Ramazzini Foundation Cancer Program

History and Major Projects, Life-Span Carcinogenicity Bioassay Design, Chemicals Studied, and Results

MORANDO SOFFRITTI, FIORELLA BELPOGGI, FRANCO MINARDI, AND CESARE MALTONI†

Cancer Research Center, European Ramazzini Foundation for Oncology and Environmental Sciences, Bologna, Italy

. . . I have dreamed of man's state, of his courteous and enlightened social state: beyond which, in the temple, the horrible blood-sacrifice was consummated.

—THOMAS MANN
The Magic Mountain

ABSTRACT: The Ramazzini Foundation research program was started over thirty years ago. The features of this program are: (1) systematic and integrated project design; (2) consistency over time; (3) homogeneity of approach: key members of the team remain unchanged; and (4) choice to work on new frontiers of scientific research. The program centers mainly on three projects: Project 1: experimental carcinogenicity bioassays; Project 2: experimental anticarcinogenesis assays to identify factors and active principles (compounds) capable of opposing the onset of tumors while being suitable for preventive/chemopreventive intervention; Project 3: epidemiological studies, both descriptive and analytical, on tumor incidence and mortality in persons professionally and environmentally exposed to industrial carcinogenic risks. The project involving experimental carcinogenicity bioassays for the identification of exogenous carcinogens (environmental and industrial above all) began in 1966. This project has included 398 experimental bioassays on 200 compounds/agents using some 148,000 animals monitored until their spontaneous death. Among the studies already concluded, 47 agents have shown "clear evidence" of carcinogenicity. The results have demonstrated for the first time that (1) vinyl chloride can cause liver angiosarcoma as well as other tumors; (2) benzene is carcinogenic in experimental animals for various tissues and organs; (3) formaldehyde may produce lymphomas and leukemias; and (4) methyl-*tert*-butyl ether (MTBE), the most common oxygenated additive used in gasolines, can cause lymphomas/leukemias. Many of the results achieved have led to the introduction of norms and measures of primary prevention.

KEYWORDS: Ramazzini Foundation; carcinogenicity; long-term bioassay; rat; benzene; vinyl chloride; MTBE; xylenes

Address for correspondence: Morando Soffritti, M.D., Cancer Research Center, European Ramazzini Foundation for Oncology and Environmental Sciences, Bentivoglio Castle, 40010 Bentivoglio (BO), Italy. Voice: +39-051-6640460; fax: +39-051-6640223.
crcfr@tin.it.
†Deceased.

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INTRODUCTION

Cancer is one of the most important current health problems. One epidemiological datum is enough: in industrialized countries, cancer represents about 30% of all deaths. The problem will get worse because the disease is expected to increase: cancer is related to the aging of the population, to environmental pollution, and to the modified life styles which characterize our times, all of which will increase in coming years with the diffusion of the industrial model of development.

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 studies play a central role. Those who have known and followed the reality of oncological research are aware that most progress in oncology derives from experimental studies.

THE RESEARCH PROGRAM OF THE RAMAZZINI FOUNDATION

The Ramazzini Foundation (RF), founded in 1992, is a nonprofit private institution with governmental recognition. The aims of the Foundation include: (1) descriptive and analytical epidemiology; (2) experimental identification of toxic and carcinogenic risks; (3) experimental research on chemoprevention; (4) clinical monitoring of high-risk groups; and (5) terminal care of cancer patients.

The cancer research program of the Ramazzini Foundation was started 30 years ago as part of the research activity conducted by the "F. Addarii" Institute of Oncology in the town of Bologna.

The features of the program are: (1) systematic and integrated program design; (2) consistency over time; (3) homogeneity of approach, the key members of the team remaining as far as possible unchanged; (4) the decision to work on new frontiers of cancer research.

The program focuses mainly on three projects:

- Project 1: long-term carcinogenicity bioassays for the identification of xenobiotic carcinogenic agents—in particular, those of industrial origin;
- Project 2: experimental bioassays of anticarcinogenesis for the identification of factors, agents (also drugs) effective in contrasting the onset/progression of tumors (chemoprevention);
- Project 3: descriptive and analytical epidemiological studies on cancer incidence and mortality in people from various geographic areas of Italy environmentally and/or occupationally exposed to carcinogenic risks.

Project of Long-Term Carcinogenicity Bioassays

This 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, 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. Very few agents have been selected from those already proven to be carcinogenic in other laboratories.

Most CRC/RF long-term carcinogenicity bioassays are planned and conducted following a basic design protocol which is summarized as follows:

(1) *Aim*: to detect the chronic toxic and carcinogenic effects of chemical and physical agents.
(2) *Animals*: Sprague-Dawley rats from the colony of the CRC/RF, now used for more than 30 years. The basic tumor incidences of this strain are well known, and the cancer susceptibility is not very different from the human counterpart. Wistar rats and Swiss mice (and other strains), as well as golden hamsters, are also used on some occasions.
(3) *Experimental groups and group sizes*: two or more (depending on the importance of the agent for public health) experimental groups (comprehensive control groups) are employed in the various experiments; each group contains 50–60 or more animals for each sex.
(4) *Routes of exposure*: typically those mimicking human exposure. The most frequently used are: inhalation, injection, ingestion, and external exposure (radiation).
(5) *Concentration/dose/intensity of the agent studied*: for each agent studied, at least two, but generally three, dose levels are tested. The dose levels are the maximum tolerated level, the order of magnitude to which humans may be exposed, and one intermediate level. Data on maximum tolerated dose levels, when not available from the scientific literature, are determined by range-finding experiments.
(6) *Starting of treatment*: during embryonal life (12 days)/perinatal/6–8 weeks old/exceptionally at other ages.
(7) *Duration of treatment*: 104 weeks/life-span/other lengths infrequently.
(8) *Duration of experiment*: until spontaneous death.
(9) *Pathology*: on dying, animals undergo systematic necropsy. Histopathology is routinely performed on the following organs and tissues: skin and subcutaneous tissue, brain, pituitary gland, Zymbal glands, parotid glands, submaxillary glands, Harderian glands, cranium (with oral and nasal cavities and external and internal ear ducts) (5 sections of head), tongue, thyroid and parathyroid, pharynx, larynx, thymus, and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach (fore and glandular), intestine (four levels), urinary bladder, prostate, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes, and any other organs or tissues with pathological lesions.

In some cases long-term carcinogenicity bioassays are planned and conducted following a special design protocol. These experiments are called "mega-experiments" and are characterized as follows: (1) *aims*: to detect low/diffuse carcinogenic risks, defined as the exposure to single or multiple agents or mixtures that

TABLE 1. The Ramazzini Foundation Cancer Program

PROJECT OF LONG-TERM CARCINOGENICITY BIOASSAYS: AGENTS STUDIED

No.	Compounds/agents	No. of Bioassays	Animals		Route of Exposure[a]
			Species	No.	
Plastic monomers					
1.	Vinyl chloride	29	Rat, mouse, hamster	8,293	Ing,Inh,Ip,Sc,Tr
2.	Vinylidene chloride	7	Rat, mouse, hamster	3,164	Ing,Inh,Tr
3.	Vinylidene fluoride	1	Rat	190	Ing
4.	Acrylonitrile	4	Rat	1,361	Ing,Inh,Tr
5.	Vinyl acetate	3	Rat, mouse	1,672	Ing,Tr
6.	Ethylene	1	Rat	200	Ing
7.	Propylene	2	Rat	1,680	Inh
8.	Styrene	4	Rat	980	Ing,Inh,Ip,Sc
9.	Styrene oxide	1	Rat	240	Ing
10.	p-methylstyrene	4	Rat, mouse	1,422	Ing
Polymers					
	Plastic disks				
11.	- Ivoclar	2	Rat	84	Imp
12.	- Acronite	2	Rat	84	Imp
13.	- Lucitone	2	Rat	84	Imp
14.	- Stellon	2	Rat	84	Imp
15.	- Teflon	4	Rat	179	Imp
	Polymeric fibres				
16.	- Kevlar	2	Rat	680	Ip,Ipl
17.	- Farlosa C-2-OM	1	Rat	240	Ip,Ipl,Sc
18.	PVC (granules)	1	Rat	230	Ing
19.	Water in PVC bottles	2	Rat	2,200	Ing
Chlorinated organic intermediates					
20.	Dichloroethane	3	Rat, mouse	2,626	Inh
Chlorinated solvents					
21.	Methylene chloride	3	Rat, mouse	1,346	Ing,Inh,Tr
22.	Carbon tetrachloride	1	Rat	160	Ing
23.	Methylchloroform	1	Rat	180	Ing
24.	Trichloroethylene	8	Rat, mouse	3,948	Ing,Inh
25.	Tetrachloroethylene	1	Rat	180	Ing
Ethers					
26.	Phenylether-biphenyl ("Dowtherm")	1	Rat	360	Ing
Aldehydes					
27.	Formaldehyde	4	Rat	1,447	Ing
28.	Acetaldehyde	4	Rat	870	Ing
	Propellants				
29.	Trichlorofluoromethane (FC11)	2	Rat, mouse	1,170	Inh
30.	Dichlorodifluoromethane (FC12)	2	Rat, mouse	1,080	Inh
31.	Chlorodifluoromethane (FC22)	2	Rat, mouse	720	Inh
Detergents					
32.	Carbonate	3	Rat	400	Ing
33.	Tripolyphosphate	2	Rat	360	Ing
34.	Nitrilotriacetic acid (NTA)	3	Rat	400	Ing
35.	Zeolite MS 4A	3	Rat	580	Ing,Int,Ip
Pesticides					
36.	N-(3,5-dichlorophenyl)-5-methyl-5-carbetoxy-1-3-oxazolydin-2,4-dione ("Serinal")	2	Rat, mouse	1,080	Ing
37.	Methyl 2-[n-phenylacetyl-N-(2,6-dimethylphenyl)amino]propanoate ("Galben")	3	Rat, mouse	960	Ing
38.	Phenthoate ("Cidial")	2	Rat, mouse	880	Ing
39.	Ethylene bisdithiocarbamate ("Mancozeb")	2	Rat	850	Ing
Compounds used in leather industry					
	Chromium compounds				
40.	- Chromitan NA	1	Rat	80	Sc
41.	- Chromitan B	1	Rat	80	Sc
42.	- Chromitan MS	1	Rat	80	Sc
43.	- Chromorganik	1	Rat	80	Sc
44.	- Chromium alum	1	Rat	80	Sc
45.	- Baychrom	1	Rat	80	Sc
46.	- Chromopol	1	Rat	80	Sc
47.	- Coripol	1	Rat	80	Sc

—Continued

TABLE 1. *Continued*

PROJECT OF LONG-TERM CARCINOGENICITY BIOASSAYS: AGENTS STUDIED

No.	Compounds/agents	No. of bioassays	Animals		Route of Exposure[a]
			Species	No.	
	Natural and man-made tannins				
48.	- Chestnut tannin	1	Rat	80	Sc
49.	- Quebraco	1	Rat	80	Sc
50.	- Mimosa	1	Rat	80	Sc
51.	- Tupasol	1	Rat	80	Sc
52.	- Tannigan BN	1	Rat	80	Sc
53.	- Tannigan P2 PLV	1	Rat	80	Sc
54.	- Tannigan 3LR	1	Rat	80	Sc
55.	- Tannigan CLS	1	Rat	80	Sc
56.	- Tannigan POL PAK	1	Rat	80	Sc
57.	- Tannesco HN	1	Rat	80	Sc
58.	- Blancoral	1	Rat	80	Sc
Drugs					
59.	Vitamin A	5	Rat	5,100	Ing
60.	Vitamin C	5	Rat	3,680	Ing
61.	Vitamin E	5	Rat	3,680	Ing
62.	N-(4-hydroxyphenyl)-retinamide	4	Rat	840	Ing
63.	Tamoxifen	17	Rat	6,008	Ing
64.	ICI 182780	1	Rat	450	Sc
65.	Toremifen	2	Rat	2,045	Ing
66.	Leuprolide	7	Rat	3,560	Sc
67.	Medroxyprogesterone acetate	4	Rat	1,710	Ing
68.	4-hydroxyandrostenedione	2	Rat	790	Sc
69.	Anastrozole	2	Rat	750	Ing
70.	Conjugated natural oestrogens	1	Rat	340	Ing
71.	Oestradiol	1	Rat	270	Sc
72.	Cyclophosphamide + methotrexate+ 5-fluorouracil (CMF)	1	Rat	300	Ip
73.	Alpha–interpheron	1	Rat	300	Sc
74.	Adriamycin	1	Rat	160	Sc
75.	Epirubicin	1	Rat	160	Sc
76.	Idarubicin	1	Rat	80	Sc
77.	MAK 4	3	Rat	1,000	Ing
78.	MAK 5	3	Rat	1,000	Ing
79.	MA631	3	Rat	1,000	Ing
Fuels: mixtures					
80.	Unleaded gasoline (1984)	1	Rat	300	Ing
81.	Unleaded gasoline (1993)	1	Rat	240	Ing
82.	Leaded gasoline	1	Rat	300	Ing
83.	Gasoline containing 3% methyl alcohol	1	Rat	240	Ing
84.	Gasoline containing 5% ethyl alcohol	1	Rat	240	Ing
85.	Gasoline containing 15% MTBE	1	Rat	240	Ing
86.	Gasoline containing 15% ETBE	1	Rat	240	Ing
87.	Kerosene	1	Rat	300	Ing
88.	Diesel fuel	1	Rat	300	Ing
89.	Naphtha	1	Rat	200	Ing
Fuels: aromatic hydrocarbons					
90.	Benzene	8	Rat, mouse	1,950	Ing,Inh,Tr
91.	Toluene	4	Rat	440	Ing
92.	Xylenes	2	Rat	380	Ing
93.	Ethylbenzene	2	Rat	380	Ing
94.	Trimethylbenzene	1	Rat	200	Ing
Fuels: isoparaffins					
95.	2,2,4-trimethylpentane	2	Rat	408	Ing
Fuels: oxygenated additives					
96.	Methyl alcohol	3	Rat	1,340	Ing
97.	Ethyl alcohol	4	Rat, mouse	1,458	Ing
98.	Methyl-tert-butyl ether (MTBE)	1	Rat	360	Ing
99.	Ethyl-tert-butyl ether (ETBE)	1	Rat	360	Ing
100.	Ter-amil-methyl ether (TAME)	1	Rat	600	Ing
101.	Di-isopropyl ether (DIPE)	1	Rat	600	Ing
Combustion Products (CP)					
102.	Automobile exhausts	3	Rat, mouse	400	Sc
103.	CP of domestic heating with oil	2	Rat, mouse	160	Sc
104.	CP of oil energy plant	2	Rat, mouse	160	Sc
105.	CP of carbon energy plant (including ashes)	2	Rat, mouse	240	Sc
106.	Welding fumes	1	Rat	880	Sc
107.	Ashes from waste incinerator	1	Rat	240	Int,Sc

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TABLE 1. ***Continued***

No.	Compounds/agents	No. of Bioassays	Animals		Route of Exposure[a]
			Species	No.	
Beverages and diet					
108.	"Coca-Cola"	4	Rat	1,999	Ing,Tr
109.	"Pepsi-Cola"	1	Rat	400	Ing
110.	Sucrose	1	Rat	400	Ing
111.	Caffeine	1	Rat	800	Ing
112.	Aspartame	1	Rat	1,800	Ing
Inorganic compounds					
113.	Chlorine (sodium hypochlorite)	2	Rat	480	Ing
114.	Arsenic (sodium arsenate)	2	Rat	823	Ing
115.	Arsenious anhydride	1	Rat	80	Sc
116.	Cadmium sulphide (cadmium yellow)	1	Rat	100	Sc
	Trivalent and hexavalent chromium compounds				
117.	- Lead chromate (chromium yellow)	1	Rat	100	Sc
118.	- Basic lead chromate (chromium orange)	1	Rat	100	Sc
119.	- Lead chromate sulphate and molybdate (molybdenum orange)	1	Rat	100	Sc
120.	- Chromite	1	Rat	100	Sc
121.	- Chromium alum	1	Rat	100	Sc
122.	- Basic chromium sulphate (Neochromium)	1	Rat	100	Sc
123.	- Silica-coated lead chromate	1	Rat	80	Sc
	Iron oxides				
124.	- Iron oxide hydrate (iron yellow)	1	Rat	100	Sc
125.	- Iron oxide (iron red)	1	Rat	100	Sc
126.	Magnesium oxide	1	Rat	120	Sc
127.	Silicone	2	Rat	600	Imp,Sc
	Titanium oxides				
128.	- Sample 1	1	Rat	80	Sc
129.	- Sample 2	1	Rat	80	Sc
130.	- Sample 3	1	Rat	80	Sc
	Vanadium				
131.	- Vanadium bioxide	1	Rat	120	Sc
132.	- Vanadium pentaoxide	1	Rat	120	Sc
133.	- Vanadium chloride	1	Rat	120	Sc
134.	- Ash A	1	Rat	120	Sc
135.	- Ash B	1	Rat	120	Sc
136.	Vitallium disks	3	Rat, mouse	252	Imp
137.	Vitallium disks (holed)	1	Rat	60	Imp
138.	Vitallium fragments	1	Rat	60	Imp
139.	Basic zinc chromate (yellow zinc)	1	Rat	120	Sc
Natural and man-made mineral particles					
	Asbestos				
140.	- Crocidolite	5	Rat	860	Ing,Int,Ip,Ipl,Sc
141.	- Chrysotile (Canada)	5	Rat, mouse	1,260	Int,Ip,Ipl,Sc
142.	- Chrysotile (Rhodesia)	1	Rat	80	Ip
143.	- Chrysotile (California)	1	Rat	80	Ip
144.	- Amosite	1	Rat	80	Ip
145.	- Anthophyllite	1	Rat	80	Ip
146.	- Asbestos-cement	1	Rat	240	Ip,Ipl,Sc
	Modified asbestos				
147.	- Compound 1	1	Rat	160	Ip, Ipl
148.	- Compound 2	1	Rat	160	Ip, Ipl
149.	- Compound 3	1	Rat	160	Ip, Ipl
150.	- Compound 4	1	Rat	160	Ip, Ipl
151.	- Compound 5	3	Rat, mouse	860	Ip, Ipl
152.	- Compound 6	3	Rat, mouse	860	Ip, Ipl
153.	- Compound 8	3	Rat, mouse	860	Ip, Ipl
154.	Wollastonite	1	Rat	240	Ip,Ipl,Sc
155.	Rock wool	3	Rat	640	Int,Ip,Ipl
156.	Ceramic fibres	1	Rat	440	Ip,Ipl
157.	Glass fibres	1	Rat	200	Int,Ip
158.	Crystalline silica	1	Rat	160	Ip,Sc
159.	Amorphous silica	1	Rat	160	Ip,Sc
160.	Alumina	1	Rat	160	Ip,Sc
161.	Talc (pure)	1	Rat	160	Ip,Sc
162.	Talc (industrial)	1	Rat	240	Ip,Ipl,Sc
163.	Kaolin	1	Rat	160	Ip,Sc
164.	Bentonite	1	Rat	240	Ip,Ipl
165.	Erionite	2	Rat, mouse	400	Ip,Ipl,Sc

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ABLE 1. ***Continued***

PROJECT OF LONG-TERM CARCINOGENICITY BIOASSAYS: AGENTS STUDIED

No.	Compounds/agents	No. of Bioassays	Animals		Route of Exposure[a]
			Species	No.	
	Other natural zeolites				
166.	- Mordenite (sed)	1	Rat	240	Ip,Ipl,Sc
167.	- Phillipsite	1	Rat	240	Ip,Ipl,Sc
168.	- Clinoptilolite	1	Rat	160	Ip,Sc
169.	- Cabasite	1	Rat	160	Ip,Sc
170.	- Ferrierite	1	Rat	160	Ip,Sc
171.	- Mordenite (crystalline)	1	Rat	240	Ip,Ipl,Sc
172.	- Heulandite	1	Rat	160	Ip,Sc
173.	- Mesolite	1	Rat	160	Ip,Sc
174.	- Natrolite	1	Rat	160	Ip,Sc
175.	- Solecite	1	Rat	160	Ip,Sc
176.	- Stilbite	1	Rat	160	Ip,Sc
177.	- Thomsonite	1	Rat	160	Ip,Sc
	Man-made zeolites and precursors				
178.	- TE-16460	1	Rat	360	Ip,Ipl,Sc
179.	- TE-16461	1	Rat	360	Ip,Ipl,Sc
180.	- TE-16462	1	Rat	360	Ip,Ipl,Sc
181.	- WGB 1189-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
182.	- WGB 1189-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
183.	- WGB 1190-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
184.	- WGB 1190-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
185.	- WGB 1191-2-1 (catalyst)	1	Rat	360	Ip,Ipl,Sc
186.	- WGB 1191-2-1 (elutriated)	1	Rat	360	Ip,Ipl,Sc
187.	- WGB 1192-1-1	1	Rat	360	Ip,Ipl,Sc
188.	- WGB 1193-1-1	1	Rat	360	Ip,Ipl,Sc
189.	- WGB 1194-1-1	1	Rat	360	Ip,Ipl,Sc
190.	- Paulsboro (catalyst)	1	Rat	360	Ip,Ipl,Sc
191.	- Paulsboro (catalyst fines)	1	Rat	360	Ip,Ipl,Sc
192.	- Kaiser alumina	1	Rat	360	Ip,Ipl,Sc
193.	- Mobil Joliet	1	Rat	360	Ip,Ipl,Sc
194.	- Joliet fresh	1	Rat	360	Ip,Ipl,Sc
195.	- MS 4A	1	Rat	160	Ip,Ipl,Sc
196.	- MS 5A	1	Rat	160	Ip,Ipl,Sc
197.	- MS 13X	1	Rat	160	Ip,Ipl,Sc
198.	Carbon fibers (disks)	1	Rat	140	Imp
Ionizing radiation					
199.	Gamma radiation	11	Rat	19,904	Total body irradiation by Co external source
Non-ionizing radiation					
200.	Extremely low frequency electromagnetic fields (50 Hz)	5	Rat	9,883	Total body irradiation

Abbreviations: Imp = subcutaneous implantation; Ing = ingestion; Inh = inhalation; Int = intratracheal instillation; Ip = intraperitoneal injection; Ipl = intrapleural injection; Sc = subcutaneous injection; Tr = transplacental route.

are expected to have a limited carcinogenic potential because of agent type (weak carcinogen) and/or low dose/concentration/intensity, yet involving large population groups and, in some cases, all of mankind; (2) *specific prerequisites*: they must reproduce as far as possible the various conditions of human exposure; they must include large groups of animals (at least 400 to 1,000) in order to express variations in effects; the study must be protracted for the life-span of the animals; animals should have as similar a basic tumorigram as possible to the human counterpart.

The results provided by "mega-experiments" serve to identify the risks and measure their levels; if negative, they do not necessarily mean absence of risk, but they then serve to determine the existence of a safeguard limit.

The number of compounds/agents and the long-term bioassays conducted per agent, the type of animal used, the route of exposure, and the numbers of animals used are described in TABLE 1.

In all, 398 long-term bioassays have been conducted on 200 compounds/agents using a total of 148,164 animals monitored until spontaneous death. Among the studies already concluded, the 47 agents showing "clear evidence" of carcinogenicity are detailed in TABLE 2.

The results obtained with this project have proved for the first time:

(1) the capacity of vinyl chloride to induce liver angiosarcomas, and other types of tumor;

TABLE 2. The Ramazzini Foundation Cancer Program

PROJECT OF LONG-TERM CARCINOGENICITY BIOASSAYS: RESULTS[a]

The 47 agents with "clear" evidence of carcinogenicity

- Plastic monomers
  - Vinyl chloride[1, 2]
  - Vinylidene chloride[3]
  - Acrylonitrile[4]
  - Vinyl acetate[5,6]
  - Styrene oxide[7]
- Plastic polymers
  - Ivoclar[8]
  - Acronite[8]
  - Lucitone[8]
  - Teflon[8]
- Chlorinated solvents
  - Trichloroethylene[9]
- Pesticides
  - Mancozeb[12]
- Inorganic compounds
  - Cadmium sulphide (cadmium yellow)[8]
  - Lead chromate (chromium yellow)[8]
  - Basic lead chromate (chromium orange)[8]
  - Lead chromate sulphate and molybdate (molybdenum orange)[8]
  - Chromium alum[8]
  - Basic chromium sulphate (Neochromium)[8]
  - Silica-coated lead chromate[8]
  - Vitallium disks[8]
  - Vitallium disks (holed)[8]
  - Basic zinc chromate (yellow zinc)[8]
- Drugs
  - Adriamycin[8]
  - Epirubicin[8]
  - Idarubicin[8]
- Fuels: aromatic hydrocarbons
  - Benzene[13, 14]
- Fuels: oxygenated additives
  - Methyl alcohol[15]
  - Ethyl alcohol[5]
  - Methyl tert-butyl ether (MTBE)[16]
  - Tertiary-amyl-methyl ether (TAME)[17]
  - Di-isopropyl ether (DIPE)[17]
- Aldehydes
  - Formaldehyde[10,11]
- Compounds used in leather industry
  - Chromitan B[8]
  - Baychrom[8]
  - Quebraco[8]
  - Tupasol[8]
- Natural & man-made mineral particles
  - Crocidolite[18]
  - Chrysotile (3 samples)[18]
  - Amosite[18]
  - Anthophyllite[18]
  - Asbestos-cement[18]
  - Modified asbestos (6 samples)[19]
  - Rock wool[20]
  - Ceramic fibers[21]
  - Talc (industrial)[22]
  - Erionite[18]
  - Carbon fibers (disks)[8]
- Ionizing radiation
  - Gamma radiation[23, 24]

[a] up to the year 2002

(2) the capacity of formaldehyde to cause lymphomas and leukemias;
(3) the carcinogenicity of benzene in experimental animals, producing various types of tumors in different tissues and organs;
(4) the capacity of methyl alcohol and ethyl alcohol to induce various types of tumor in different tissues and organs;
(5) the capacity of methyl tert-butyl ether (MTBE), the most utilized gasoline oxygenated additive, to cause lymphomas and leukemias;
(6) the carcinogenicity of Mancozeb, tertiary-amyl-methyl ether (TAME) and di-isopropyl ether (DIPE).

The results obtained have formed the basis of rules and regulations for primary prevention.

One distinctive characteristic of the CRC/RF long-term carcinogenicity bioassays is to keep experimental animals under observation until spontaneous death. The neoplastic response depends not only on the kind of agent, its physicochemical and toxicologic properties, the mode of exposure, and the type of animal, but also to a great extent, on the latency of the tumor, which varies and may be very long. Experimental findings agree that the latent neoplastic potential for causing a tumor increases with the length of the observation time or age. That is why we are convinced that experimental carcinogenicity trials should continue until spontaneous animal

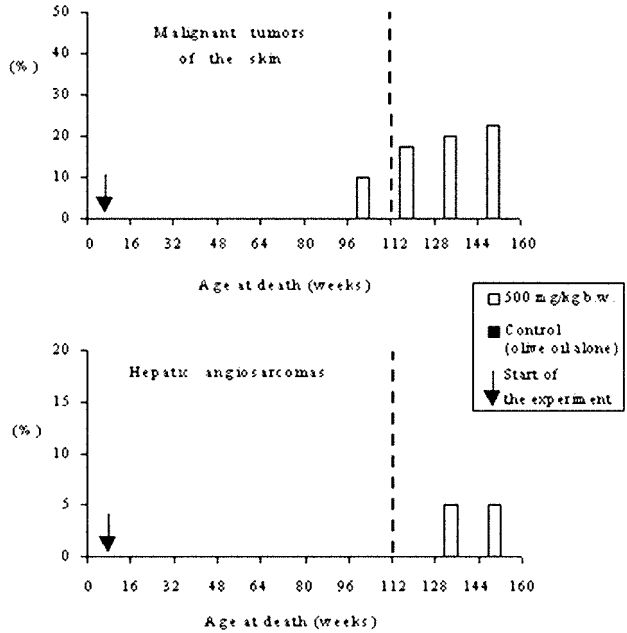

FIGURE 1. Cumulative prevalence of animals with malignant tumors of the skin and hepatic angiosarcomas, histopathologically observed, by age at death, in male Sprague-Dawley rats treated with benzene (BT 15).

death and not be cut short. Cutting short an experiment after two years may mask a possible carcinogenic response.

The following cases afford good examples.

Benzene

At the CRC/RF, benzene by ingestion and inhalation was studied . In experiment BT 902, benzene was administered by ingestion (stomach tube) in extra virgin olive oil. Benzene was administered at doses of 500 or 0 mg/kg b.w. once daily 4 or 5 days per week for 104 weeks to 40 male and 40 female Sprague-Dawley rats beginning at age 7 weeks. Animals were observed until spontaneous death. The control group received olive oil alone. An increase was observed in total malignant tumors; Zymbal gland, oral and nasal cavity, skin, forestomach carcinomas; and hepatic angiosarcomas. Skin carcinomas and hepatic angiosarcomas were observed after 112 weeks of age (FIG. 1).[25]

Xylenes

Xylenes (experiment BT 904) were administered by stomach tube at concentrations of 500 or 0 mg/kg b.w. using the protocol described above for benzene. The control group received olive oil alone. An increase was observed in total malignant tumors, mammary and oral cavity carcinomas, and hemolymphoreticular neoplasias. The increase in total malignant tumors, oral cavity carcinomas, and hemolymphoreticular neoplasias was only observed after 112 weeks of age (FIG. 2).[26] It should be

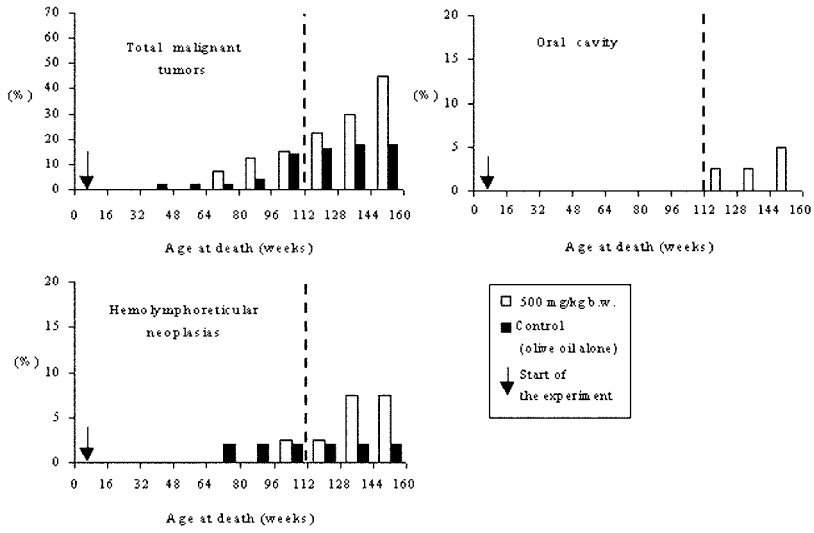

FIGURE 2. Cumulative prevalence of total malignant tumors, oral cavity carcinomas and hemolymphoreticular neoplasias, histopathologically observed, by age at death, in male Sprague-Dawley rats treated with xylenes (BT 904).

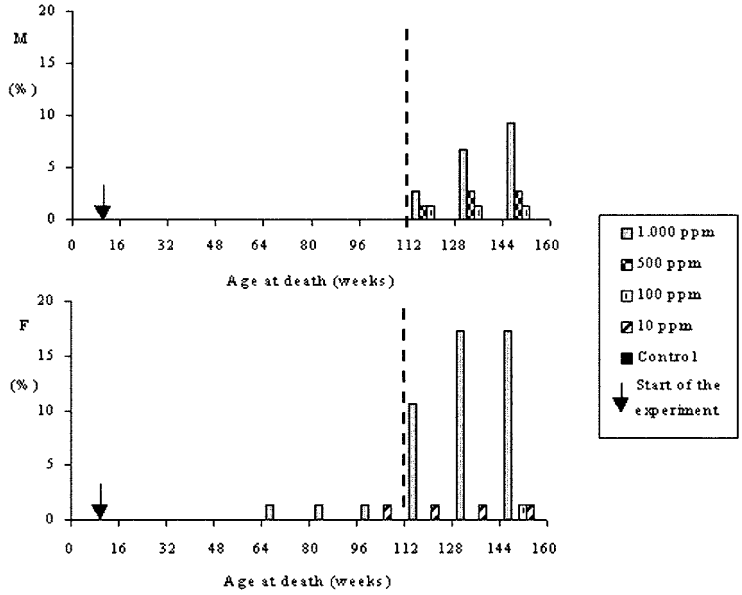

FIGURE 3. Cumulative prevalence of thyroid gland malignant tumors, histopathologically observed, by age at death, in male and female Sprague-Dawley rats treated with Mancozeb (BT 5007).

noted that the experiment with xylene performed by the NTP sacrificed rats after 104 weeks of treatment; no carcinogenic effect was found.[27]

Mancozeb

Mancozeb (experiment BT 5007) was administered to Sprague-Dawley rats with feed at concentrations of 1,000, 500, 100, 10, or 0 ppm supplied *ad libitum* for 104 weeks to 75 males and 75 females, 8 weeks old at start. All animals were observed until spontaneous death. Control animals received standard feed. Among other things, a strong increase in malignant tumors of the thyroid gland in males and females was observed after 112 weeks of age (FIG. 3).[12]

Vinyl Acetate Monomer

Vinyl acetate monomer (experiment BT 51) was administered in drinking water supplied *ad libitum*, at doses of 5,000, 1,000, and 0 ppm (v/v), to Sprague-Dawley rats, 17-week-old (breeders) or 12-day embryos (offspring) at the start of the experiment. Treatment lasted 104 weeks after which time the animals were kept under control conditions until spontaneous death. The increase in carcinomas of the oral cavity, tongue, esophagus and forestomach (considered altogether), in male and female offspring, was only clearly observed after 112 weeks of age (FIG. 4).[6]

In an other experiment (BT 52), vinyl acetate monomer was administered in drinking water, supplied *ad libitum*, at doses of 5,000, 1,000, and 0 ppm (v/v), to

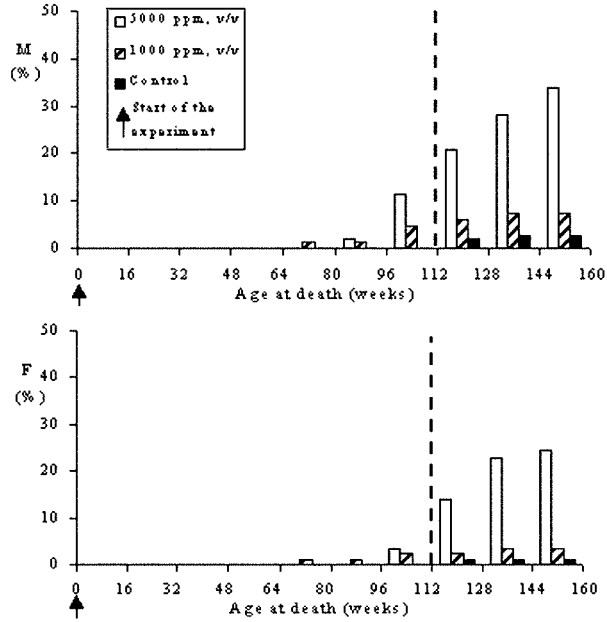

FIGURE 4. Cumulative prevalence of animals with tumors of the oral cavity, tongue, esophagus and forestomach, histopathologically observed, by age at death, in male and female offspring Sprague-Dawley rats treated with vinyl acetate monomer (BT 51).

Swiss mice, 17-week-old (breeders) or 12-day embryos (offspring) at the start of the experiment. The treatment lasted 78 weeks, the animals were kept under control conditions until spontaneous death. The increase in total malignant tumors in male and female breeders and offspring was again clearly observed after 112 weeks of age (FIGS. 5 and 6).[5]

Project of Experimental Anticarcinogenesis (Chemopreventive) Bioassays

By interventive prevention, we mean the use of factors and agents to contrast, block or inhibit the onset of tumors of different types and sites. When these factors or agents are chemicals, and in particular drugs, the interventive prevention is properly known as chemoprevention.

The project started in 1985. The aims of this project are: (1) to set up adequate animal models and experimental protocols; (2) to conduct experimental studies on compounds potentially eligible for chemoprevention; (3) to determine the minimal effective drug dose, the effect of the drug administration schedule, the effect of the interruption of drug administration, side effects (in particular carcinogenic), efficacy, and tolerability of the drug with or without other drugs.

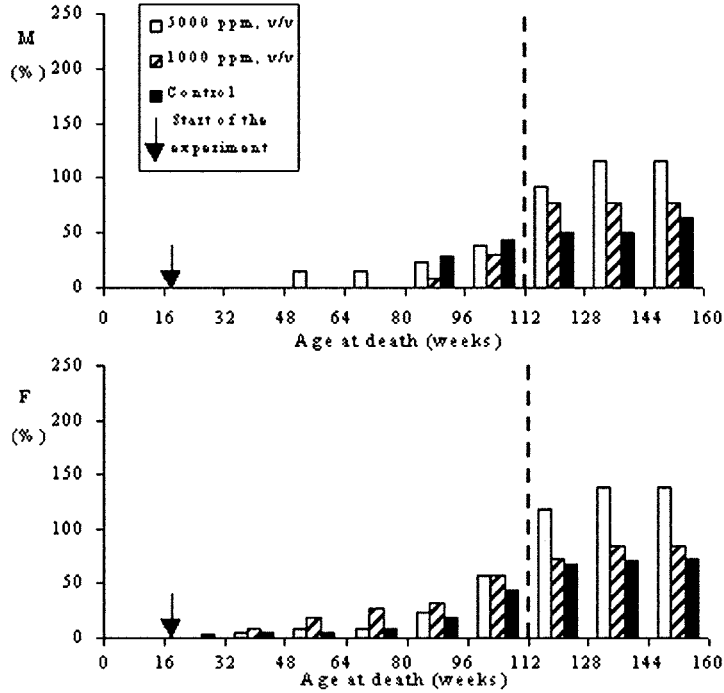

FIGURE 5. Cumulative prevalence of total malignant tumors *per* 100 animals, histopathologically observed, by age at death, in male and female breeder Swiss mice treated with vinyl acetate monomer (BT 52).

Chemopreventive bioassays are planned and conducted following two types of protocols:

- *Protocol I:* studies on limited groups of animals (90–150), at high risk (by virtue of age and other factors), with limited treatment and observation period (35–40 weeks), so as to reproduce clinical trials.
- *Protocol II:* studies on larger groups of animals (200–400), starting at a young age, with a varying duration of treatment (often life span), and with observation until spontaneous death, thus reproducing epidemiological studies on populations.

All studies are performed on Sprague-Dawley rats and conducted according to Good Laboratory Practices.

Twenty factors/compounds have been studied on Sprague-Dawley rats from the colony of the CRC/RF, which represents a human-equivalent model for various different tumors, and in particular mammary cancer. The factors/compounds studied are given in TABLE 3. Over 33,000 animals have been used for the study of these factors/compounds.

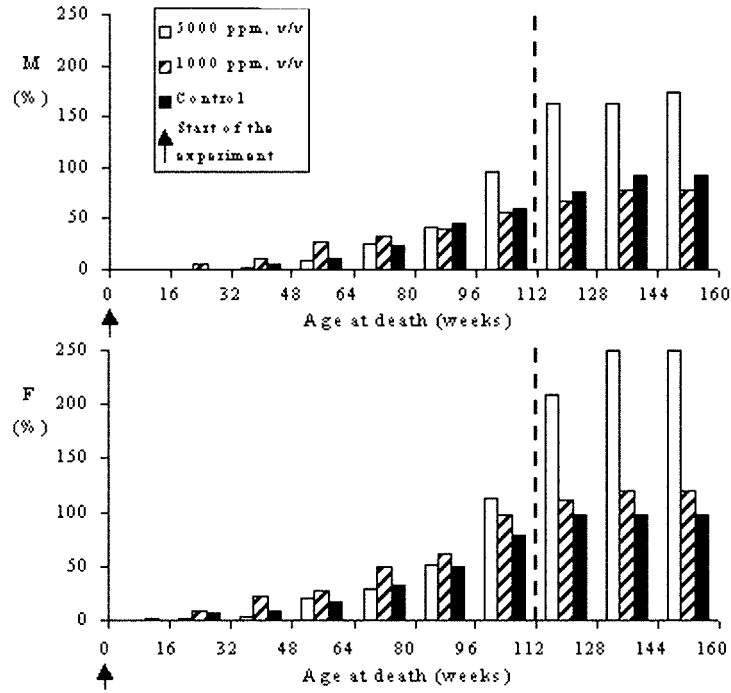

FIGURE 6. Cumulative prevalence of total malignant tumors per 100 animals, histopathologically observed, by age at death, in male and female offspring of Swiss mice treated with Vinyl acetate monomer (BT 52).

With these experiments the preventive effects of pregnancy, ovarectomy, leuprolide,[28] tamoxifen,[29,30] faslodex, medroxyprogesterone acetate,[31] and 4-hydroxyandrostenedione on mammary carcinogenesis have been shown.

In contrast, our experimental studies on Vitamin A (retinol palmitate and acetate) have shown that this vitamin increases the incidence of mammary malignant tumors.[32]

The results of this project not only indicate the chemopreventive effects of the factors/compounds studied, but may provide important information on their side effects. Furthermore they may be useful, as in the case of tamoxifen, to determine the lowest doses still effective for chemoprevention: this is an important datum in the case of trials on women, to avoid unnecessary overtreatment.

Project of Descriptive and Analytical Epidemiology

This project encompasses: (1) registries of nominative mortality, with particular regard to tumors, in the province of Bologna and other Italian geographic areas; and (2) analytical epidemiology studies on the tumor incidence and mortality in people professionally and environmentally exposed to carcinogens of environmental, industrial origin or both.

TABLE 3. The Ramazzini Foundation Cancer Program

PROJECT OF EXPERIMENTAL CHEMOPREVENTIVE BIOASSAYS: FACTORS / AGENTS STUDIED

1. Pregnancy
2. Ovarectomy

Vitamins
3. Vitamin A
4. Vitamin C
5. Vitamin E

Retinoids
6. N-(4-hydroxyphenil)retinamide (HPR)

Antiestrogens
7. Tamoxifen
8. Faslodex
9. Toremifene
10. Raloxifene

LHRH antagonists
11. Leuprolide (LEU)

Progestins
12. Medroxyprogesterone acetate (MPA)

Aromatase inhibitors
13. 4-hydroxyandrostenedione (4-OHA)
14. Anastrozole

Estrogens
15. Conjugated estrogens (Premarin)

Chemotherapeutic drugs
16. Cyclophosphamide, methotrexate, 5-fluorouracil (CMF)

Immunomodulants
17. Alpha-interpheron

Drug Combinations
18. Tamoxifen-HPR
19. Tamoxifen-MPA
20. Tamoxifen-LEU-MPA

The aims of the registry of nominative mortality are: (1) to produce information on the trends of tumor mortality and other causes of death; (2) to identify possible areas of higher mortality due to tumors and trace the causes; (3) to establish a database available for prevention and clinical practice; and (4) to furnish data for health programs.

All mortality data are acquired from photocopies of the original death certificate. Data are ordered, completed, recodified, elaborated, and published according to a controlled protocol, applied to the registries of all areas studied. The results of every geographic area are published in a consistent manner.

Data dealing with the project design and state-of-the-art are presented in TABLE 4. Mortality data for a 40-year period from Bologna, its province, and some other areas will be available in the next few years.

The nominative mortality registries, with particular regard to tumors, are precise instruments that define the current dimension of neoplastic disease and can be used to forecast future trends and to evaluate the social burden of tumors and the efficacy of current control strategies.

The studies on analytical epidemiology began in the early 1960s. The main areas of interest are tumors of the urinary tract due to aromatic amines,[33] hepatic angiosarcomas due to vinyl chloride,[34] mesotheliomas due to asbestos used in the railroads[35] and sugar refineries,[36] and to asbestos exposure in other circumstances.

Our data on pathologies related to exposure to asbestos are reported in TABLE 5.

TABLE 4. The Ramazzini Foundation Cancer Program

PROJECT OF DESCRIPTIVE AND ANALYTICAL EPIDEMIOLOGY: THE REGISTRIES OF NOMINATIVE MORTALITY WITH PARTICULAR REGARD TO TUMORS

	Geographical Area	Population	Years Considered
1.	Province of Bologna	908,631	1959-2000
2.	Province of Reggio Emilia	429,865	1959-1988
3.	LHU 32 of Portomaggiore (FE)	53,701	1986-1987
4.	Faenza (RA)	48,419	1960-1998
5.	LHU 75 of Acqui Terme (AL)	44,587	1959-1994
6.	Albignasego (PD)	18,452	1960-1996
7.	Cittadella (PD)	18,415	1960-1997
8.	LHU 5 of Urbino		1960-1994
	Province of Pesaro and Urbino	340,830	1995-2000
9.	Rocca di Papa (RM)	13,242	1960-1996
10.	S. Giovanni Rotondo (FG)	25,121	1960-2000
11.	Manfredonia (FG)	58,623	1960-2000
12.	Sannicandro Garganico (FG)	18,643	1960-2000
13.	Mountain Communities Appennino Dauno Settentrionale (FG)	24,296	1960-2000
14.	Foggia	155,237	1980-2000
15.	Bari	338,949	1980-2000
16.	S. Vito dei Normanni (BR)	20,877	1936-2000
17.	Brindisi	94,429	1990-2000
18.	Catanzaro	96,886	1980-1999
19.	Molise Region	327,987	1990-1999
	TOTAL	**3,037,190**	

Studies in these areas are ongoing. A mortality study has recently started on a cohort of over 1,400 workers in the vinyl chloride/polyvinyl chloride industries.

The results of these studies help to identify and quantify the risk of populations exposed to carcinogenic risks and can be used for preventive strategies and to establish the occupational origin in cases of compensation for neoplasias. They are an important area of study.

CONCLUSION

All the projects herein reported have, for the past 35 years, borne the strong leadership of Professor Cesare Maltoni, Director of the Institute of Oncology "F. Addarii" (1964–1997) and thereafter Scientific Director of the Ramazzini Foundation until his untimely death in January 2001. The commitment of his group of coworkers continues.

TABLE 5. The Ramazzini Foundation Cancer Program

PROJECT OF DESCRIPTIVE AND ANALYTICAL EPIDEMIOLOGY: REGISTRY OF THE PATHOLOGIES OF PEOPLE EXPOSED TO ASBESTOS

Category	Pathology			
	Mesothelioma	Lung cancer	Other tumors	Asbestosis[a]
Railway workers	**207**	**146**	**157**	**173**
- FS workers	92	26	27	37
- Workers of rolling-stock workshops not belonging to FS	97	119	128	132
- Workers not belonging to FS, exposed to work environment pollution	4	0	1	1
- Family members of FS workers	7	0	0	3
- Family members of workers in rolling-stock workshops not belonging to FS	3	1	1	0
- Environmental pollution in FS	4	0	0	0
Shipyard and port workers	**17**	**14**	**15**	**2**
- Workers	17	13	15	2
- Family members	0	1	0	0
Workers of asbestos-cement industry	**18**	**7**	**3**	**2**
- Workers	17	7	3	2
- Family members	1	0	0	0
Workers of sugar refineries	**19**	**2**	**1**	**1**
- Workers	18	2	0	1
- Family members	1	0	1	0
Seamen and sailors	**18**	**1**	**0**	**1**
- Workers	17	1	0	1
- Family members	1	0	0	0
Other categories[b]	**238**	**45**	**28**	**114**
TOTAL	**517**	**215**	**204**	**293**

[a] Pleural plaques, pleural thickenings, pleural asbestosis, pleuro-pulmonary asbestosis and pulmonary asbestosis are included.

[b] Over 60 work categories are included.

The project of primary experimental research into industrial carcinogenesis, anti-carcinogenesis, and epidemiology performed by Ramazzini Foundation has been conducted in highly standardized control conditions by a team largely unchanged over time. For these reasons, it represents an unusual entity in oncological research, in our country and the world. Projects such as these involve considerable cost.

The meaning of this cost was very clearly expressed by Cesare Maltoni at the end of his presentation at the Conference on Vinyl Chloride in Bethesda in 1980, as reported in the proceedings published in the EHP.

> The cost of our project of long-term carcinogenicity bioassays on vinyl chloride includes the cost of (1) the planning and setting-up of experimental apparatus, including inhalation facilities, of a type uncommon in 1971, and the working out of a protocol for long-term bioassays; (2) the study of nearly 7,000 animals up to the point of their natural death; (3) ten years of work; (4) the routine examination of some 200,000 histological slides; (5) a financial commitment equivalent to more than $2,000,000 U.S. at present prices; (6) the availability of the same team of scientists throughout the entire 10 years of the project, a prerequisite which may be difficult or even impossible to ensure in many countries at the present time; (7) the highly motivated commitment of those scientists to a type of work which is long-lasting, onerous and often tedious; (8) the effort involved in maintaining the consistency of the methodology, which has as its reverse side the limits placed on the exercise of imagination (the most positive element in scientific life); (9) the effort involved in establishing and preserving objectivity and balance in the evaluation and interpretation of data; (10) and finally, the strength required to withstand the sense of loneliness arising from the lack of co-operation of many of those bodies which should properly be concerned with the progress of science in this field, not excluding part of the scientific community whose indifference sometimes degenerates into frank hostility.
>
> The high costs probably represent the reason why, in the field of experimental and environmental carcinogenesis, words overlap facts, opinions overlap data, and meetings and commissions reports submerge good laboratory work.

Twenty-two years after the paper appeared in EHP, our entire project, in number of animals, investments and commitment, is about 20 times greater than the research program on vinyl chloride; we can still only reconfirm those words. Indeed, we wish to state, clearly, that:

(1) primary research in the field of oncology continues to be disregarded, almost everywhere, including in Italy and the rest of Europe
(2) innovative research is understood and accepted with difficulty; and
(3) when primary and innovative research reveals that agents of fundamental importance for technological development, and therefore of great economic and political interest, may be hazardous for health and, specifically, may present a risk of cancer, obstacles will be put in its way, and the public is unlikely to know or understand.

Two clear perceptions give us cause for optimism however: (1) history teaches us that the search for truth, and the habit of bearing witness to it, in the end prevails; and (2) culture and science induce in their disciples a sort of mithridatization: in the words of our great poet Giacomo Leopardi, they come to learn an "aristocratic pleasure in displeasing," in the defense of truth.

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