Annual Ramazzini Days
LIVING IN A CHEMICAL WORLD, IV EDITION

WELCOME!!!
The CMCRC (1969)

Cesare Maltoni (1930-2001)

Castle built in 1475
Domus Jucunditatis
of Bentivoglio family
The Cancer Research Center of the Ramazzini Institute dedicated to Professor Cesare Maltoni, September 20°, 2005
The Ramazzini Institute (RI) is a non-profit independent cooperative; it is located in Bologna, Italy.

The RI depends on support from more than 25,000 members, each year covering 30% of the RI budget.

Our work principally involves strategies based on prevention of environmental diseases, in particular cancer.

Besides member support, funding in 2014 came from associations, banking foundations, private individuals and public and private international organizations (EU Commission, NIEHS, MSSM, and others).
2015: NIEHS and Ramazzini Institute general agreement
Cesare Maltoni: peculiarities of his experimental research

Among the different projects for cancer prevention, Professor Cesare Maltoni favored experimental research. He started in 1969 and his research had the following prerequisites:

- It was a well planned and systematic research project;
- The procedures were consistent and homogeneous over time;
- The focus of research was original and innovative.
In 1982 Prof. Maltoni together with Prof. Irving J. Selikoff and other eminent scientists founded the “Collegium Ramazzini”
Cesare Maltoni: The international relationship
Since 2014, the Scientific Advisory Board assesses projects proposed by the Ramazzini Institute, highlights study opportunities of social and economic importance, possibly suggests changes to research protocols, and critically appraises the results of studies presented.
The Scientific Advisory Board

Professor PHILIP J. LANDRIGAN, President, Professor of Pediatrics, Director, Children's Environmental Health Center (Icahn School of Medicine at Mount Sinai, NY, NY, USA)
http://www.mountsinai.org/profiles/philip-j-landrigan

Dr. KRISTINA THAYER, director of the National Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT), USA.
www.niehs.nih.gov/research/atniehs/dntp/assoc/ohat/

Dr. PIETRO COMBA, Director, Unit of Environmental Epidemiology, National Institute of Health, Italy

Dr. MELISSA McDIARMID, Professor of Medicine and Director of the University of Maryland School of Medicine's Occupational Health Program, USA
http://findadoctor.umm.edu/Details/23375

Professor ELLEN SILBERGELD, Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MA, USA

Dr. LENNART HARDELL, Professor of Oncology and Epidemiology, Orebro, Sweden.
http://www.lennarthardellenglish.wordpress.com/

Dr. MARIÉ-NOEL BRUNE DRISSE, Uruguay. Children Health Department, WHO
http://www.excellence-in-paediatrics.org/content/page/292/childrens-environmental-health
The human equivalent model

Sprague-Dawley rats of the CMCRC Colony

- Body weight and survival trends at 104 weeks of age in 40 years
- Diet composition is the same from 1969
The human equivalent model

CMCRC colony: body weight and 104-week survival compared to other facilities
Comparing distribution by age at death of:

- **1,114 people** (1/2 both sexes) with malignant tumors (out of 2,560 autopsied men and women deceased at the Hospital of Trieste, in 1989)

- **1,212 Sprague-Dawley rats** (1/2 both sexes) with malignant tumors (out of 3,051 necropsied male and female untreated rats, monitored until spontaneous death, used as control groups 1984-1994)

- **10 years of humans** are equivalent to **16 weeks in a rat**
The human equivalent model

Both in humans and in rats 80% of tumors arise after age 65 years/104 weeks

Cumulative prevalence of animals/humans with malignant tumors, histopathologically observed, by age at death

Age: 16 weeks of age in Sprague Dawley rats are considered equivalent to 10 years in humans

Data from the Hospital of Trieste were kindly made at our disposal by Professor Luigi Giarelli
LIFE SPAN /130 WEEKS

Survival

Cumulative prevalence of total malignant tumors histopathologically observed, by age at death

Total malignant tumors in Sprague-Dawley, male rats

Xylenes
VINYL CHLORIDE: comparison of the effects of neonatal exposure (offspring) and adult exposure (breeders)

SPRAGUE-DAWLEY RATS (Exp. BT14, 1976)*
Treatment: 4hd/5dw/5w

Cytochrome P450 2E1 **

- Adult exposure (breeders)
- Neonatal exposure (offspring)

N. for 100 animals


10000 ppm
0 10 20 30 40 50 60

6000 ppm

* Maltoni et al, 1981
** El Ghissassi, Barbin, and Bartsch, 1998; Novak and Woodcroft, 2000
Results of the RI experimental project

- Studied compounds/agents: 210
- Elaborated/published results: 120

- Carcinogenicity:
  - Clear evidence (44%)
  - Borderline evidence (16%)
  - No evidence (40%)
## Predictivity of the RI study results

<table>
<thead>
<tr>
<th>Agent/Compound</th>
<th>CMCRC-RI*</th>
<th>IARC Group/year</th>
<th>Predictivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinyl Chloride</td>
<td>1974</td>
<td>1 /1979</td>
<td>5 years</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>1989</td>
<td>1 /2012</td>
<td>23 years</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>1986</td>
<td>1 /2014</td>
<td>28 years</td>
</tr>
<tr>
<td>Benzene</td>
<td>1979</td>
<td>1 /2012</td>
<td>33 years</td>
</tr>
<tr>
<td>MTBE</td>
<td>1995</td>
<td>3 /1999</td>
<td>?</td>
</tr>
<tr>
<td>Aspartame</td>
<td>2005</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

*first evidence as multipotent carcinogen*
In order to implement efficient primary prevention by experimental bioassays we should consider all the various components of risk which were until a few years ago underestimated or ignored. Our challenges at CMCRC are now to understand:

- the role of **early exposures**
- the role of **low concentrations**
- how **combined environmental exposures** affect disease pathogenesis
- systematic reviews and **evidence based toxicology**

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In conclusion, to reach these goals, what is the lesson from 40 years of research to detect environmental risks?
50 years of long-term studies in rodents have been the accepted benchmark for assessing chronic long-term toxic effects, particularly carcinogenic, of chemicals.

The EFSA and WHO have pointed out that the current set of internationally utilized test methods capture only some of the potential adverse effects associated with exposures to these agents over the lifetime.

Our model is sensitive, predictive for human health, very consistent over the time.
Integrative experimental model

The adaptation of the carcinogenicity bioassay to integrate additional protocols for comprehensive long-term toxicity assessment that includes developmental exposures and long-term outcomes, capable of generating information on a broad spectrum of different endpoints.
Integrating a comprehensive set of relevant toxicological endpoints in a single protocol represents an efficient opportunity, reducing animal use in accordance with the 3Rs (replacement, reduction and refinement).

This strategy has the potential to provide sufficient data on multiple windows of susceptibility of specific interest for risk assessments and public health decision making, by including prenatal, lactational, neonatal exposures and evaluating outcomes over the lifespan.
Integrated experimental model

- **Same generational cohort of rats** is used for evaluating long-term outcomes and for measuring biomarkers and other parameters related to system-specific responses including metabolic alterations and endocrine disturbances.

- **2,140 animals per study**, with a **reduction up to 62%** as compared to using separate test protocols (OECD guidelines) representing an opportunity for investigating multiple toxicological endpoints at once, **sparing animal lives**.

- **Reduction in terms of time (and costs):** shorter time for design, approval, performance and analysis if compared with multiple and sequential ones.
Integrated experimental model

- The *in vivo* biophase would be responsibility of one laboratory in order to consistency and quality of methods

- information on each category of parameters might be evaluated by different topic-expert scientists/laboratories

- Experience will be required in order to reach the *same level of confidence* that has been achieved for the standard carcinogenicity bioassays

- A priori establishment of criteria and consensus on relevant endpoints of interest is also *good starting point for evidence based evaluations* and following systematic review of the obtained results

- My Colleagues Drs Manservisi Panzacchi and Mandrioli will present more details regarding some of our new methodologies
Conclusions

“The reward of great men is that, long after they have died, one is not quite sure that they are dead”

Jules Renard, 1864 - 1910