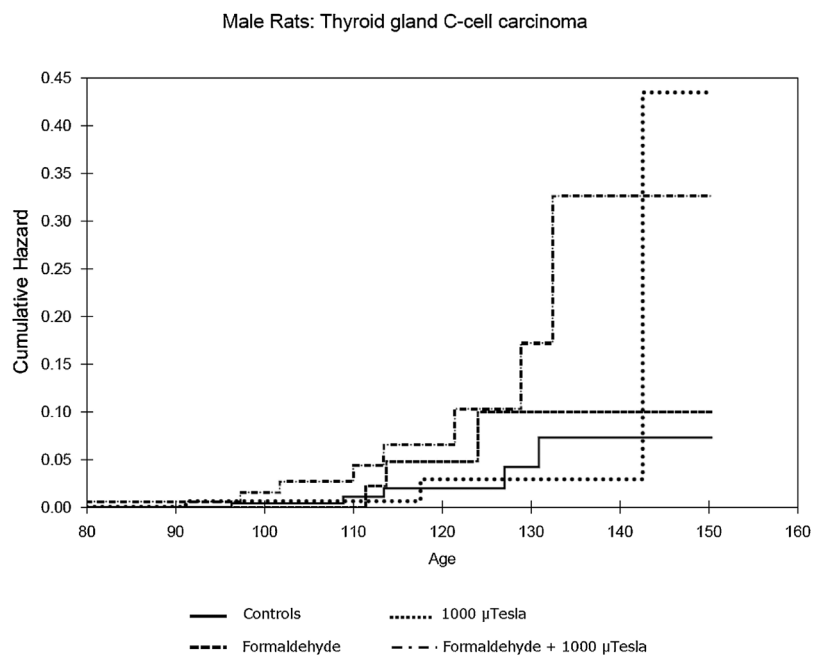

FIGURE 6. Cumulative hazard for Kaplan–Meier estimation for thyroid gland C-cell carcinoma in male Sprague–Dawley rats.

for the first time shown a statistically significant increased incidence of total malignant tumors in males ($P \leq 0.05$) and, in particular, a statistically significant ($P \leq 0.05$) increased incidence of C-cell thyroid carcinomas and of hematological neoplasias ($P \leq 0.05$). These effects were observed both comparing EMF plus formaldehyde to negative controls and in comparison to the group exposed to 1,000 μT alone. The same effects were not observed comparing combined exposure to S-50 Hz MF plus formaldehyde to formaldehyde alone.

Upon histopathological evaluation of the organs and tissues of the males and females exposed to 1,000 μT alone, no statistically significant differences were observed with the negative control group. This confirms the results of other studies in which exposure to 50/60 Hz MF alone does not increase the carcinogenic risk, including thyroid C-cell carcinomas, mammary cancer, brain tumors, and hematological neoplasias [Boorman et al., 1999].

Concerning C-cell thyroid carcinomas, it must be recognized that this is a very rare malignant tumor. In humans, C-cell carcinoma comprises 5–10% of all thyroid malignancies. Among our untreated historical controls of the past 10 years, out of 1,814 male Sprague–Dawley rats, we

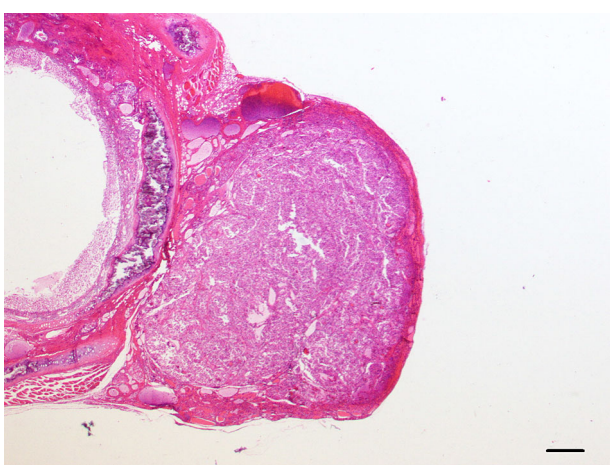

FIGURE 7. C-cell carcinoma fill almost the entire lobe of the thyroid. Staining HE, magnification 25×, bar = 500 μm.

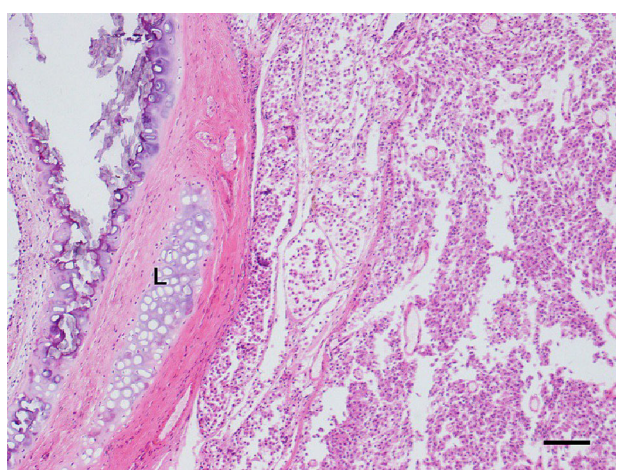

FIGURE 8. C-cell carcinoma spreads beyond the capsule and invades the surrounding tissue adjacent to the larynx (L). Staining HE, magnification 100×, bar = 100 μm.

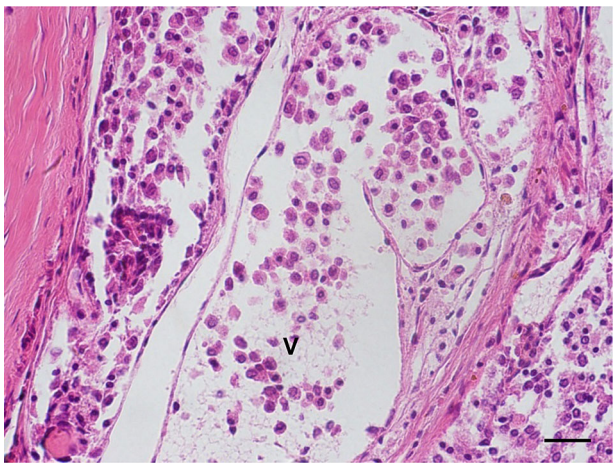

FIGURE 9. The neoplastic C-cells are permeating the blood vessels (V). Staining HE, magnification 400×, bar = 20 μm.

observed an overall incidence of C-cell carcinomas of 1.1% (0.1–2.1%).

Among chemical and physical agents evaluated at the Cesare Maltoni Cancer Research Center, no agent was shown to induce this type of tumor. In the USA National Toxicology Program (NTP), among the long-term carcinogenicity bioassays conducted up to now, only two compounds, Ziram and 2,4 Diaminoanisole sulfate, have shown clear evidence of increasing C-cell carcinomas. This is out of 589 studies, which means that this tumor type is very rarely linked with chemical treatment.

In this respect, it is interesting to note that in the long-term bioassays performed by the NTP, and published in 1999 [Boorman et al., 1999], in which one control group and 4 groups of 100 male and 100 female F344/N rats were treated from 6 weeks of age for 104 weeks at the dose levels of 1,000 (intermittent), 1,000 (continuous), 200 and 2 μT, a statistically significant increased incidence ($P \leq 0.01$) of C-cell carcinomas plus adenomas was found among male rats treated at the doses of 2 and 200 μT (31% and 30%, respectively) compared to 25% in rats treated at 1,000 μT continuously and 16% in controls. In our case, the incidence of C-cell adenomas and carcinomas combined among males exposed to S-50 Hz MF and formaldehyde did not significantly exceed the incidence of other groups; it may be because of the progression of some adenoma to carcinoma due to the life span duration of the experiment. Indeed, observing the cumulative hazard of C-cell carcinomas (Fig. 6), the curves start to differentiate sharply after 120 weeks of age. It may be speculated that in the NTP study if the rats had been observed until 120–130 weeks of age and not sacrificed at 110 weeks of age when more than 50% of animals were still alive, the results might have been different. However, these results were considered by the authors as an equivocal finding providing "little support for the hypothesis that exposure to power frequency magnetic fields may be associated with increased cancer risk."

Of course that study cannot be compared with ours for various reasons: (i) a different goal and consequently different design; (ii) a different strain of rats; (iii) in our

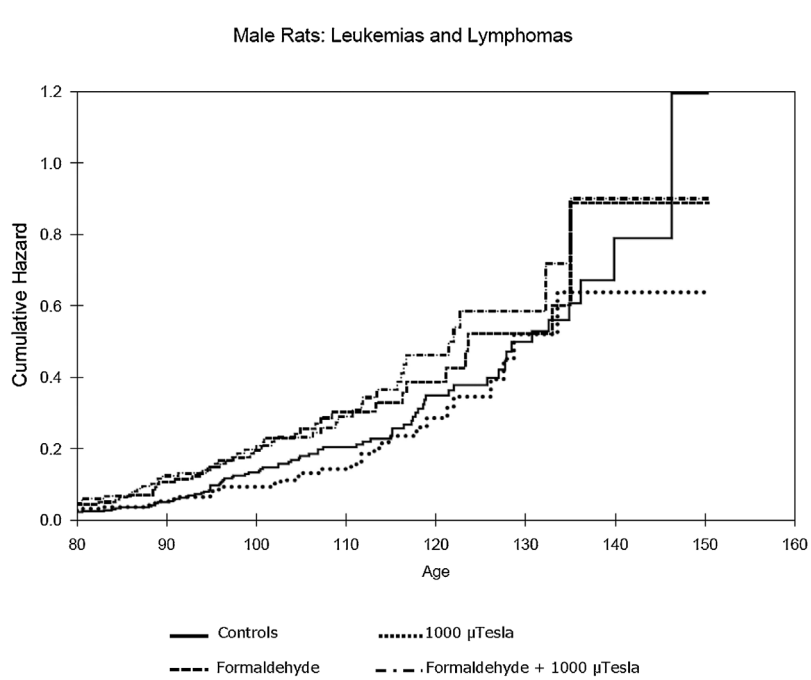

FIGURE 10. Cumulative hazard for Kaplan–Meier estimation for leukemias and lymphomas in male Sprague–Dawley rats.

TABLE V. Hazard Ratios (HR) of Total Malignant Tumors, Thyroid C-Cell Tumors and Hemolymphoreticular Neoplasias in Male and Female Sprague–Dawley Rats Exposed to S-50 Hz MF Plus Formaldehyde Compared to Rats Exposed to Formaldehyde Alone (1,000/50 vs. 0/50) or to S-50 Hz MF Alone (1,000/50 vs. 1,000/0)

		1,000/50 versus 0/50			1,000/50 versus 1,000/0		
Tumors	**Sex**	**HR**	**95%CI**	***P*-value**	**HR**	**95%CI**	***P*-value**
Total malignant tumors	M	1.18	0.88–1.58	0.28	1.49	1.11–1.99	0.08
	F	0.85	0.63–1.15	0.29	0.81	0.61–1.09	0.17
Thyroid C-cell carcinomas	M	2.66	0.70–10.09	0.15	5.01	1.06–23.60	**0.04**
	F	0.61	0.15–2.56	0.50	0.81	0.16–4.17	0.81
Thyroid C-cell carcinomas plus adenomas	M	1.01	0.51–2.03	0.97	1.83	0.95–3.94	0.12
	F	0.39	0.16–0.93	**0.03**	0.44	0.18–1.08	0.07
Hemolymphoreticular neoplasias	M	1.06	0.70–1.60	0.79	1.67	1.08–2.58	**0.02**
	F	1.14	0.64–2.00	0.66	0.73	0.44–1.19	0.20

In bold are reported the statistically significant p-values using Cox proportional hazard model.

study the exposure to EMF started prenatally and lasted all life long; and (iv) our animals were observed until natural death (probably prolonging observation of the NTP study rats might have allowed some C-cell adenomas to progress to carcinomas).

However, the fact that in these different experimental situations, a statistically significant increased incidence of the same rare type of tumor was induced in the same gender, in our opinion raises concern regarding the safety of ELFMF.

Concerning hematopoietic neoplasias, concurrent exposure to S-50 Hz MF and formaldehyde induced a statistically significant increase in lymphomas/leukemias among males compared to negative controls. The same evidence of a statistically significant increased incidence ($P \leq 0.05$) of lymphomas/leukemias in males was observed in a concurrent study performed in our laboratory in which Sprague–Dawley rats were exposed to 1,000 μT of S-50 Hz MF from prenatal life until death and to an acute exposure of 0.1 Gy at 6 weeks of age [Soffritti et al., 2016]. In this case too, the male rats exposed only to 1,000μT alone did not develop a significant increased incidence of lymphomas/leukemias compared to negative controls, or indeed for other types of tumor.

Regarding evaluation of the cancer hazard ratios for males concurrently exposed to S-50 Hz MF and formaldehyde, there does seem to be a multiplicative interaction for C-cell carcinomas but not much else for total malignant tumors and lymphomas/leukemias (Table IV). In females, no statistically significant increase was observed in the incidence of total malignant tumors and other specific targets among the groups. The fact that in this case, a different gender sensitivity was observed cannot be considered unusual in carcinogenicity bioassays. For instance, among the carcinogenic bioassays performed by US NTP, in 65 agents out of 589 tested in rats, a different expression of carcinogenicity by gender was observed: 38 agents proved clearly carcinogenic only in males and 27 only in females [NTP, 2016].

CONCLUSIONS

The results of this study have shown that, compared to negative controls, the concurrent exposure to S-50 Hz MF from prenatal life until natural death, and to formaldehyde in drinking water for 104 weeks starting at 6 weeks of age, induces statistically significant increased carcinogenic effects in male Sprague–Dawley rats, namely an increased incidence of total malignant tumors, C-cell carcinomas of the thyroid and lymphomas/leukemias. Combined exposure to MF and formaldehyde compared to magnetic field alone still induces a statistically significant increased incidence of C-cell carcinomas and HLRN. No statistically significant differences in malignant tumors were observed when combined exposure to MF and formaldehyde was compared to formaldehyde alone.

Concerning C-cell carcinomas, our results reinforce the biological significance of the increased incidence of combined C-cell adenomas and C-cell carcinomas observed among males in the rat bioassay conducted by the US/NTP and classified as an equivocal finding [Boorman et al., 1999].

All in all, the results of this study, together with the demonstration by us that exposure to S-50 Hz MF significantly enhances the carcinogenic effects of γ radiation in male and female Sprague–Dawley rats [Soffritti et al., 2016], confirm the carcinogenic potential of the interaction between ELFMF and known carcinogenic agents. Considering the widespread diffusion of 50/60 Hz MF and of carcinogenic agents in the general and occupational environment, this issue must be taken to heart by public health.

AUTHORS' CONTRIBUTIONS

All authors have contributed in various degrees to the research concept, the experiment design, the conduct, analysis, and interpretation of data for the work. In particular, Dr. Morando Soffritti made the design of the study and had the responsibility in conducting the experiment and producing the draft of the paper; the others members of the Ramazzini group performed the most part of the study in monitoring the system of exposure and conducting the biophase and histopathological evaluation of the experiment; Dr. Hoel performed the statistical analysis of the data; and Dr. Giuliani revised critically the exposure system before starting the study. All authors have read the manuscript and agree that this version of the work is ready for submission to American Journal of Industrial Medicine. Moreover authors accept responsibility for the manuscript's contents ensuring that questions related to the accuracy or integrity of the work is appropriately investigated and resolved.

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ETHICS APPROVAL AND INFORMED CONSENT

The experiments were conducted according to the Italian law regulating the use and humane treatment of animals for scientific purposes [Decreto Legislativo, 1992].

CONFLICTS OF INTEREST

The authors declare no conflict of interest in relation to this work. We also declare that our funding sources had no direct role in the study design, data collection, analysis and interpretation of the data, in the writing of the manuscript, or in the decision to publish the work.

DISCLOSURE BY AJIM EDITOR OF RECORD

Steven Markowitz declares that he has no competing or conflicts of interest in the review and publication decision regarding this article.

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