Cesare Maltoni Cancer Research Center
Ramazzini Institute, Bologna Italy

PAN Europe Conference

Our Disrupted Food: Endocrine Disrupting Chemicals In Pesticides Residues

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European Parliament
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THE RAMAZZINI INSTITUTE
The Ramazzini Institute (RI) is a non-profit, independent organization located in Bologna, Italy. It is a social cooperative with more than 24,000 active associates.

Facilities include:
- A Cancer Research Center where one of the world’s largest and longest-existing programs of carcinogenesis bioassays is performed
- A GLP Laboratory
- A Clynical facility for oncological surveillance

In 40 years, long-term carcinogenicity studies have been conducted at the CMCRC on more than 200 agents present in the industrial and general environment performing more than 500 bioassays.
The **aims** of the Ramazzini Institute are:

- Implementing schemes of tumor prevention by a strategy based on **promotion of scientific research**
- Training specialized staff
- Circulating information on environmental and work-related cancer risks and other diseases
- to set up clinical programs of early tumor diagnosis
THE ENVIRONMENT AND CANCER
Factors which involve the carcinogenicity process

We can’t control ageing and genetic factors, to protect people we can just prevent the exposure risk.
The causes of cancer: genetic susceptibility

DNA as the tale of the 3 little pigs

DNA structure image

DNA as the tale of the 3 little pigs images
Why are children more vulnerable?

Because they aren’t little adults...!
THE HUMAN-EQUIVALENT MODEL
THE ANIMAL MODEL OF THE RI

Sprague-Dawley rats
Compared 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 tumor (out of 3,051 necropsied male and female untreated rats, under control until spontaneous death, used as control groups 1984-1994)

- **10 years of humans** are equivalent to **16 weeks in a rat**
A HUMAN-EQUIVALENT MODEL

Both in humans and rats 80% of tumors arise after the 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.
A HUMAN-EQUIVALENT MODEL

Distribution of animals/humans with mammary malignant tumors, histopathologically observed, by age at incidence in 1264 out of 130000 women and 226 out of 2274 female rats

Age: 16 weeks of age in Sprague Dawley rats are considered equivalent to 10 years in humans
Mancozeb
Mancozeb: Thyroid malignant tumors in male Sprague-Dawley rats
Mancozeb: Thyroid malignant tumors in female Sprague-Dawley rats
ENDOCRINE INTERFERENCE
This study is part of the NIH founded project “Breast Cancer Genomics in Windows of Susceptibility to Endocrine Disruptors.”

It combines animal experiments and epidemiologic investigations using a bi-directional translation approach.

Epidemiologic data were drawn from the population-based Long Island Breast Cancer Study Project (LIBCSP).
Aims

Explore whether environmental endocrine disrupting chemicals (EDs) act in specific developmental windows and whether they exert their biological effects independently or synergistically/antagonistically in breast tissue leading to breast cancer development.
Animal experimental phase at RI

Female Sprague-Dawley rats were daily treated with 3 EDs:
- diethylphthalate (DEP)
- methylparaben (MPB)
- triclosan (TRC), and
- a mixture of the three EDs.
Dose-calibration study

Oral dose of each ED which would result in rat urinary metabolite concentrations comparable to the concentrations detected in the LIBSCP population.

Main study

in order to explore whether environmental EDs act on six mammary cancer susceptibility windows (prenatal, postnatal, pre-puberty, pubertal, parous, nulliparous)
DOSE-CALIBRATION STUDY: RESULTS ON DIETHYL PHTHALATE

Mono-Ethyl-phtalate = metabolite of Diethylphtalate (DEP)

ppb

1000000

100000

10000

1000

100

10

1

vehicle olive oil

Low NOEL/100.000
0,01735 mg/Kg/bw

Medium NOEL/10.000
0,1735mg/Kg/bw

High NOEL/200
8,675 mg/Kg/bw

LI max

LI 95%

LI median
Dose-calibration study: results on Methy paraben

- Vehicle (olive oil)
  - NOEL/100,000: 0.0105 mg/kg/bw

- Low dose (NOEL/10,000: 0.105 mg/Kg/bw)

- Medium dose
  - NOEL/10,000: 0.105 mg/Kg/bw

- High dose
  - NOEL/200: 5.25 mg/Kg/bw

LI max
LI 95%
LI median
Dose-calibration study: results on Triclosan

![Graph showing Triclosan levels and NOEL values](image)

- **Vehicle**: Olive oil
- **Low**: NOEL/10,000 mg/Kg/bw
- **Medium**: NOEL/1,000 mg/Kg/bw
- **High**: NOEL/200 mg/Kg/bw

**LI Max.**
**LI 95%**
**LI median**
## MAIN STUDY

<table>
<thead>
<tr>
<th>Windows of susceptibility</th>
<th>Treatment</th>
<th>Administration (by oral gavage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>1. Pre-natal</td>
<td>matching</td>
<td>delivery</td>
</tr>
<tr>
<td>2. Neo-natal</td>
<td>Post Natal Day (PND)1</td>
<td>PND20</td>
</tr>
<tr>
<td>3. Pre-puberty</td>
<td>PND21</td>
<td>PND40</td>
</tr>
<tr>
<td>4. Pubertal</td>
<td>PND42</td>
<td>PND62</td>
</tr>
<tr>
<td>5. Adult-parous</td>
<td>PND1</td>
<td>PND180</td>
</tr>
<tr>
<td>6. Adult-nulliparous</td>
<td>PND1</td>
<td>PND180</td>
</tr>
</tbody>
</table>
ED exposure results in profound changes in both gross phenotypes (e.g. reproductive mortality and mammary gland morphology) as well as in molecular genome profiles, at levels comparable to those of human scenario.

More specifically, ED exposure appeared to hamper normal breast development and resulted in increased mortality in the offspring, possibly due to reduced milk production.

Whole genome expression profiling of mammary tissue also revealed that in the course of development, the number of differentially expressed genes was lower in ED-treated rats compared to controls, suggesting developmental delay or suppression by ED exposure.
CONCLUSIONS
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✓ Some pesticides or their metabolites have been demonstrated endocrine disrupting chemicals (EDCs)

✓ Studies on EDCs chemicals effects cannot be performed with conventional protocols

✓ OECD or EFSA guidelines do not establish criteria for studying EDCs and actions are needed (Collegium Ramazzini statement)
CONCLUSIONS

✓ In 40 years of activity in environmental and cancer research, the RI is now able to indicate feasible models and protocols

✓ This protocol covers not only general toxicity/carcinogenicity end-points, but also different biological mechanistic parameters, including endocrine interference
CONCLUSIONS

- Protocol includes:
  - Satellite groups from the same generation of the concurrent long-term bioassays (OECD TG 453)
  - Starting the exposition during prenatal life or after weaning
  - Different schedule of treatment to evidence WOS
  - Possibility of comparison and integration with the long-term concurrent bioassay
  - The adoption of our protocols helps sparing animals and resources