Cesare Maltoni Cancer Research Center
Ramazzini Institute, Bologna Italy

PAN Europe Conference

Our Disrupted Food: Endocrine Disrupting Chemicals In Pesticides Residues

Dr. Fiorella Belpoggi

European Parliament

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THE RAMAZZINI INSTITUTE

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- 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

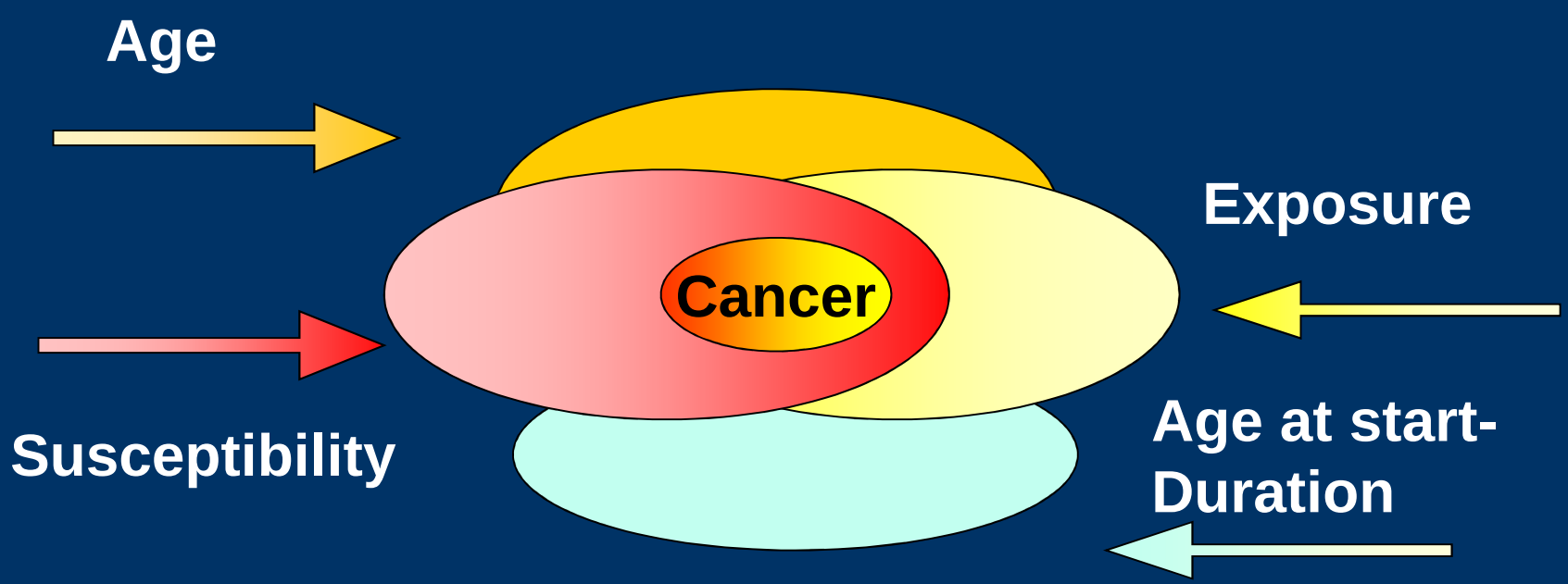
RAMAZZINI INSTITUTE: THE AIMS

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

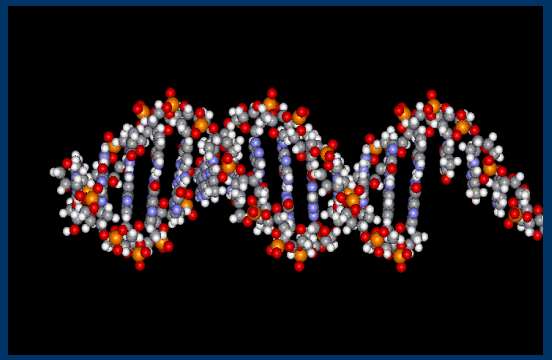
THE ENVIRONMENT AND CANCER

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

Why are children more vulnerable?

Because they aren't little adults...!

THE HUMAN-EQUIVALENT MODEL

THE ANIMAL MODEL OF THE RI

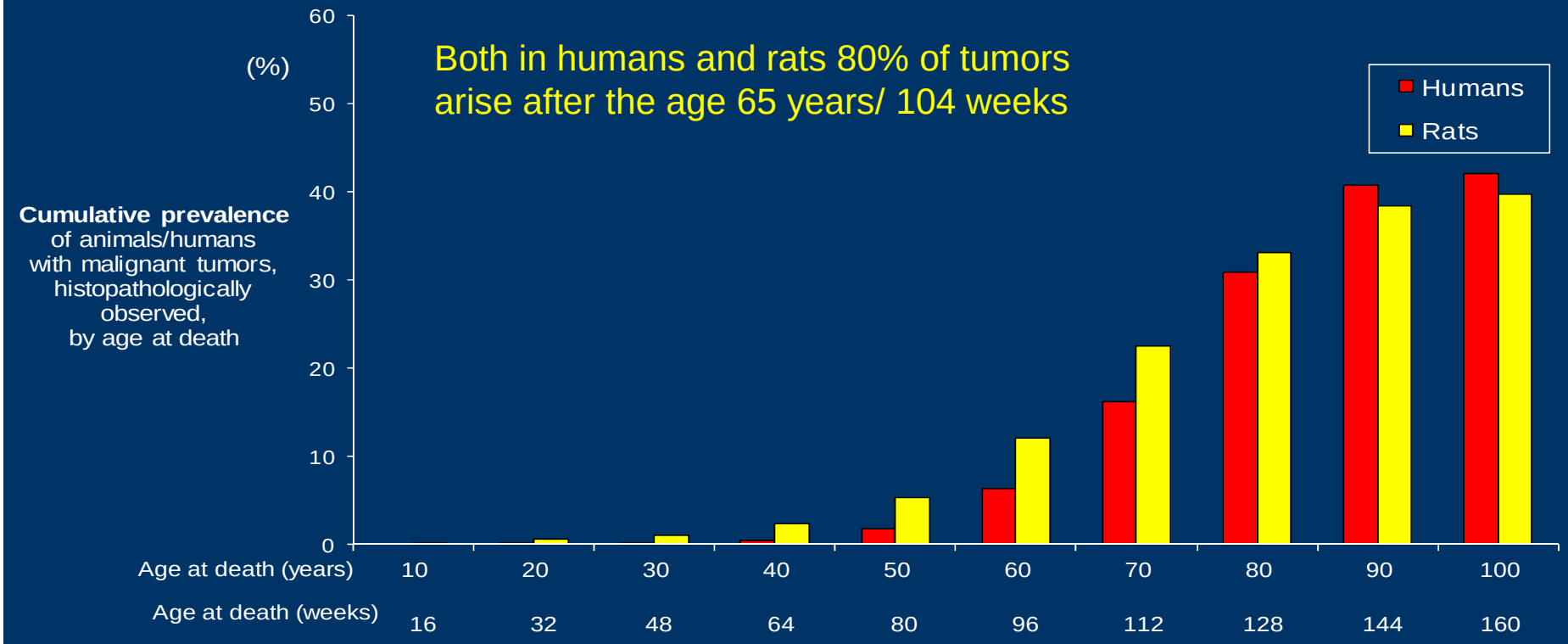
Sprague-Dawley rats

A HUMAN-EQUIVALENT MODEL

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

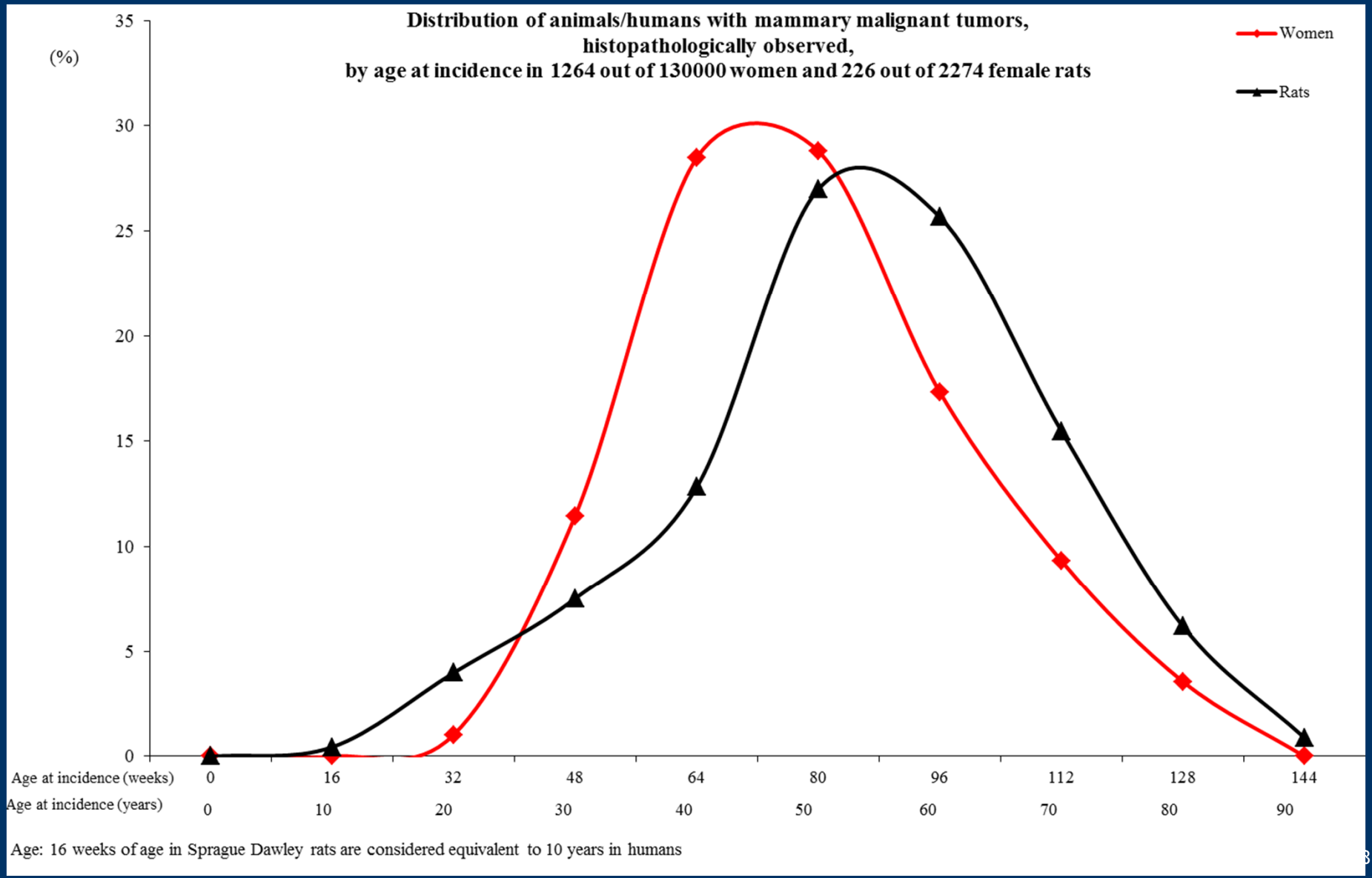
A HUMAN-EQUIVALENT MODEL



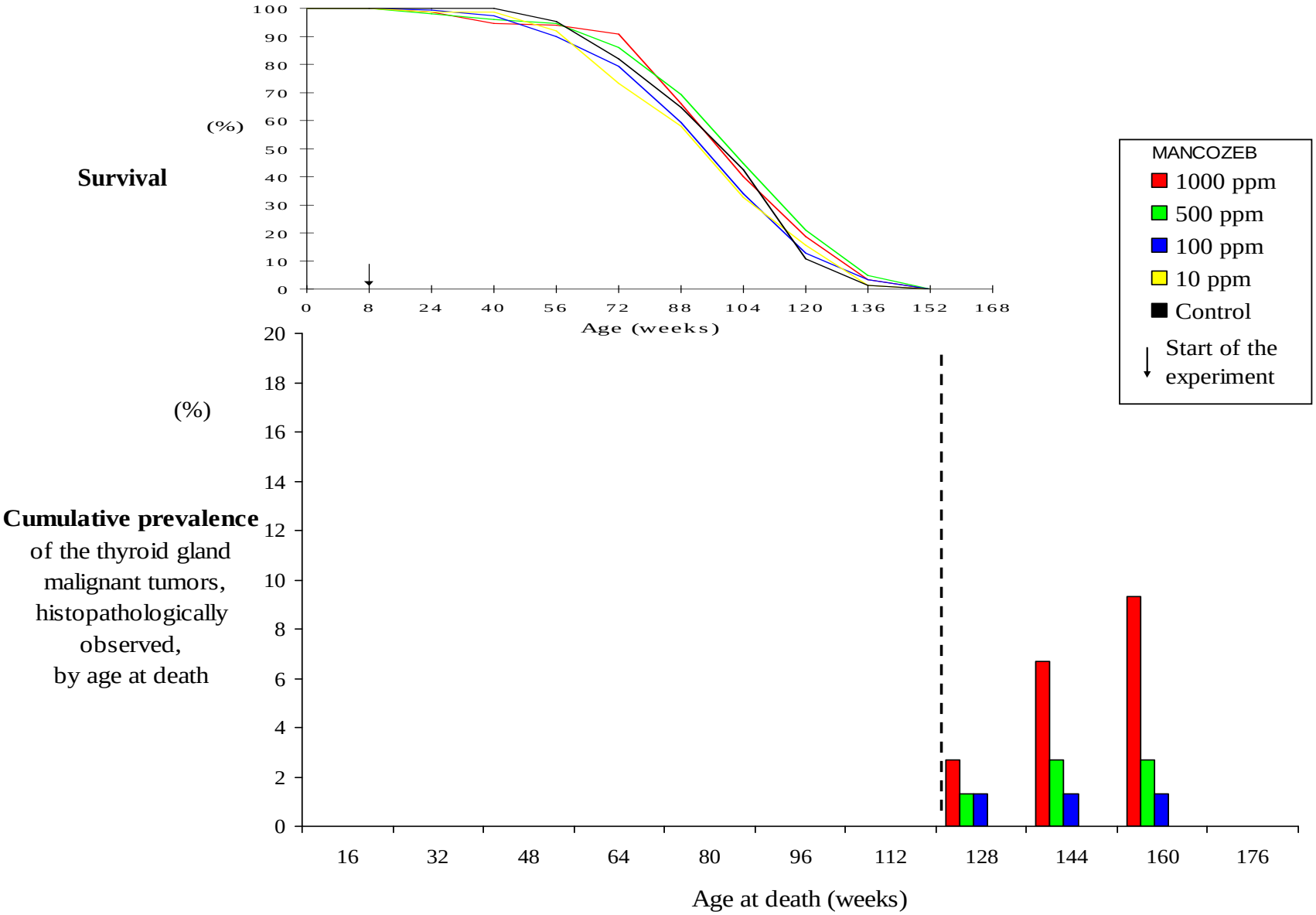
Age: 16 weeks of age in Sprague Dawley rats are considered equivalent to 10 years in humans

Data from the Hospital of Trieste were kindly made at our disposal by Professor Luigi Giarelli

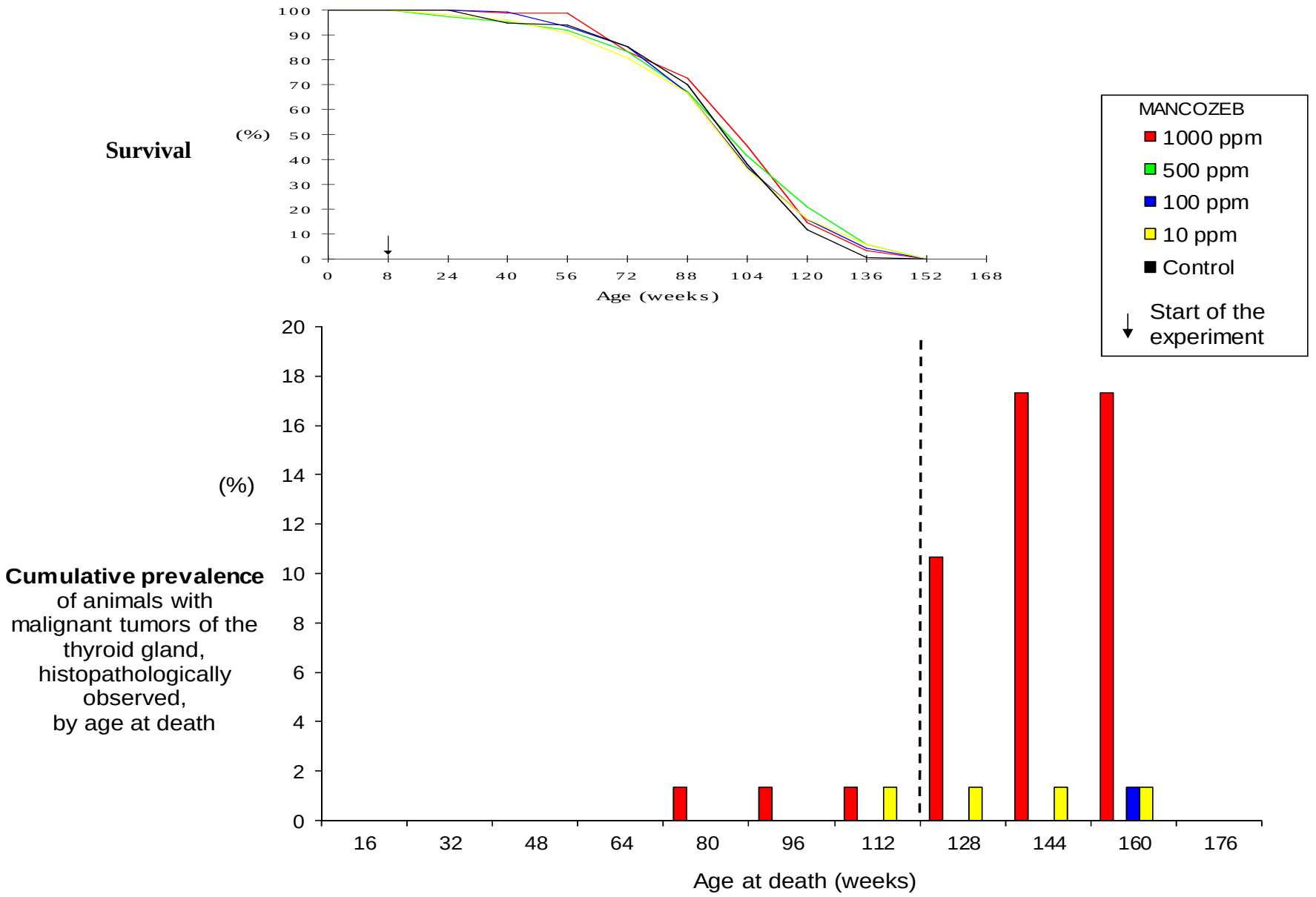
A HUMAN-EQUIVALENT MODEL



Mancozeb



Mancozeb: Thyroid malignant tumors in male Sprague-Dawley rats



Mancozeb: Thyroid malignant tumors in female Sprague-Dawley rats

ENDOCRINE INTERFERENCE

PROJECT
MOUNT SINAI SCHOOL OF MEDICINE - RAMAZZINI INSTITUTE

- 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)

PROJECT
MOUNT SINAI SCHOOL OF MEDICINE - RAMAZZINI INSTITUTE

- 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.

PROJECT
MOUNT SINAI SCHOOL OF MEDICINE - RAMAZZINI INSTITUTE

- **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.

PROJECT
MOUNT SINAI SCHOOL OF MEDICINE - RAMAZZINI INSTITUTE

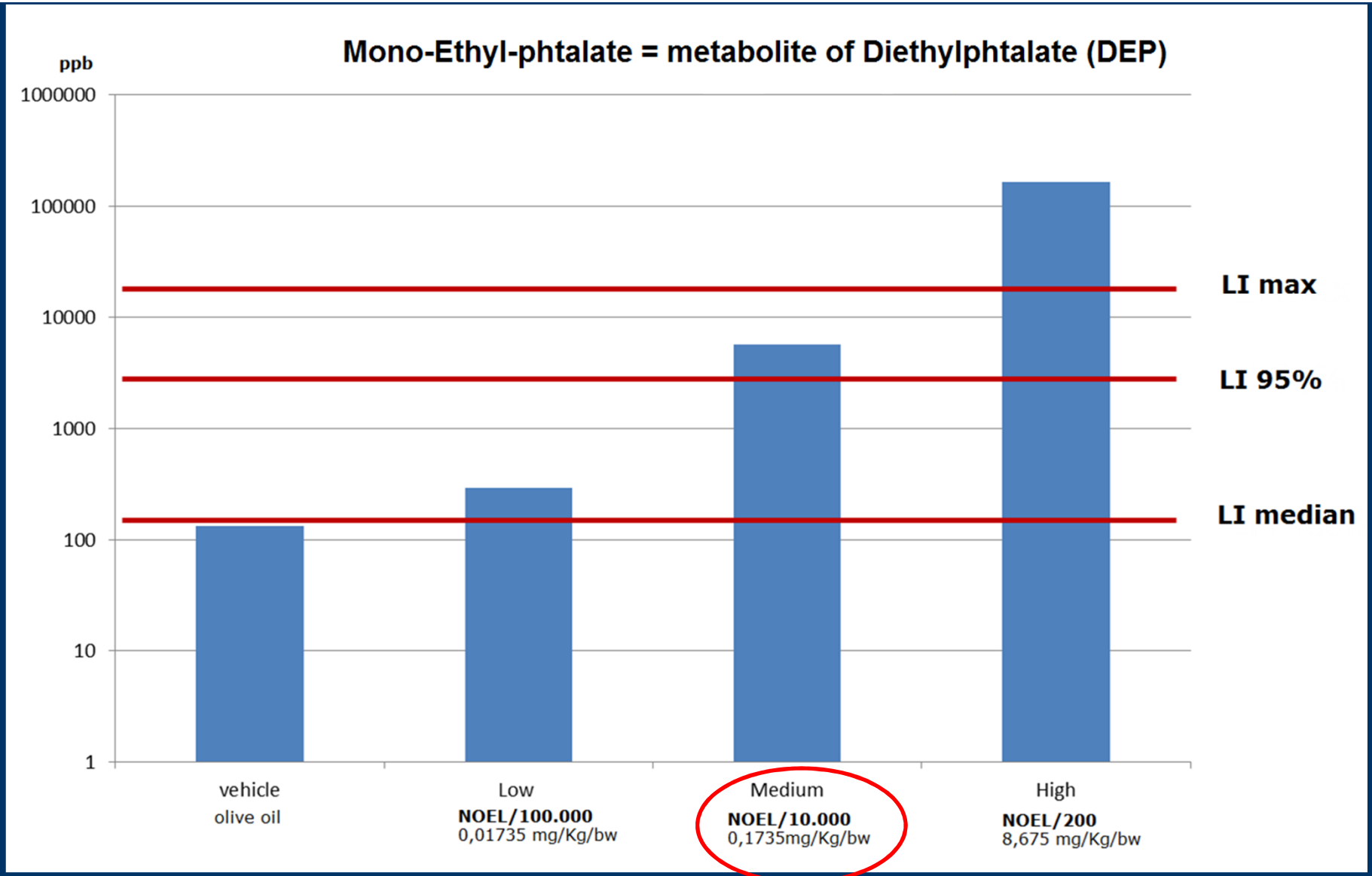
- **Dose-calibration study**

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

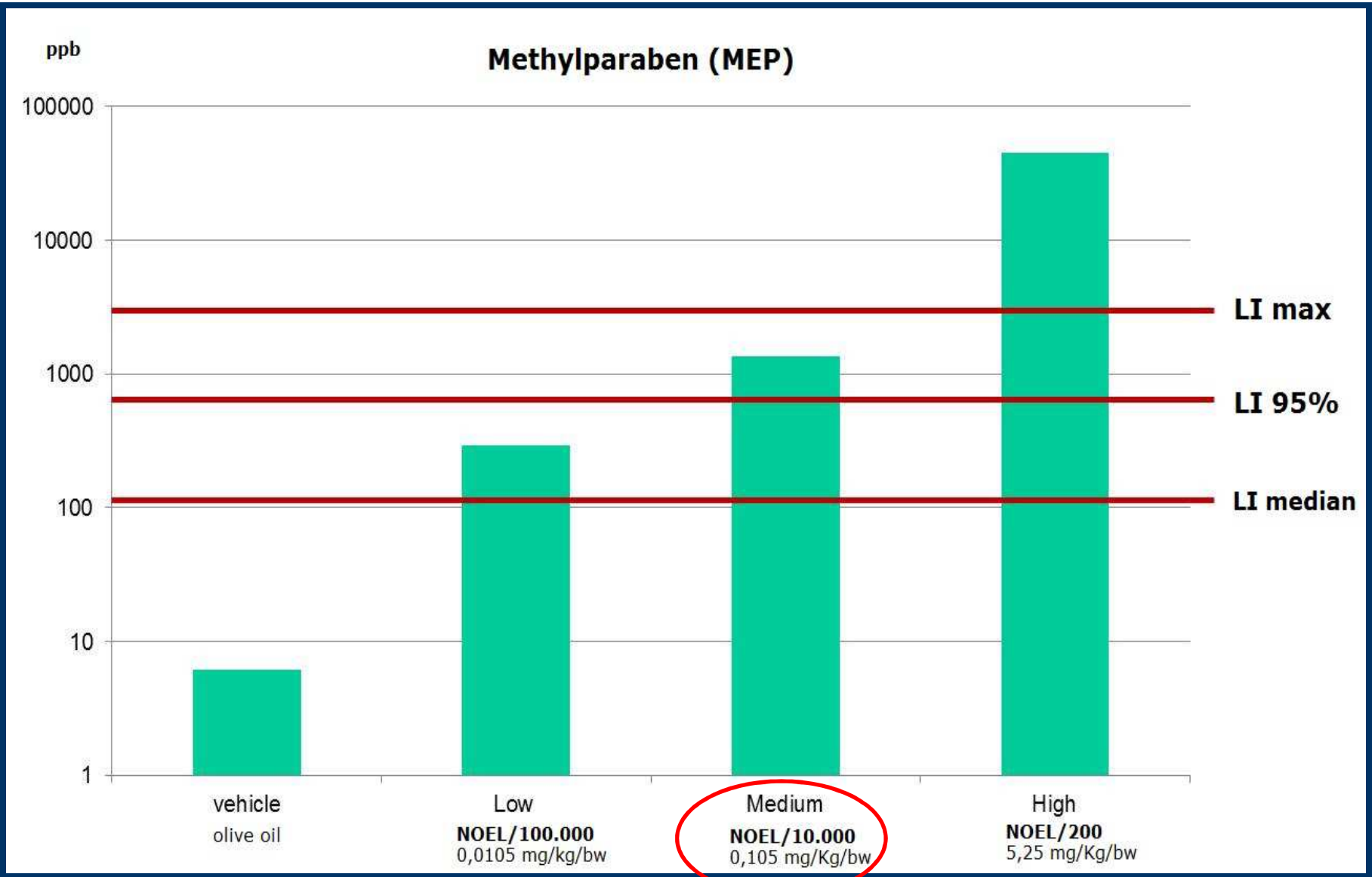
- **Main study**

  in order to explore whether environmental EDs act on six mammary cancer susceptibility windows (prenatal, postnatal, pre-puberty, pubertal, parous, nulliparous)

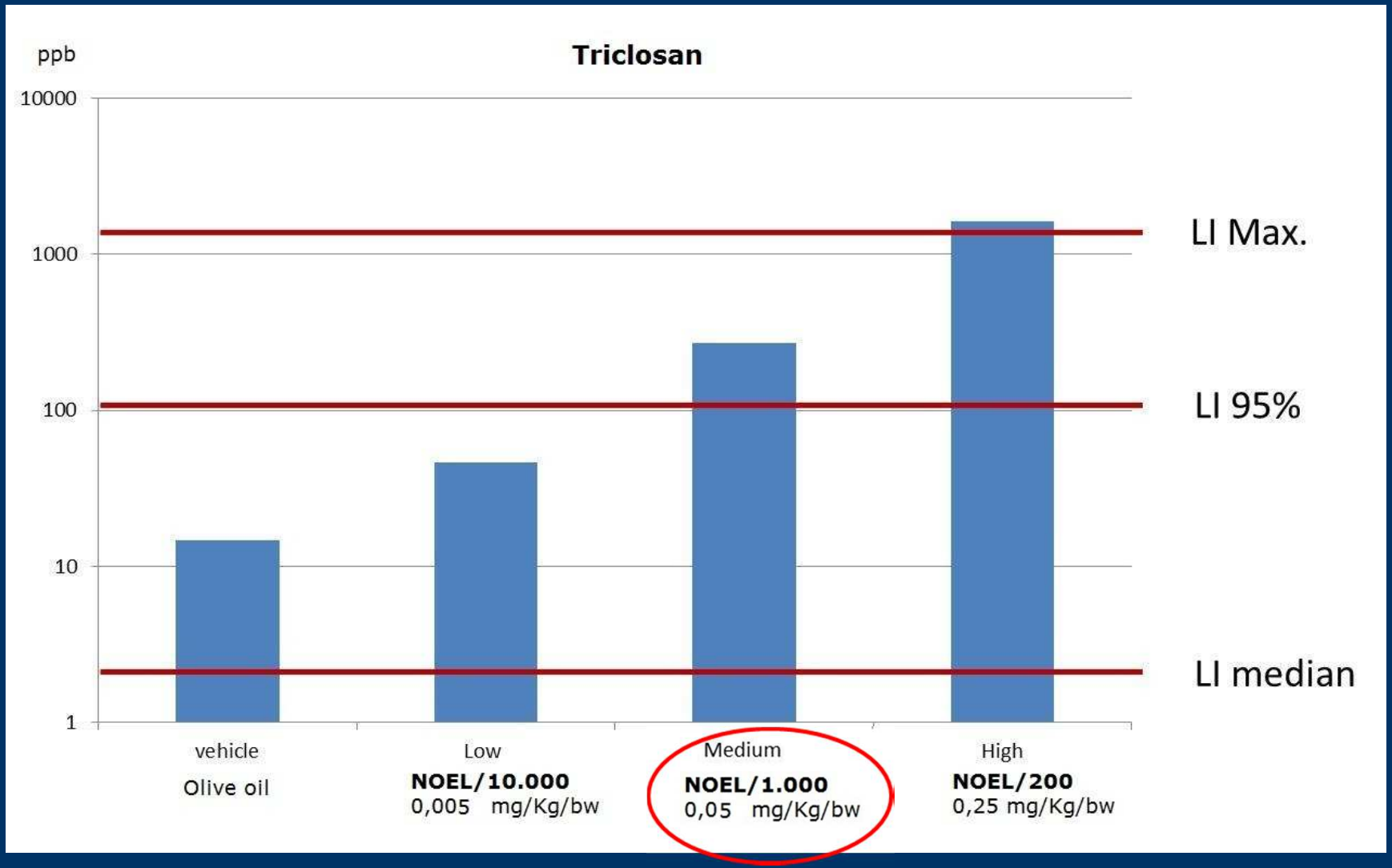
DOSE-CALIBRATION STUDY: RESULTS ON DIETHYL PHTHALATE



Dose-calibration study: results on Methyparaben



Dose-calibration study: results on Triclosan



MAIN STUDY

Windows of susceptibility	Treatment		
	Start	End	Administration (by oral gavage)
1. Pre-natal	matching	delivery	dams
2. Neo-natal	Post Natal Day (PND)1	PND20	dams
3. Pre-puberty	PND21	PND40	Dams until weaning (4 weeks) then pups individually
4. Pubertal	PND42	PND62	pups
5. Adult-parous	PND1	PND180	Dams until weaning (4 weeks) then pups individually. Once adult, at 14 weeks old, female pups are matched and continued to be treated until their PND 180
6. Adult-nulliparous	PND1	PND180	Dams until weaning (4 weeks) then pups individually. Once adult, female pups are not matched

MAIN STUDY: PRELIMINARY RESULTS

- ✓ 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 cuncurrent 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 cuncurrent bioassay
- ✓ The adoption of our protocols helps sparing animals and resources

THE RAMAZZINI STAFF