«Large scale projects of the Ramazzini Institute carcinogenic effects from ionizing and non-ionizing radiations on Sprague-Dawley rats.»

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Ramazzini Institute, Bologna

Ramazzini Days, October 23rd 2015
Carcinogenic effects of ionizing radiations
Some unresolved questions related to ionizing radiation

- Differences in risk between acute and chronic exposure
- Effects of low dose
- What cancer effects, if any, may result from preconception exposure or in utero exposure
- The possible interaction between ionizing radiation and other well-known or potential carcinogenic agents
- Identification of possible radio-sensitive subgroups in the population and how these groups can be identified
The three large scale experiments on γ-radiation at the Ramazzini Institute

Plan of the experiments

• **Acute and fractionated exposure**: 3 groups of M and F rats irradiated with a single dose of 3, 1, 0.1 Gy at 6 weeks of age (Total: 2081); 3 groups of M and F rats exposed to 3, 1, 0.1 Gy fractionated into 10 shots, starting at 6 weeks of age (Total: 884)

• **Prenatal exposure**: 3 groups of M and F rats irradiated one-off prenatally (12th day) at 1, 0.5, 0.1 Gy (Total: 2799)

• **Father exposure**: 3 groups of M and F rats born from parents whose fathers were irradiated one-off by 3, 1, 0.1 Gy before mating (Total: 2557)

• The experiments started in sequence and involved **8321** rats
Experiment on gamma radiation

acute and fractionated exposures
Acute exposure at 6 weeks of age: summary of the carcinogenic effects

- The data have been already published in the AJIM in January 2015
- Total sites with significant increased incidence of cancer endpoints:

<table>
<thead>
<tr>
<th>Number of cancer sites per dose in males (M) and females (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Gy</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

Sites: skin, subcutaneous tissues, mammary glands, head and neck, liver, pancreas, kidney, thyroid, adrenal glands, circulatory system, lymphomas/leukemias
Life-span carcinogenicity bioassay on $\gamma$-radiation delivered at 6 weeks of age as acute (A) or fractionated (Fr) treatment to M and F Sprague-Dawley rats (BT1R)

### Results: TOTAL MALIGNANT TUMORS AND MAMMARY ADENOCARCINOMAS

<table>
<thead>
<tr>
<th>Group / Schedule (Gy)</th>
<th>Sex No of rats</th>
<th>Malignant tumors (%)</th>
<th>Mammary Adenocarcinomas (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A (3)</td>
<td>M (211)</td>
<td>73.9**</td>
<td>3.3**</td>
</tr>
<tr>
<td></td>
<td>F (205)</td>
<td>66.3**</td>
<td>44.4**</td>
</tr>
<tr>
<td>II Fr (3)</td>
<td>M (83)</td>
<td>51.8**</td>
<td>2.4 *</td>
</tr>
<tr>
<td></td>
<td>F (107)</td>
<td>60.7**</td>
<td>27.1**</td>
</tr>
<tr>
<td>III A (1)</td>
<td>M (318)</td>
<td>49.4**</td>
<td>2.2**</td>
</tr>
<tr>
<td></td>
<td>F (301)</td>
<td>47.2**</td>
<td>28.6**</td>
</tr>
<tr>
<td>IV Fr (1)</td>
<td>M (126)</td>
<td>41.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>F (133)</td>
<td>45.9</td>
<td>18.8**</td>
</tr>
<tr>
<td>V A (0.1)</td>
<td>M (524)</td>
<td>48.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>F (522)</td>
<td>53.6</td>
<td>14.9</td>
</tr>
<tr>
<td>VI Fr (0.1)</td>
<td>M (220)</td>
<td>43.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>F (215)</td>
<td>41.9</td>
<td>15.3</td>
</tr>
<tr>
<td>VII Control (0)</td>
<td>M (514)</td>
<td>46.9##</td>
<td>0.6##</td>
</tr>
<tr>
<td></td>
<td>F (537)</td>
<td>52.1##</td>
<td>14.2##</td>
</tr>
</tbody>
</table>

** Statistically significant $p \leq 0.01$ using Cox Proportional Hazard Model

## Near the control incidence are the p-values ($p \leq 0.01$) associated with the Cox Regression Model for the analysis of the trend
Results: TUMORS OF ENDOCRINE PANCREAS AND LIPOSARCOMAS

<table>
<thead>
<tr>
<th>Group / Schedule (Gy)</th>
<th>Sex No. of rats</th>
<th>Islet cell Carcinoma (%)</th>
<th>Liposarcomas (all sites) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A (3)</td>
<td>M (211)</td>
<td>11.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (205)</td>
<td>2.0**</td>
</tr>
<tr>
<td>II</td>
<td>Fr (3)</td>
<td>M (83)</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (107)</td>
<td>0.0</td>
</tr>
<tr>
<td>III</td>
<td>A (1)</td>
<td>M (318)</td>
<td>1.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (301)</td>
<td>2.3**</td>
</tr>
<tr>
<td>IV</td>
<td>Fr (1)</td>
<td>M (126)</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (133)</td>
<td>0.0</td>
</tr>
<tr>
<td>V</td>
<td>A (0.1)</td>
<td>M (524)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (522)</td>
<td>2.1**</td>
</tr>
<tr>
<td>VI</td>
<td>Fr (0.1)</td>
<td>M (220)</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (215)</td>
<td>1.4**</td>
</tr>
<tr>
<td>VII</td>
<td>Control (0)</td>
<td>M (514)</td>
<td>0.8##</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F (537)</td>
<td>0.0**</td>
</tr>
</tbody>
</table>

** Statistically significant $p \leq 0.01$ or $p \leq 0.05$ using Cox Proportional Hazard Model
*** $p$-values $p \leq 0.01$ or $p \leq 0.05$ using Mantel-Haenszel Model for the analysis; there are insufficient data to use Cox Model
## Near the control incidence are the $p$-values ($p \leq 0.01$) associated with the Cox Regression Model for the analysis of the trend
**Results: HEMANGIOSARCOMAS AND HEMOLYMPHORETICULAR NEOPLASIAS**

<table>
<thead>
<tr>
<th>Group / Schedule (Gy)</th>
<th>Sex</th>
<th>Hemangiosarcomas (%)</th>
<th>Lymphomas/Leukaemias</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A (3)</td>
<td>M (211)</td>
<td>7.6**</td>
<td>26.1**</td>
</tr>
<tr>
<td></td>
<td>F (205)</td>
<td>5.4**</td>
<td>8.3*</td>
</tr>
<tr>
<td>II Fr (3)</td>
<td>M (83)</td>
<td>0.0</td>
<td>24.1*</td>
</tr>
<tr>
<td></td>
<td>F (107)</td>
<td>0.9</td>
<td>14.0</td>
</tr>
<tr>
<td>III A (1)</td>
<td>M (318)</td>
<td>1.9**</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>F (301)</td>
<td>1.0</td>
<td>7.3</td>
</tr>
<tr>
<td>IV Fr (1)</td>
<td>M (126)</td>
<td>1.6</td>
<td>19.8</td>
</tr>
<tr>
<td></td>
<td>F (133)</td>
<td>1.5</td>
<td>14.3</td>
</tr>
<tr>
<td>V A (0.1)</td>
<td>M (524)</td>
<td>1.0*</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>F (522)</td>
<td>1.5</td>
<td>13.4</td>
</tr>
<tr>
<td>VI Fr (0.1)</td>
<td>M (220)</td>
<td>1.8*</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>F (215)</td>
<td>1.4</td>
<td>7.9</td>
</tr>
<tr>
<td>VII Control (0)</td>
<td>M (514)</td>
<td>0.0**</td>
<td>23.3##</td>
</tr>
<tr>
<td></td>
<td>F (537)</td>
<td>0.7##</td>
<td>17.9</td>
</tr>
</tbody>
</table>

**Statistically significant p ≤ 0.01 or * p ≤ 0.05 using Cox Proportional Hazard Model**

**p-values p ≤ 0.01 or * p ≤ 0.05 using Mantel-Haenszel Model for the analysis; there are insufficient data to use Cox Model**

**Near the control incidence are the p-values (p ≤ 0.01) associated with the Cox Regression Model for the analysis of the trend**
Experiment on gamma radiation
embryonal exposure
Life-span carcinogenicity bioassay on IONIZING RADIATION, delivered in one shot prenatally (12° day of gestation) to embryo male (M) and female (F) Sprague-Dawley rats (Experiment BT2R)

Results: TOTAL MALIGNANT TUMORS

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment (γ rays)</th>
<th>Dose (Gy)</th>
<th>Animals</th>
<th>Animal bearing malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>M</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>575</td>
</tr>
<tr>
<td>II</td>
<td>0.5</td>
<td></td>
<td>M</td>
<td>363</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>728</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td></td>
<td>M</td>
<td>737</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>759</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>1496</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>M</td>
<td>514</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>537</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>1051</td>
</tr>
</tbody>
</table>

* Significantly greater than controls (p≤0.05); ** (p≤0.01); # trend test p-values (p≤0.05); ## (p≤0.01)
Life-span carcinogenicity bioassay on IONIZING RADIATION, delivered in one shot prenatally (12° day of gestation) to embryo male (M) and female (F) Sprague-Dawley rats (Experiment BT2R)

### Results: MAMMARY GLAND THYROID AND OVARY MALIGNANT TUMORS

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment (γ rays)</th>
<th>Dose (Gy)</th>
<th>Animals</th>
<th>Animals bearing mammary adenocarcinomas %</th>
<th>Animals bearing thyroid follicular adenocarcinoma</th>
<th>Animals bearing ovary adenocarcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>2.1*</td>
<td>M 286</td>
<td>2.1*</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 289</td>
<td>23.2**</td>
<td>1.0</td>
<td>1.7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F 575</td>
<td>12.7**</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>0.5</td>
<td>1.1</td>
<td>M 363</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 365</td>
<td>18.1**</td>
<td>1.6*</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F 728</td>
<td>9.6**</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td>1.2</td>
<td>M 737</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 759</td>
<td>11.1*</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F 1496</td>
<td>6.2**</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0.6 #</td>
<td>M 514</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 537</td>
<td>14.2 ##</td>
<td>0.2 ##</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F 1051</td>
<td>7.5 ##</td>
<td>0.4</td>
<td>-</td>
</tr>
</tbody>
</table>

* Significantly greater than controls (p≤0.05); ** (p≤0.01); # trend test p-values (p≤0.05); ## (p≤0.01)
Life-span carcinogenicity bioassay on IONIZING RADIATION, delivered in one shot prenatally (12° day of gestation) at various doses to embryo male (M) and female (F) Sprague-Dawley rats (Experiment BT2R)

Results: ADRENAL GLAND MALIGNANT TUMORS

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment (γ rays)</th>
<th>Animals Sex</th>
<th>Animals Dose (Gy)</th>
<th>Animals bearing adrenal cortical adenocarcinoma</th>
<th>Animals bearing adrenal feocromoblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>M 286</td>
<td>0.0</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 289</td>
<td>2.1*</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 575</td>
<td>1.0</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>II</td>
<td>0.5</td>
<td>M 363</td>
<td>0.0</td>
<td></td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 365</td>
<td>0.5</td>
<td></td>
<td>3.0**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 728</td>
<td>0.2</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td>M 737</td>
<td>0.1</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 759</td>
<td>0.3</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 1496</td>
<td>0.2</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>M 514</td>
<td>0.4</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 537</td>
<td>1.1 **</td>
<td></td>
<td>1.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 1051</td>
<td>0.7</td>
<td></td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Significantly greater than controls (p≤0.05); ** (p≤0.01); # trend test p-values (p≤0.05); ## (p≤0.01)
Experiment on gamma radiation
Father exposure before mating
Carcinogenic bioassay on ionizing radiation delivered in one shot to male parents Sprague-Dawley rats before mating (BT3R) and following up the offspring for the life-span

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment (γ rays)</th>
<th>Animals</th>
<th>Animals bearing malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (Gy)</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>M</td>
<td>154</td>
</tr>
<tr>
<td></td>
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<td>F</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>321</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>M</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>398</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>799</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td>M</td>
<td>743</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>694</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>1437</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>M</td>
<td>514</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>537</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>1051</td>
</tr>
</tbody>
</table>

* Statistically significant decrease (p≤0.05) or ** (p≤0.01) using Cox Proportional Hazard Model
Conclusions

- Acute exposure to γ radiation causes carcinogenic effects at all tested doses and even at the lowest dose of 0.1 Gy
- When the same acute doses are fractionated in 10 shots, the carcinogenic effects are reduced but the incidence of some tumors is still significantly increased at 0.1 Gy
- Carcinogenic effects have been observed after pre-natal exposure, in particular in females
- Father exposure to γ radiation before mating does not induce carcinogenic effects in the descendants
Carcinogenic effects of non-ionizing radiation
1.8 GHz RFEMF: Life-span carcinogenicity bioassay on 1.8 GHZ RFEMF alone administered at various intensities to males (M) and females (F) Sprague-Dawley rats from prenatal life until natural death

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Dose (V/m)</th>
<th>Animals</th>
<th>Total malignant Tumors %</th>
<th>Mammary adenocarcinoma %</th>
<th>Brain malignant tumors %</th>
<th>Lymphomas/leukemias %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sex No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>M 207</td>
<td>34.3</td>
<td>0.5</td>
<td>-</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 202</td>
<td>42.1</td>
<td>8.9</td>
<td>1.5</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 409</td>
<td>38.1</td>
<td>4.6</td>
<td>0.7</td>
<td>14.9</td>
</tr>
<tr>
<td>II</td>
<td>25</td>
<td>M 209</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 202</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 411</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>M 401</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 410</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 811</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>M 412</td>
<td>29.6</td>
<td>0.5</td>
<td>0.2</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F 405</td>
<td>42.5</td>
<td>7.9</td>
<td>0.2</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F 817</td>
<td>36.0</td>
<td>4.2</td>
<td>0.2</td>
<td>10.6</td>
</tr>
</tbody>
</table>

**Early results**
In 2002 the IARC classified ELFMF as a possible human carcinogen on the basis of epidemiological evidence in children.

Experimental bioassays on rats and mice performed up to now on ELFMF alone or in association with known carcinogens have failed to provide conclusive confirmation.

However, these studies suffer from a number of limitations, in particular regarding:

> Limited number of animals tested per sex / per group
> Limited time of observation
> Few studies have been carried out starting exposure during gestation
This background motivated the RI to embark on a project of life-span experimental studies designed to evaluate carcinogenic effects of ELFMF alone and also in association with other known carcinogens as well as when MF was administered to Sprague-Dawley rats from embryonal life until spontaneous death.
The integrated project on S-50 Hz MF: overall design

<table>
<thead>
<tr>
<th>Experiments</th>
<th>No. of animals</th>
<th>Treatment $^a$ (µ-Tesla)</th>
<th>Other treatment</th>
<th>Duration</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>5029</td>
<td>1000; 100; 20; 2; 0</td>
<td></td>
<td>Life-span</td>
<td>50 Hz MF</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>805</td>
<td>1000; 0</td>
<td>Formaldehyde, 50 ppm in drinking water from 6 weeks of age for 104 weeks</td>
<td>Life-span</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>657</td>
<td>1000; 20; 0</td>
<td>$\gamma$-radiation, 10 rads one shot at 6 weeks of age</td>
<td>Life-span</td>
<td>Promotional</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The treatment with ELFMF started from fetal life and lasted until spontaneous death

$^b$ The first experiment control group of over 500 M and 500 F was repeated in experiments 2-3
S-50-Hz Magnetic Fields exposure system
Experiment on S-50 Hz Magnetic Field alone
Life-span carcinogenicity bioassay on S-50Hz Magnetic Field alone administered at various intensities to M and F Sprague-Dawley rats (BT1CEM)

### Results: MALIGNANT TUMORS

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose S-50Hz MF (µT)</th>
<th>Animals (No.)</th>
<th>Animals bearing malignant tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>253</td>
<td>270</td>
</tr>
<tr>
<td>II</td>
<td>1000 O/O</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>III</td>
<td>100 C</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>IV</td>
<td>20 C</td>
<td>501</td>
<td>502</td>
</tr>
<tr>
<td>V</td>
<td>2 C</td>
<td>500</td>
<td>502</td>
</tr>
<tr>
<td>VI</td>
<td>0 (control) (b)</td>
<td>500</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>2504</strong></td>
<td><strong>2525</strong></td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C) or intermittent On/Off (O/O)

(b) The control group is common among experiments BT1-3 CEM
Experiment on S-50 Hz Magnetic Field plus Formaldehyde
Life-span syncarcinogenic bioassay in Sprague-Dawley rats exposed to S-50Hz Magnetic Field (S-50Hz MF) and Formaldehyde (BT2CEM)

Results: total malignant tumors compared to negative controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Animal bearing malignant tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MF S-50 Hz (µT)&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde (mg/l)&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>M</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>403</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>M</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>402</td>
</tr>
<tr>
<td>III</td>
<td>0 (control)&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>M</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M+F</td>
<td>1001</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12<sup>th</sup> day of pregnancy
(b) Formaldehyde was administered in drinking water for 104 weeks, starting at 6 weeks of age
(c) The control group is common among the experiments BT1-3 CEM

** Statistically significant p≤0.01 using Cox Regression Test

Near the control incidence is the p-value # (p≤0.05) associated with Cox Regression Model for trend analysis
## Results: thyroid c-cell carcinomas and lymphomas/leukemias compared to negative controls

<table>
<thead>
<tr>
<th>Group</th>
<th>S-50Hz MF (µT)</th>
<th>Formaldehyde (mg/l)</th>
<th>Animals</th>
<th>Animal bearing C-cell carcinomas %</th>
<th>Animal bearing Lymphomas/Leukemias %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µT)(^{(a)})</td>
<td>(mg/l)(^{(b)})</td>
<td>Sex</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>50</td>
<td>M</td>
<td>200</td>
<td>4.0 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>203</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>403</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23.5 *</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>50</td>
<td>M</td>
<td>200</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>202</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>402</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22.0</td>
</tr>
<tr>
<td>III</td>
<td>0 (control)(^{(c)})</td>
<td>-</td>
<td>M</td>
<td>500</td>
<td>1.0 ##</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>501</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>1001</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.6 #</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12\(^{th}\) day of pregnancy

(b) Formaldehyde was administered in drinking water for 104 weeks, starting at 6 weeks of age

(c) The control group is common among the experiments BT1-3 CEM

* Statistically significant \( p \leq 0.05 \) or ** \( p \leq 0.01 \) using Cox Regression Test

Near the control incidence is the p-value # \( p \leq 0.05 \) or ## \( p \leq 0.01 \) associated with Cox Regression Model for trend analysis
Experiment on S-50 Hz Magnetic Field plus γ radiation
### Results: total malignant tumors and mammary adenocarcinomas compared to negative controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Animal bearing malignant tumors</th>
<th>Animal bearing mammary Adenocarcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-50 Hz MF (µT)</td>
<td>γ-radiation (Gy)</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>0.1</td>
<td>M</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>222</td>
</tr>
<tr>
<td>II</td>
<td>20 C</td>
<td>0.1</td>
<td>M</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>212</td>
</tr>
<tr>
<td>III</td>
<td>0 (+ control)</td>
<td>0.1</td>
<td>M</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>223</td>
</tr>
<tr>
<td>IV</td>
<td>0 (- control)(c)</td>
<td>-</td>
<td>M</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>1001</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12\textsuperscript{th} day of pregnancy
(b) Administered one-off ad 6 weeks of age
(c) The control group is common among experiments BT1-3 CEM

* Statistically significant \( p \leq 0.05 \) or ** \( p \leq 0.01 \) using Cox Regression Test

Near the negative control incidence is the p-value # \( p \leq 0.05 \) or ## \( p \leq 0.01 \) associated with Cox Regression Model for trend analysis
**Life-span initiation/promotion bioassay on S-50Hz Magnetic Field (S-50Hz MF) and γ-radiation administered to M and F Sprague-Dawley rats (BT3CEM)**

**Results: aggregated animals bearing mammary cancers or their atypical precursors compared to negative controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Aggregated animals bearing mammary cancers or their atypical precursors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-50 Hz MF (µT)</td>
<td>γ-radiation (Gy)</td>
<td>Sex</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>0.1</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
</tr>
<tr>
<td>II</td>
<td>20 C</td>
<td>0.1</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
</tr>
<tr>
<td>III</td>
<td>0 (+ control)</td>
<td>0.1</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
</tr>
<tr>
<td>IV</td>
<td>0 (- control)</td>
<td>-</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12th day of pregnancy
(b) Administered one-off ad 6 weeks of age
(c) The control group is common among experiments BT1-3 CEM

♦ Statistically significant p≤0.05 or □□ p≤0.01 using the Mantel-Haenszel Model for the analysis (used for incidental lesions)

Near the negative control incidence is the p-value ♦ p≤0.05 or ♦♦ p≤0.01 using the Mantel-Haenszel Model for incidental lesions for trend analysis
## Results: Malignant Schwannomas of the heart and Lymphomas/Leukemias compared to negative controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Animals bearing heart mal. Schwannomas</th>
<th>Animals bearing Lymph/Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-50 Hz MF (μT)</td>
<td>γ-radiation (Gy)</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>0.1</td>
<td>M</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>222</td>
</tr>
<tr>
<td>II</td>
<td>20 C</td>
<td>0.1</td>
<td>M</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>212</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0.1</td>
<td>M</td>
<td>118</td>
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<tr>
<td>(+ control)</td>
<td></td>
<td></td>
<td>F</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>223</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>M</td>
<td>500</td>
</tr>
<tr>
<td>(- control)</td>
<td></td>
<td></td>
<td>F</td>
<td>501</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M+F</td>
<td>1001</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12th day of pregnancy
(b) Administered one-off ad 6 weeks of age
(c) The control group is common among experiments BT1-3 CEM
* Statistically significant $p \leq 0.05$ or ** $p \leq 0.01$ using Cox Regression Test
Near the negative control incidence is the $p$-value # $p \leq 0.05$ or ## $p \leq 0.01$ associated with Cox Regression Model for trend analysis
### Results: significantly different incidences of malignant tumors compared to 0.1 Gy

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Total Malignant Tumors</th>
<th>Animals bearing Lymph/Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-50 Hz MF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(µT)</td>
<td>γ-radiation</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>(µT)</td>
<td>(Gy)</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>0.1</td>
<td>M</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>112</td>
</tr>
<tr>
<td>II</td>
<td>20 C</td>
<td>0.1</td>
<td>M</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>107</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0.1 (+ control)</td>
<td>M</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>105</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/day, continuous (C), started 12th day of pregnancy
(b) Administered one-off at 6 weeks of age
* Statistically significant $p \leq 0.05$ using Cox Regression Test
† Statistically significant $p \leq 0.05$ or ‡ $p \leq 0.01$ using the Mantel-Haenszel Model for the analysis (used for incidental lesions)
Near the negative control incidence is the p-value † $p \leq 0.05$ or ‡‡ $p \leq 0.01$ using the Mantel-Haenszel Model for incidental lesions for trend analysis
Results: significantly different incidences of malignant mammary tumors compared to 0.1 Gy

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Animals</th>
<th>Mammary ADCA plus atypical lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-50 Hz MF (µT)(a)</td>
<td>Sex</td>
<td>No.</td>
</tr>
<tr>
<td>I</td>
<td>1000 C</td>
<td>M</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>112</td>
</tr>
<tr>
<td>II</td>
<td>20 C</td>
<td>M</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>107</td>
</tr>
<tr>
<td>III</td>
<td>0 (+ control)</td>
<td>M</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>105</td>
</tr>
</tbody>
</table>

(a) Treatment for 19 hours/die, continuous (C), started 12<sup>th</sup> day of pregnancy
(b) Administered one-off ad 6 weeks of age

* Statistically significant compare to 0.1 Gy group (Group II) p≤0.05 using Cox Regression Test
† Statistically significant p≤0.05 or ‡ p≤0.01 using the Mantel-Haenszel Model for the analysis (used for incidental lesions)

Near the negative control incidence is the p-value ◊ p≤0.05 or ◊◊ p≤0.01 using the Mantel-Haenszel Model for incidental lesions for trend analysis
Results: Hazard for mammary gland adenocarcinomas in females compared to 0.1 Gy treated group.
Conclusions (Part I)

- The exposure to 50 Hz MF alone for the life-span showed no carcinogenic effects and confirmed the negative results of other authors;
- Significant increased incidences of animals bearing malignant tumors, thyroid C-cell carcinomas and Lymphomas/leukaemias were observed in males exposed to 1000μT and formaldehyde compared to negative controls;
In animals exposed to S-50Hz MF and γ-radiation compared to negative controls, significant increased incidences were observed in:
- males bearing malignant tumors
- males and females bearing mammary cancers;
- males bearing heart-malignant schwannomas;
- males bearing lymphomas /leukaemias;

- when compared to positive controls (0.1 Gy) the differences were still significant for males bearing malignant tumors, lymphomas /leukaemias and mammary cancers in females exposed to 1000µT plus 0.1 Gy.
Moreover, our results on mammary cancer in male and female rats seem to support the hypothesis reported in early epidemiological studies that EMF may be involved in the carcinogenesis process both in women (Wetheimer and Leeper, 1979) and men (Matanoski and Breysse, 1989; Tynes et al, 1992) as well as leukemia in adults (Milham, 1982).

Finally, it cannot be underestimated that a child can receive 30 mGy for each CT scan and that for each study at least 3-4 scans are needed (which corresponds at least to 120 mGy, which is > 0.1 Gy) and that this exposure has been demonstrated to increase the cancer risk in children (Pearce et al., 2012).
In conclusion, in my opinion these results call for a re-evaluation of the safety of non-ionizing radiation, particularly at this time when the pressure to move from conventional fuel-based mobility to electric mobility deserves high priority in the EU and US and other industrialized countries.
I would appreciate an opportunity to review your report.
Thank you.

On November 3, 2014 10:35:00 AM EST, "Dott. Morando Soffritti" <soffrittim@ramazzini.it> wrote:

Dear Colleagues,

I am sending you the paper on the effects of gamma radiation which has been now online on American Journal of Industrial Medicine.

Any comments are welcome.

Best regards,

Morando Soffritti