

WG5

THE RAMAZZINI INSTITUTE ANIMAL MODEL FOR STUDYING ADVERSE HEALTH OUTCOMES RELATED TO THE ENVIRONMENTAL EXPOSURES

DiMoPEX



Fabiana Manservigi¹, Simona Panzacchi¹, Daniele Mandrioli¹, Fiorella Belpoggi¹

¹ Cesare Maltoni Cancer Research Center, Ramazzini Institute, Italy



Istituto Ramazzini
COOPERATIVA SOCIALE ONLUS

Introduction

The Ramazzini Institute (RI) is a non-profit, independent organization located in Bologna, Italy. It is a social cooperative with more than 25,000 active associates. In 40 years, long-term carcinogenicity studies have been conducted at the Cesare Maltoni Cancer Research Center (CMCRC) of the RI on more than 200 agents present in the industrial and general environment performing more than 500 bioassays.

The RI project was started in 1966 and has been conducted with a systematic and integrated approach aimed at identifying exogenous carcinogens and quantifying their effects. This project is second only to that of the United States' National Toxicology Program (NTP), and has studied a greater number of agents than in any other single laboratory. Most agents have been selected on the basis the amount produced, their diffusion in the environment, and the number of people potentially exposed.

Methods

The features of the program are:

- ✓ **Human-equivalent animal model:** our animal model is the Sprague-Dawley (SD) rats, basing on the evidence that they are adequately sensitive, have a long historical base, and are also recommended by the Organisation for Economic Co-operation and Development (OECD), NTP and used by many other Universities and Organizations. SD rats are a known and accepted human equivalent model for cancer. The lifecourse of rats can be considered in a human equivalent model: with the expected times in utero, pre-puberty, puberty, etc. With regard to cancer studies, on average, 80% of spontaneous tumors occur by 104 weeks in rats and by age 65 in humans¹⁻².
- ✓ **Dose ranges:** under current testing procedures relatively high doses of a chemical are given to animals, generally higher than the doses humans are exposed. However this is not always the case, especially for various workplaces/occupations and high dose drug and cancer chemotherapies. When conducting exposure studies with low doses (many orders of magnitude lower than the No Observed Adverse Effect Level NOAEL), a systematic dose-calibration study should be performed in an appropriate rodent model in order to identify the administered oral dose of the test substance that result in biomarker concentrations (e.g. urine, serum) comparable to the ones observed in human population³.
- ✓ **Timing of exposure:** there is ample evidence demonstrating that exposures during early developmental phases, produce an overall increase of malignant tumors and increases of specific organ site neoplasms related to exposures to specific carcinogens as in the case of vinyl chloride and benzene. Lifelong and early exposure models provide a greater opportunity to construct comprehensive mechanistic models of disease³.
- ✓ **Additional endpoints and adverse effects of the test compound: the Ramazzini Institute integrated experimental design:** many examined chemicals have shown to also cause complex effects in animals, affecting organ development, functional and behavioral changes. We have recently proposed the adaption 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.

Results

- ✓ For our model we use Sprague-Dawley rats whose basic tumorigram is well known and whose cancer susceptibility is not too different from the human model. These rats come from the colony used for more than 40 years by the CMCRC laboratory. It is a human-equivalent model (Fig. 1-2).

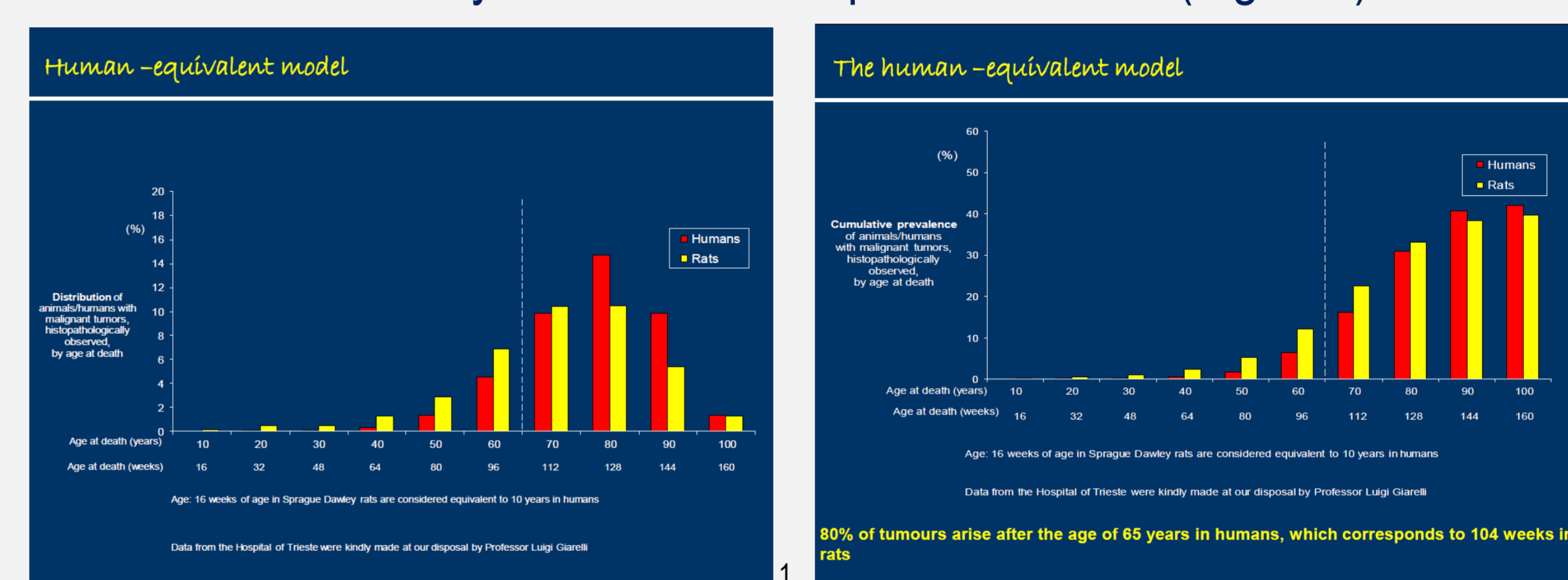


Fig.1-2. Comparison between the human and rat population counterpart. In 2002 we studied a large population of SD rats from our colony, representative of both sexes, in order to study the total cancer incidence in necropsied animals (malignant cancers being lethal). We also studied their distribution by age at death. In the same period we analysed a human population the same size as the town of Trieste, Northern Italy, who, on death, were autopsied at the public hospital of Trieste. Professor Luigi Giarelli, Director of the Pathological Anatomy Unit of the Hospital, had permission from the local authorities to autopsy each patient dying during hospitalization.

- ✓ Numerous long-term carcinogenicity studies have been conducted at the CMCRC that demonstrate the life-time consequences of chemical/physical exposures beginning during developmental life and lasting for life (Fig. 3-4).

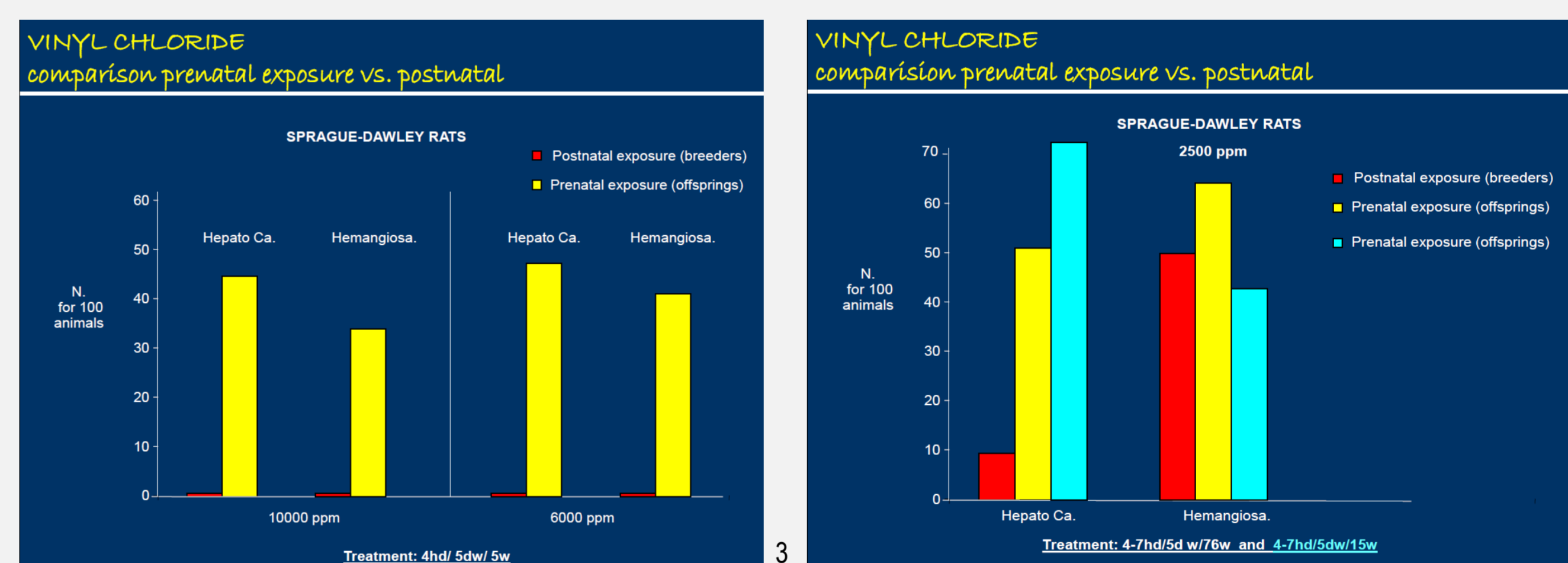


Fig.3-4. The same dose of VCM administered to adult SD rats did not increase liver malignant tumours when the exposure started in adult life; when administered starting from foetal life, a statistically significant increase in liver tumours was observed.

- ✓ The results obtained have formed the basis of rules and regulations for primary prevention, even if sometimes many years have passed over before having the confirmation of multipotent carcinogenicity (Fig.5-6)

AGENT/COMPOUND	CMCRC-RI ⁴	IARC GROUP/YEAR	PREDICTIVITY
VINYL CHLORIDE	1974	1 /1979	5 years
FORMALDEHYDE	1989	1 /2012	23 years
TRICHLOROETHYLENE	1986	1 /2014	28 years
BENZENE	1979	1 /2012	33 years
MTBE	1995	3 /1999	?
ASPARTAME	2005	?	?

AGENT	YEAR	BEFORE	AFTER
VINYL CHLORIDE	1974	Limit 500 ppm	Limit 1 ppm
BENZENE	1979	Not classified	Carcinogenic (IARC 1)
TRICHLOROETHYLENE	1988	Not classified	Carcinogenic (IARC 1)
FORMALDEHYDE	1989	Not classified	Carcinogenic (IARC 1)
ASBESTOS	1991	Associated only to lung cancer and mesothelioma	Also associated with larynx, ovary, pharynx, stomach, colon and rectum cancer.
MANCOZEB	2002	Not classified	Carcinogenic (Prop 65)
FLUORO-EDENITIC FIBRES	2004	Not classified	Carcinogenic (IARC 1)

Fig.5-6. The contribution of experimental (animal) studies to the control of chemical carcinogenesis: the Ramazzini Institute's first experimental evidence of carcinogenicity and the confirmed classification by the Regulatory Agencies

Conclusions

Cancer is an extremely complex disease, not easy to control, and one about which there is insufficient knowledge. Clinical, psychological, and economic aspects of the disease should all be taken into consideration: they cause heavy individual, familial, and social costs. To face the problem, it is necessary to increase our knowledge to provide solid scientific bases for the prevention and clinical control of cancer.

Basic as well as preventive and clinical research should be developed. In this research, experimental animal studies play a central role.

REFERENCES:

1. Sofritti M, Belpoggi F, Degli Esposti D. Cancer prevention: The lesson from the lab. In: Biasco G, Tanneberger S, editors. Cancer Medicine at the Dawn of the 21st Century: The view from Bologna. Bologna: Bononia University Press; 2006, p. 49-64.
2. Silvestri F, Bussani R, Giarelli L. Changes in underlying causes of death during 85 years of autopsy practice in Trieste. IARC Sci Publ 1991; 112:3-23
3. Teitelbaum SL, Li Q, Lambertini L, Belpoggi F, Manservigi F, Falcioni L, Bua L, Silva MJ, Ye X, Calafat AM, Chen J. Paired Serum and Urine Concentrations of Biomarkers of Diethyl Phthalate, Methyl Paraben, and Triclosan in Rats. Environ Health Perspect. 2016 Jan;124(1):39-45.