

## Mega-experiments on the carcinogenicity of $\gamma$ -radiation on Sprague-Dawley rats at the Cancer Research Centre of the European Ramazzini Foundation of Oncology and Environmental Sciences: plan and report of early results on mammary carcinogenesis

M. Soffritti, F. Belpoggi, F. Minardi, L. Bua and C. Maltoni

European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italy

M. Soffritti, F. Belpoggi, F. Minardi, L. Bua and C. Maltoni: Mega-esperimenti sulla cancerogenicità delle radiazioni  $\gamma$  al Centro di Ricerca sul Cancro della Fondazione Europea di Oncologia e Scienze Ambientali B. Ramazzini: piano e primi risultati sulla cancerogenesi mammaria. Eur. J. Oncol., 4 (5), 509-522, 1999

M. Soffritti, F. Belpoggi, F. Minardi, L. Bua e C. Maltoni: Mega-esperimenti sulla cancerogenicità delle radiazioni  $\gamma$  al Centro di Ricerca sul Cancro della Fondazione Europea di Oncologia e Scienze Ambientali B. Ramazzini: piano e primi risultati sulla cancerogenesi mammaria. Eur. J. Oncol., 4 (5), 509-522, 1999

### Summary

This report deals with the aims, plan, material and methods, as well as the peculiarities, and some early results on mammary carcinogenesis, of the project on mega-experiments being conducted by the Cancer Research Centre (CRC) of the European Ramazzini Foundation of Oncology and Environmental Sciences (RF) into the carcinogenic effects of  $\gamma$ -radiation, as studied on Sprague-Dawley rats from the colony of the CRC/RF. The project sets out to evaluate the carcinogenic potentiality of these radiations with reference to their effects on various tissues and organs, to the doses (especially low doses), to the calendar of exposure, to biological factors and to the potential protective effect, mainly on mammary carcinogenesis, of an antioestrogen (tamoxifen) and an LH-RH agonist-antagonist (leuprolide). The project includes 7 experiments: 1) BT 1R, which studies the effects at the three dose levels, 300, 100 and 10 rads, delivered in one shot or in 10 shots (1 every 4 weeks) to 4016 six week-old (starting age) male and female animals; 2) BT 2R, which studies the effects on male and female offspring of three doses, 100, 50 and 10 rads, delivered in one shot to pregnant breeders, irradiated on the 12th day of pregnancy (3850 animals); 3) BT 3R, which studies the effects on male and female offspring of three doses, 300, 100 and 10 rads, delivered, in one shot to male breeders before mating

### Riassunto

In questo resoconto vengono riferite le finalità, il piano, i materiali e metodi, le peculiarità e alcuni risultati preliminari del progetto di mega-esperimenti del Centro di Ricerca sul Cancro (CRC) della Fondazione Europea di Oncologia e Scienze Ambientali B. Ramazzini (FR), sugli effetti cancerogeni delle radiazioni  $\gamma$ , studiati su ratti Sprague-Dawley della colonia CRC/FR. Il progetto ha come finalità la valutazione delle potenzialità cancerogene di queste radiazioni, con riferimento ai loro effetti su vari tessuti ed organi, alle dosi (con particolare riguardo a quelle più basse), al calendario delle esposizioni, a fattori biologici ed agli eventuali effetti protettivi, soprattutto sulla cancerogenesi mammaria, di un antiestrogeno (il tamoxifen) e di un LH-RH agonista-antagonista (il leuprolide). Il progetto si articola su 7 esperimenti: 1) il BT 1R, che studia gli effetti di tre livelli di dose, 300, 100 e 10 rad, erogati una tantum o in 10 dosi (1 ogni 4 settimane) ad animali maschi e femmine di 6 settimane di età (4016 animali); 2) il BT 2R, che studia gli effetti, sui figli maschi e femmine, di tre livelli di dose, 100, 50 e 10 rad, erogati una tantum alle madri il 12° giorno di gravidanza (3850 animali); 3) il BT 3R, che studia gli effetti, sui figli maschi e femmine, di 3 livelli di dose, 300, 100 e 10 rad, erogati una tantum ai padri prima dell'accoppiamento (3608 animali); 4) il BT 4R, che studia gli effetti della irradiazione con 4 Mrad e 1 Mrad del cibo somministrato alle madri dal 12° giorno di gravidanza, e quindi ai figli maschi e femmine fino a morte spontanea (2190 animali); 5) il BT 5R, che studia gli effetti protettivi sulla cancerogenesi da 300 rad erogati una tantum a ratte di 6 settimane di età, del tamoxifen, somministrato con calendari diversi rispetto alla irradia-

Indirizzo/Address: Morando Soffritti, Centro di Ricerca sul Cancro, Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini", Castello di Bentivoglio, 40010 Bentivoglio (BO), Italia

(3608 animals); 4) BT 4R, which studies the effects of feed irradiated by 4 Mrad and 1 Mrad administered to pregnant breeders from the 12th day of pregnancy, and then to their male and female offspring until spontaneous death (2190 animals); 5) BT 5R, which studies the protective effects, on 6 week-old female rats exposed one off to 300 rads, of tamoxifen, administered by differing calendars, with reference to radiation treatment (800 animals); 6) BT 6R, which studies the protective effects, on 6 week-old female rats exposed one off to 300 rads, of leuprolide (400 animals); 7) BT 7R (first part), which studies the protective effects on 56 week-old female rats exposed one off to 300 rads, of tamoxifen (480 animals); and BT 7R (second part), which studies the protective effects on 56 week-old female rats exposed one off to 300 rads, of leuprolide (480 animals). The animals in the various experimental groups of the first 4 experiments were predestined; the parents were randomized before mating, which was strictly out-bred. In the other three experiments the randomization was by litter. Experiments BT 1R-BT 6R lasted for the life-span; only experiment BT 7R was truncated at 96 weeks of animal age. The peculiarities of the project are: 1) the use of animals with well-known expected pathologies (from available histopathological data on more than 15,000 historical controls); 2) the high number of animals in the experimental groups; 3) the duration of experiments; 4) the high standardization of the experimental methodologies, which are in line with the Good Laboratory Practices (GLP); and 5) the systematic observation and registration of pathological effects. The report provides some early results on mammary carcinogenesis from  $\gamma$ -radiation as seen in the females of the first experiment (BT 1R) which were treated with the 3 dose levels, delivered in one shot. These results confirm the carcinogenic effects of  $\gamma$ -radiation on the mammary gland; they also show a sharp parallelism between dose and response; and finally they indicate quite clearly that exposure to 10 rads entails a carcinogenic risk for the breast.

**Key words:**  $\gamma$ -radiation, low doses, rat, mammary carcinogenesis

## Introduction

### *Radioactivity and ionizing radiation*

Ionizing radiation comprises electromagnetic radiation (X- and  $\gamma$ -rays) and corpusculate radiation (electrons, protons, neutrons,  $\alpha$ -particles, heavy nuclei). Both kinds of radiation are thought to exert their biological effect primarily through ionization of the cell constituents by which they are absorbed (targets). Hence in either case the mode of action and the basic biological effects produced are expected to be similar. Nonetheless, the effects of the two types of radiation do differ quantitatively, having different powers of penetration and degrees of absorption, as well as transferring their energy to different cell sites. They may, and indeed do, represent a problem for human health: in particular they can trigger the onset of cancer.

Such radiation is natural, but also artificial, in origin. The natural kind divides into cosmic and terrestrial (rocks, soil and bios-

phere); the artificial kind is man-made for war- or peacetime purposes. As far as is known, radioactive materials and the radiation from them formed part of the big bang which gave rise to the universe some 20 billion years ago, since which time they have pervaded the cosmos. They were present in the universe before the world was formed. Radioactive matter subsequently became part of the Earth (and its biosphere) from the very outset, and long before life appeared thereon. The element uranium was discovered in 1789 in pitchblende from Johann-Georgenstadt. Thereafter it was used to manufacture yellow glass and luminous or uranium pigments. The phenomenon of radioactivity, the radiation deriving from it, and how this is generated, is knowledge mankind has possessed for little more than a century. In 1895 the German physicist Wilhelm Conrad Röntgen discovered X-rays and a method for producing them. In 1896 the Frenchman A. Henri Becquerel realized that uranium generated

**Parole chiave:** radiazioni  $\gamma$ , basse dosi, ratto, cancerogenesi mammaria

phere); the artificial kind is man-made for war- or peacetime purposes.

As far as is known, radioactive materials and the radiation from them formed part of the big bang which gave rise to the universe some 20 billion years ago, since which time they have pervaded the cosmos. They were present in the universe before the world was formed. Radioactive matter subsequently became part of the Earth (and its biosphere) from the very outset, and long before life appeared thereon.

The element uranium was discovered in 1789 in pitchblende from Johann-Georgenstadt. Thereafter it was used to manufacture yellow glass and luminous or uranium pigments.

The phenomenon of radioactivity, the radiation deriving from it, and how this is generated, is knowledge mankind has possessed for little more than a century.

In 1895 the German physicist Wilhelm Conrad Röntgen discovered X-rays and a method for producing them. In 1896 the Frenchman A. Henri Becquerel realized that uranium generated

radiation by the chance observation that the mineral left an impression on photographic plates (the sample that Becquerel observed came from Joachimsthal, which lies on the Czechoslovak side of a mountain area bordered on the North by Schneeberg, Aue and Oberschlema in Saxony). Schmidt discovered that thorium gives a similar radiation to that of uranium. The research leapt forward with Marie Skłodowska Curie, who first coined the word “radioactivity”. Together with her husband Pierre Curie, she discovered in 1898 that, on emitting radiation, uranium transformed into other elements: polonium (Marie was Polish) and radium. Ernest Rutherford studied the gas-like decay products of thorium, and coined the term “emanation”. Dorn found gas-like emanations from radium. These few years resulted not only in the discovery of X-rays and the radioactivity of uranium, thorium, polonium, radium and actinium, but also of some of the gaseous decay products in the form of emanation. By the end of the Thirties, Otto Hahn, Friedrich Strassmann and Lisa Meitner had discovered the fission of uranium: seven years later, in August 1945, an atom bomb based on uranium was used to destroy Hiroshima. The catastrophe of Chernobyl dates from 1986<sup>(a)</sup>.

Nowadays it is thought that the most important of all natural sources of radiation is radon, an invisible, odourless, tasteless gas seven-and-a-half times heavier than air, together with its “daughter” radionuclides, which form as it decays. Radon has two main forms, radon-222 (one of the radionuclides in the sequence formed by the decay of uranium-238) and radon-220 (produced during the decay series of thorium-232).

### *Exposure scenarios*

The sources of radioactivity and ionizing radiation have gone on multiplying and spreading across the earth’s crust, waters and biosphere, as radioactive materials have been extracted and new sources of radioactivity created (X-rays and new radioactive isotopes), and the latter used and spread about the environment in the form of various products and waste materials.

The levels of natural and artificial radioactivity and ionizing radiation are particularly raised by certain situations/scenarios, leaving in their wake an exposure risk for various categories of the population (Table 1).

The magnitude of ionizing radiation as a pollutant, and worry as to its effect on our planetary life and human health, have grown alongside the expansion of nuclear industry and mushrooming exposure scenarios. A number of exposure situations are currently, and will be in future, causing especial concern and attention: 1) occupational exposure (by those who extract, handle or use radioactive materials); 2) the widespread risk of radioactive weapons being used; 3) the imminence of catastrophe; 4) maintenance and control systems in existing military nuclear arsenals; 5) the dispersal of growing amounts of nuclear waste; 6) the growing number of outlets in industry; 7) expanding medical outlets in the fields of diagnosis and therapy; 8) pollution of consumer goods, including foodstuffs; 9) the potential build-up of background radiation in the biosphere, surface and ground water, and the soil; and 10) radioactivity in confined environments such as the home.

### *The carcinogenicity of ionizing radiation*

Ionizing radiation acts on biological systems and destroys them. As long ago as 1900, O. Walkhoff observed that radium emissions killed cells.

Since then it has been proved that ionizing radiation causes regressive alterations to the tissues, leading to necrosis, phlogistic alterations and fibrosis, mutation, pathology of the gonads and reproductive life, and embryotoxic, teratogenic and above all carcinogenic effects, which form the object of the present project and the results it sets out to report.

#### *A) Milestones in the history of carcinogenicity due to ionizing radiation*

Research into radioactivity, industrial use of radioactive materials and the release of these into the environment have all proceeded hand in hand, and with them our knowledge of the carcinogenic effect of ionizing radiation, both electromagnetic and corpuscular.

In 1902, seven years after Röntgen discovered the X-rays, Frieben reported to the Hamburg Medical Society on the first-ever case of X-ray-related tumour found in a worker at a firm manufacturing radiogenic bulbs. The employee had been using his hand to show customers how the skeleton was highlighted, and the result was a carcinoma. In 1909, Wolbach described the histological sequences behind chronic dermatitis and skin carcinoma from X-rays. In 1908 Clunet exposed four albino rats to an X-ray dose sufficient to produce skin ulcers: one of the two surviving animals developed a local sarcoma (Clunet, 1910). In 1918 Lazarus-Barlow reported that injecting radium sulphate into subcutaneous rat tissue caused the onset of skin carcinoma.

In 1879 Harting and Hesse had announced that the fatal lung disease among Schneeberg miners – described by Agricola in his “*De re metallica*”, 1556 – was in fact a malignant tumour responsible for 75% of all deaths among the miners (the authors described it as a lymphosarcoma; only later was it ascertained to be a carcinoma). In 1913 a layman called H.E. Müller, a mine manager, gave his view that radium radiation and emanations caused the disease in question. In 1926 Rostowski, Saupe and Schmorl found by autopsy that 13 out of 21 uranium miners (62%) had died of lung cancer. Only in 1939 would Rajewsky indicate that emanations present in the air of the mine might be an important cause of the miners’ lung cancer. Rajewsky, Schraub and Kahlau (1943) were to prove the association by experiment: mice made to live in a radioactive atmosphere had a high incidence of pulmonary tumours: adenoma, sometimes in multiple form, and also carcinoma.

In 1929 and 1931 respectively, Martland and Humphries and Martland reported on bone sarcomas arising in factory girls painting signs with a luminous compound containing radium and mesothorium. With their lips the girls would wet the tip of brushes already dipped in compound; swallowed over the years, it fixed in the bones. It was in 1933 that Oberling and Guerin first showed how injecting rats with thorotrast produced peritoneal sarcoma. Thorotrast is a solution of thorium dioxide stabilized with dextrin: this fixed in the reticulo-endothelial system of organs like the liver or spleen, to highlight which organs it had indeed been in use in diagnosis since the outset of the Thirties. In 1947 the first case was reported of a liver angiosarcoma in a patient, twelve years after thorotrast in-

<sup>(a)</sup> While this article was going to press, the news was received of the Tokaimura power station accident (Japan) on September 30, 1999.

**Table 1** - Exposure to ionizing radiation: type of exposure, exposure scenario and populations exposed

Type of exposure	Exposure scenarios	Populations exposed
Occupational	Radiology and nuclear medicine services	Workers
	Nuclear power stations	Workers
	Military arsenal depots	Workers, soldier
	Radioactive waste disposal	Workers
	Disaster zone reclaiming	Workers, soldiers
	Industries where radiation and/or radioactive materials are used	Workers
Medical	Therapy	Patients
	Diagnostic techniques	Patients
	Screening	Sectors of population
Environmental	Confined spaces (houses, public buildings) polluted by radon	Resident, workers
	Nuclear testing grounds	Residents
	Areas for nuclear waste dumping	Residents
Wartime	Area bombed by nuclear weapons	Residents
	Participation in nuclear wars	Soldiers
	Participation in nuclear tests	Workers, soldiers
Consumer-related	Tobacco smoking	Smokers
	Food polluted by radioactive elements	Consumers
	Radioactive goods	Consumers
Peacetime calamities	Nuclear power station accidents	Workers, soldiers, residents
	Radioprotection mistakes	Workers

jection (MacMahon, Murphy and Bates, 1947).

In 1944 March documented an increase in leukaemia in an occupational category with a clear-cut exposure to ionizing radiation: radiologists.

By the end of the Forties a wealth of results was available from anatomico-clinical, epidemiological and experimental studies. The data were collected and reviewed with exemplary precision by Lacassagne in two classic monographs, one devoted to corpusculata, the other to electromagnetic radiation (Lacassagne, 1945a, b). These showed clearly that ionizing radiation of various kinds, emitted by radioactive sources outside or inside the human body and to which humans may be exposed in different ways, produced a whole range of tumours in various tissues and organs of humans and experimental animals (multipotent carcinogen).

#### B) *The present state of knowledge*

Research into the carcinogenicity of ionizing radiation has continued into this latter half-century. There have been three main branches of study: 1) epidemiological, 2) experimental and 3) biostatistical. Such studies have set out to define the carcinogenic potential of the various radioactive sources and the rays they emit; to establish the relationship between dose and response; to measure the carcinogenic effect of low-dose ionizing radiation; to assess the effect on carcinogenesis of factors linked to exposure (e.g. the calendar) or host (age, hormonal status, reproductive history); and lastly to supply information on the mechanisms of action.

It is our opinion that biostatistical studies have tended to outweigh epidemiological or experimental research. Important epidemiological studies have been, and are still being, conducted in various population groups exposed, like the survivors of Nagasaki and Hiroshima, or patients treated by ra-

diotherapy, workers in nuclear power stations, residents near nuclear plants or testing grounds, and miners in uranium mines (Ron, 1998); they fall, in any case, short of the need. Experimental research has marked time; clearly the amount of experimental research is derisory when compared to the gravity of the issue of ionizing radiation carcinogenesis, and it is anyway confined to sectorial spheres of interest.

By any current assessment it is evident that animal research has proved and confirmed the capacity of ionizing radiation to cause various kinds of tumour (Upton, 1968; Upton *et al.*, 1986). It has emerged with no less clarity how human beings in a range of exposure scenarios are subject to a variety of neoplasias due to radiation, although the extent of the evidence and the quantitative estimates vary from neoplasia to neoplasia (Table 2) (Ron, 1998). It has also been proved that relatively low exposures may cause carcinogenic effects in human beings: classics of this sector are the studies by Alice Stewart (Stewart, Webb and Hewitt, 1958), who demonstrated an increase in leukaemias among children whose mothers had undergone radiological pelvimetry during pregnancy. It has, lastly, been shown that radiation may act synergically with other carcinogens, e.g. tobacco (National Research Council, Committee on the Biological Effects of Ionizing Radiation, 1998) and chemotherapy agents (Curtis *et al.*, 1992), or with host-related factors, e.g. age at exposure (Thompson *et al.*, 1994) and reproductive history (Land *et al.*, 1994).

Much still remains to be discovered. The cancer effect of various exposure scenarios has gone unexplored. The data on the total carcinogenic burden of radiation still await proper definition. Data on the effect of low and very low doses have yet to be collected, since: 1) epidemiological investigations are handicapped by the difficulty of precisely establishing exposure levels and by the welter of confounding factors; while 2)

**Table 2** - Neoplasias related to ionizing radiation exposure in humans: distribution according to accuracy of knowledge<sup>(a)</sup>

Clear evidence of association	Evidence of association	Evidence but no solid	Slight evidence
Fairly precise quantitative risk estimates (association at relatively low doses: <0.2 Gy)	No precise quantitative risk estimates (at 1 Gy the relative risk generally ranges from about 1.0 to 2.5)	No quantitative risk estimates	No risk estimate
Leukaemia Cancer of the thyroid Cancer of the breast Cancer of the lung	Cancer of the skin Cancer of the salivary glands Cancer of the stomach Cancer of the colon-rectum Cancer of the bladder Cancer of the ovary Tumours of the central nervous system	Cancer of the oesophagus Cancer of the liver Multiple myeloma Non-Hodgkin's lymphoma	Chronic lymphocytic leukaemias Hodgkin's lymphoma Cancer of the pancreas Cancer of the prostate Cancer of the cervix Tumours of the testis

<sup>(a)</sup> Ron, 1998

the experimental research conducted to date has its own limitations: the scanty size of animal groups, the duration of the biophase, which is all too often arbitrarily truncated, not to mention the lack of system in collecting details of pathology.

### The project of mega-experiments on ionizing radiation ( $\gamma$ -rays) carcinogenicity conducted by the European Ramazzini Foundation of Oncology and Environmental Sciences (Ramazzini Foundation/RF), Bologna, Italy

#### Background

The need to set up a project of mega-experiments on the carcinogenic effects of  $\gamma$ -radiation arose in the immediate aftermath of the Chernobyl disaster in 1986, when it became apparent how little scientific knowledge still existed on the carcinogenicity of ionizing radiation.

The project was deemed to be feasible, thanks to the know-how on conducting mega-experiments possessed by the Cancer Research Centre of the Ramazzini Foundation (CRC/RF).

The research was initially financed in part by the Province of Bologna and its Municipalities, as well as by the Bologna section of the Italian League for the Fight against Cancer. Additional support later came from the Regional Agency for Prevention and Environment (Agenzia Regionale Prevenzione e Ambiente/ARPA) of the Emilia-Romagna Region.

The project started on December 14, 1991.

#### Aims

The project has the following aims:

- 1) to define the full carcinogenic potential of  $\gamma$ -radiation and of feed treated with  $\gamma$ -radiation in controlled conditions;
- 2) to assess the dose-response relationship;
- 3) to define the carcinogenic potential of exposure to low doses;
- 4) to evaluate the influence of the exposure calendar on carcinogenicity;
- 5) to evaluate the influence on carcinogenicity of host factors, such as family and age at start of exposure;
- 6) to evaluate the effect of treatments (antihormonal) eligible for chemoprevention of  $\gamma$ -radiation carcinogenesis with particular regard to mammary carcinogenesis;

- 7) to identify possible tumour precursors and markers of carcinogenicity; and finally
- 8) to improve our knowledge on the mechanism of  $\gamma$ -radiation carcinogenesis.

#### Distinguishing features

The project is distinguished by a number of features:

- 1) the highly standardized laboratory operating procedures adopted by CRC/RF experiments over the last 30 years;
- 2) the highly standardized rules for conducting experiments;
- 3) the same scientific team from the start of the project, mostly working at the CRC/RF for more than 20 years;
- 4) the use of a colony of Sprague-Dawley rats, employed in the CRC/RF laboratories for 30 years, with data available on expected pathology from more than 15,000 controls;
- 5) the availability of animal housing conditions allowing prolonged survival;
- 6) the availability of adequate apparatus for exposing animals to  $\gamma$ -radiation and monitoring the level of exposure;
- 7) the size of experimental animal groups;
- 8) the whole life-span duration of experiments (an usual practice at CRC/RF);
- 9) data collection on the effects of treatment extending throughout the biophase to necropsy and histopathological examination;
- 10) conformity with Good Laboratory Practices (GLP);
- 11) concentration of all the project experiments in a fairly restricted period of time.

#### Plans of experiments

The plans of the various experiments are shown in Tables 3-10.

The project experiments started in sequence:

- BT 1R: February 19, 1992
- BT 2R: December 27, 1991
- BT 3R: December 14, 1991
- BT 4R: December 27, 1991
- BT 5R: January 12, 1993
- BT 6R: September 12, 1994
- BT 7R: February 14, 1995

Experiments BT 1R, BT 2R and BT 3R study the carcinogenicity of  $\gamma$ -radiation delivered at various doses and with various calendars to 6 week-old animals, either born from female breeders

irradiated during pregnancy, or from fathers irradiated before mating, divided into different experimental groups by destined families. Experiment BT 4R studies the carcinogenicity of  $\gamma$ -radiation irradiated feed, exposing the animals from embryo development onwards. Experiments BT 5R, BT 6R and BT 7R tested the protective effect (chemoprevention) of the anti-oestrogen tamoxifen and of the LH-RH agonist-antagonist leuprolide against  $\gamma$ -radiation carcinogenesis.

## Materials and methods

### A) $\gamma$ -radiation exposure conditions and irradiation facilities

The facilities for irradiation are located on the ground floor of the Castle of Bentivoglio (where the CRC/RF is located), inside a properly shielded irradiation room (bunker), 5 m x 4 m and 3 m high, communicating with the animal housing premises through a 5 x 4 m room, where the control board

**Table 3** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the carcinogenic effects of different doses, delivered by two different schedules, on male (M) and female (F) Sprague-Dawley rats, 6 weeks-old at the start of treatment and then kept alive until spontaneous death (Exp. BT 1R). Plan of the experiment

Group	Exposure	Treatment		Age at start (weeks)	Animals		
		Doses (rads)	Schedule		M	F	M+F
I	External	300	Single	6	211	205	416
II	External	300	Fractionated <sup>(a)</sup>	6	83	107	190
III	External	100	Single	6	318	301	619
IV	External	100	Fractionated <sup>(a)</sup>	6	126	133	259
V	External	10	Single	6	524	522	1046
VI	External	10	Fractionated <sup>(a)</sup>	6	220	215	435
VII <sup>(b)</sup>	–	0	–	6	514	537	1051

<sup>(a)</sup> The whole dose was subdivided into 10 shots, one every 4 weeks, starting at 6 weeks of age

<sup>(b)</sup> The control group is common to experiments BT 1R-4R

**Table 4** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the carcinogenic effects of different doses, delivered to pregnant breeder Sprague-Dawley rats at 12th day of pregnancy, as seen in male (M) and female (F) offspring kept alive until spontaneous death (Exp. BT 2R). Plan of the experiment

Group	Exposure	Treatment		Age at start	Animals (offspring)		
		Doses (rads)	Schedule		M	F	M+F
I	External to the female breeders (N. 100)	100	Single exposure	12 day embryos	286	289	575
II	External, to the female breeders (N. 150)	50	Single exposure	12 day embryos	363	365	728
III	External, to the female breeders (N. 250)	10	Single exposure	12 day embryos	737	759	1496
IV <sup>(a)</sup>	–	0	–		514	537	1051

<sup>(a)</sup> The control group is common to experiments BT 1R-4R

**Table 5** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the carcinogenic effects of different doses, delivered to male breeder Sprague-Dawley rats immediately before mating, as seen in male (M) and female (F) offspring kept alive until spontaneous death (Exp. BT 3R). Plan of the experiment

Group	Exposure	Treatment		Animals (offspring)
		Doses (rads)	Schedule	
I	External, to the male breeders (N. 100)	300	Single exposure	M: 154, F: 167, M+F: 321
II	External, to the male breeders (N. 150)	100	Single exposure	M: 401, F: 398, M+F: 799
III	External, to the male breeders (N. 250)	10	Single exposure	M: 743, F: 694, M+F: 1437
IV <sup>(a)</sup>	–	0	–	M: 514, F: 537, M+F: 1051

<sup>(a)</sup> The control group is common to experiments BT 1R-4R

**Table 6** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the carcinogenic effects of feed, irradiated with two dose levels of  $\gamma$ -radiation, supplied to breeder Sprague-Dawley rats from 12th day of pregnancy and then to male (M) and female (F) offspring for the life-span (Exp. BT 4R). Plan of the experiment

Group	Treatment (feed)			Age at start	Animals (offspring)		
	Exposure	Doses (Mrads) <sup>(a)</sup>	Schedule		M	F	M+F
I	Ingestive, to the female breeders (N. 125)	Feed irradiated with 4 Mrads	Life -span	12 day embryos	272	258	530
II	Ingestive, to the female breeders (N. 125)	Feed irradiated with 1 Mrads	Life -span	12 day embryos	292	317	609
III <sup>(b)</sup>	–	Feed not irradiated	Life-span		514	537	1051

<sup>(a)</sup>Mrads = Megarads<sup>(b)</sup>The control group is common to experiments BT 1R-4R**Table 7** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Experiment on the chemopreventive effects of Tamoxifen, administered with differing schedules, on  $\gamma$ -radiation carcinogenesis in female Sprague-Dawley rats, with particular regard to mammary carcinogenesis (life-span experiment) (Exp. BT 5R). Plan of the experiment

Group	Treatments						Animals		No.
	Exposure	$\gamma$ -radiation Dose (rads)	Schedule	Route <sup>(a)</sup>	Tamoxifen/water Dose <sup>(b)</sup> (mg/kg b.w.)	Schedule	Age at start of treatment $\gamma$ -radiation	Tamoxifen/water	
I	External	300	Single exposure	Ingestion	3.3	Once daily, 6 days weekly for 16 weeks	6	4	200
II	External	300	Single exposure	Ingestion	3.3	Once daily, 6 days weekly for 16 weeks	6	6	200
III	External	300	Single exposure	Ingestion	3.3	Once daily, 6 days weekly for life-span	6	6	200
IV	External	300	Single exposure	Ingestion	0	Once daily, 6 days weekly for life-span <sup>(c)</sup>	6	4	200

<sup>(a)</sup> By stomach tube<sup>(b)</sup> In water suspension<sup>(c)</sup> Water only**Table 8** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Experiment on the chemopreventive effects of Leuprolide, on  $\gamma$ -radiation carcinogenesis in female Sprague-Dawley rats, with particular regard to mammary carcinogenesis (life-span experiment) (Exp. BT 6R). Plan of the experiment

Group	Treatments						Animals		No.
	Exposure	$\gamma$ -radiation Dose (rads)	Schedule	Route <sup>(a)</sup>	Leuprolide/water Dose <sup>(b)</sup> (mg/kg b.w.)	Schedule	Age at start of treatment $\gamma$ -radiation	Leuprolide/water	
I	External	300	Single exposure	Injection	0.0625	Once every 28 days for 96 weeks	6	6	200
II	External	300	Single exposure	Injection	0	Once every 28 days for 96 weeks <sup>(c)</sup>	6	6	200

<sup>(a)</sup> By subcutaneous injection<sup>(b)</sup> In water suspension<sup>(c)</sup> Water only

**Table 9** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Experiment on the chemopreventive effects of Tamoxifen, on  $\gamma$ -radiation carcinogenesis in female Sprague-Dawley rats, with particular regard to mammary carcinogenesis (40 week experiment) (Exp. BT 7R, first part). Plan of the experiment

Group	Treatments						Animals		No.
	$\gamma$ -radiation			Tamoxifen/water			Age at start of treatment		
	Exposure	Dose (rads)	Schedule	Route <sup>(a)</sup>	Dose <sup>(b)</sup> (mg/kg b.w.)	Schedule	$\gamma$ -radiation	Tamoxifen/water	
I	External	300	Single exposure	Ingestion	0.10	Once daily, 6 days weekly for 40 weeks	56	56	160
II	External	300	Single exposure	Ingestion	0	Once daily, 6 days weekly for 16 weeks <sup>(c)</sup>	56	56	160
III	–	–	–	Ingestion	0	Once daily, 6 days weekly for 40 weeks <sup>(c)</sup>	–	56	160

<sup>(a)</sup> By stomach tube<sup>(b)</sup> In water suspension<sup>(c)</sup> Water only**Table 10** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Experiment on the chemopreventive effects of Leuprolide, on  $\gamma$ -radiation carcinogenesis in female Sprague-Dawley rats, with particular regard to mammary carcinogenesis (40 week experiment) (Exp. BT 7R, second part). Plan of the experiment

Group	Treatments						Animals		No.
	$\gamma$ -radiation			Leuprolide/water			Age at start of treatment		
	Exposure	Dose (rads)	Schedule	Route <sup>(a)</sup>	Dose <sup>(b)</sup> (mg/kg b.w.)	Schedule	$\gamma$ -radiation	Leuprolide/water	
I	External	300	Single exposure	Injection	0.0625	Once every 28 days for 40 weeks	56	56	160
II	External	300	Single exposure	Injection	0	Once every 28 days for 40 weeks	56	56	160
III	–	–	–	Injection	0	Once every 28 days for 40 weeks <sup>(c)</sup>	–	56	160

<sup>(a)</sup> By subcutaneous injection<sup>(b)</sup> In water suspension<sup>(c)</sup> Water only

and exposure monitoring facilities are set up. This room houses all the equipment needed to prepare animals for irradiation.

The radiation source is a therapy unit supplying Co 60, Theratron 780 type, with an activity of around 56 TBq (1500 Ci) as measured on October 12, 1991.

During treatment, animals are located on a turntable, placed under the radioactive source, at a distance of 120 cm. The turntable is made of a 1 cm thick plexiglass sheet with dimensions of 48 cm x 48 cm, supplied with grooves for the correct positioning of 10 plexiglass holders, with 1 cm thick walls, 20.8 cm long x 6.5 cm broad, inside which the animals to be irradiated are previously inserted and immobilized. The size of the radiation field at this distance is 46 cm x 46 cm, with an absorbed dose rate of about 0.21 Gy/min. The turntable is equipped with a pneumatic system for a rotation of 180° on its axis, at right angles to that of the irradiation beam, and is controlled from outside the bunker.

Dose measurements can be made with a Nuclear Enterprise dosimeter of 2571A type, with a 0.6 cc graphite ionization

chamber, calibrated in terms of dose absorbed to water with a 4% uncertainty.

All irradiation times designed to treat animals at the planned doses are determined by inserting the dosimeter into a life-size cylindrical plexiglass model of the animals to be treated.

Treatment at the required dose is divided into two equal irradiations, performed on the ventral and dorsal side of the animals, respectively. In these conditions the animals are treated by 2 opposite irradiation fields, with an almost homogenous dose distribution. Dosimeter differences due to the differing alignment of individual animals on the turntable are about 5%.

Possible sources of operator error in carrying out treatment may be incorrect selection of irradiation times, or non rotation of the turntable after the first part of treatment. To ensure correct execution of the treatment, two controls have been devised: 1) a dosimeter, irradiated together with the animals, is permanently inserted in the turntable; and 2) this dosimeter is located on the axis of the beam, in the lower part of the turntable, about 1 cm from its rotation axis, so that dose readings in the two positions must differ by more than 2%.

**B) Ordinary and irradiated feed**

The ordinary feed is provided by “Mangimificio COMER”, Bologna, and is as used in the CRC/RF for more than 25 years. The feed to be irradiated of the ordinary type is provided by “Mangimificio Piccioni”, Gessate (MI). The feed is irradiated by “Gammatom”, Guanzate (CO).

**C) Drugs and their administration**

The tamoxifen used is that marketed by Zeneca (now Astra Zeneca), under the commercial name of <sup>®</sup>Nolvadex. The drug is administered by stomach tube in 1 ml of water suspension, once daily, 6 days per week, while water alone is administered to control animals.

The leuprolide used is that marketed by Takeda, under the commercial name of <sup>®</sup>Enantone. The drug is suspended in water and administered by subcutaneous injection, once every 4 weeks, while water alone is injected into control animals.

**D) Animals**

The animals used in all the experimental projects are Sprague-Dawley rats from the same colony used for more than 30 years in the laboratories of the CRC/RF. The basic expected tumorigram and its fluctuations are based upon data derived from more than 15,000 historical controls. These animals do not develop specific malignant tumours with any undue incidence. For the specific purposes of this report, it must be stressed that

female Sprague-Dawley rats in our colony develop mammary carcinomas with an incidence of the same order as observed in women in industrialized countries, and with an equivalent age-distribution to that of women. All malignant and benign mammary tumours show the various typical morphological patterns and biological behaviour of human types and subtypes (Maltoni, 1982). Sprague-Dawley rats are particularly responsive to mammary carcinogenesis by ionizing radiation as shown by definitive experimental evidence.

For experiments BT 1R, BT 2R, BT 3R and BT 4R, since: 1) the animals for experiments BT 2R, BT 3R and BT 4R were predestined, since the exposure, or the start thereof, was to take place before birth; 2) the same control group was to be used; and 3) the results were to be as closely comparable as possible, the production of experimental animals took place as follows: a) breeders were distributed, family by family, in four groups (BT 1Rbr, BT 2Rbr, BT 3Rbr and BT 4Rbr) and the offspring of these were assigned to the respective experiments (Table 11); b) distribution of the brother and sister breeders among groups BT 1Rbr - BT 4Rbr was as homogenous as possible; c) mating was strictly out-bred in the breeder groups; it was synchronized among groups and lasted 72 hours; d) the size of breeder groups was dictated by the number of offspring required. All the male and female breeders were sacrificed respectively 6 weeks after birth and 1 week after the weaning of offspring. At sacrifice, systematic necropsy was performed

**Table 11** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Generation, destination and number of experimental animals

Identification	Group	Breeders (BT 1Rbr-4Rbr) <sup>(a)</sup>			Treatment (rads)	Offspring (experimental animals) (BT 1R, 2R, 3R, 4R)							
		No. of animals				Exp.	Group	No. of animals planned			No. of animals in experiment		
		M	F	M+F				M	F	M+F	M	F	M+F
BT 1Rbr	I	83	83	166	–	BT 1R	I	200	200	400	211	205	416
	II	37	37	74	–		II	80	80	160	83	107	190
	III	105	105	210	–		III	300	300	600	318	301	619
	IV	37	37	74	–		IV	120	120	240	126	133	259
	V	190	190	380	–		V	500	500	1000	524	522	1046
	VI	95	95	190	–		VI	200	200	400	220	215	435
	VII <sup>(a)</sup>	203	203	406	–		VII	500	500	1000	514	537	1051
Total	750	750	1500		Total	1900	1900	3800	1996	2020	4016		
BT 2Rbr	I	100	100	200	100	BT 2R	I	200	200	400	286	289	575
	II	150	150	300	50		II	300	300	600	363	365	728
	III	250	250	500	10		III	500	500	1000	737	759	1496
	IV <sup>(a)</sup>	203	203	406	–		IV	500	500	1000	514	537	1051
	Total	703	703	1406			Total	1500	1500	3000	1900	1950	3850
BT 3Rbr	I	100	100	200	300	BT 3R	I	200	200	400	154	167	321
	II	150	150	300	100		II	300	300	600	401	398	799
	III	250	250	500	10		III	500	500	1000	743	694	1437
	IV <sup>(a)</sup>	203	203	406	–		IV	500	500	1000	514	537	1051
	Total	703	703	1406			Total	1500	1500	3000	1812	1796	3608
BT 4Rbr	I	125	125	250	Freed irradiated with 4 Mrads <sup>(b)</sup>	BT 4R	I	250	250	500	272	258	530
	II	125	125	250	Feed irradiated with 1 Mrads		II	250	250	500	292	317	609
	III <sup>(a)</sup>	203	203	406			III	500	500	1000	514	537	1051
	Total	453	453	906			Total	1000	1000	2000	1078	1112	2190

<sup>(a)</sup>The control group is common to experiments BT 1R-4R<sup>(b)</sup>Mrads = Megarads

and specimens of pathological tissues and organs were taken for histopathological examination. Pathological lesions were few, due to the young age of the breeders.

For experiments BT 5R, BT 6R and BT 7R, animals were inbred (the ordinary experimental procedure in the CRC/RF laboratory). They were randomized litter by litter in order to have no more than 1 animal from each litter in the same group.

The animals of all experiments were weaned at 5 weeks of age and identified by ear punch. After weaning, animals received food and water *ad libitum*. They were housed 5 per cage, in makrolon cages with a solid top of stainless steel. A shallow layer of white wood shavings served as bedding. The animals were kept in a temperature-controlled laboratory at 19–20°C, with a 12-h light/dark alternation.

#### E) Conduct of experiments

- a) *Housing*: animals were kept in highly standardized environmental conditions, as usual in the laboratory of the CRC/RF over the last 25 years.
- b) *Duration of experiments*: in experiments BT 1R, BT 2R, BT 3R, BT 4R, BT 5R and BT 6R the animals were kept under control until spontaneous death (life-span experiments); in experiment BT 7R the animals were kept alive until the 96th week of age (truncated experiment).
- c) *Feed and water consumption*: in experiments BT 1R, BT 2R, BT 3R and BT 4R the daily consumption of feed and water were measured in a sample of 100 animals (50 males and 50 females) from each group and each experiment, at 4 week periods, from the age of 6 weeks until 110 weeks. In experiments BT 5R, BT 6R and BT 7R the consumption of feed and water was measured in all animals weekly from the start of the experiment for the first 13 weeks and then every 2 weeks until 110 weeks of age (life-span experiments), or until 96 weeks of age (truncated experiment).
- d) *Body weight*: in experiments BT 1R, BT 2R, BT 3R and BT 4R the body weight was registered from the age of 6 weeks every 4 weeks until 110 weeks of age, then every 8 weeks until the end of the experiment. In experiments BT 5R, BT 6R and BT 7R body weight was registered weekly from the start of the experiment for the first 13 weeks, then every 2 weeks until 96 weeks of age (truncated experiment) or until 110 weeks of age (life-span experiments), and thereafter, in the latter case, every 8 weeks until the end of the bio-phase.
- e) *Health control*: the health and behaviour of animals were controlled 3 times daily throughout the experiments.
- f) *Clinical control*: checking for pathological lesions, including mammary tumours (the most frequent lesion in females), was performed every 2 weeks throughout the experiments.
- g) *Necropsy*: a systematic gross examination was performed, and all pathological changes recorded. For histopathological examination, in addition to macroscopically observed pathological lesions (with a margin of surrounding normal tissue), the following tissues and organs were taken: skin, subcutaneous tissues, mammary gland, the brain, pituitary gland, Zymbal gland and cartilaginous ear ducts, salivary glands, Harderian glands, cranium, tongue, thyroid and parathyroids, pharynx, larynx, thymus, trachea, lung, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands,

oesophagus, stomach (fore and glandular), intestine (4 levels), bladder, prostate, uterus, ovaries, testes, interscapular fat pad, and subcutaneous, mediastinal and mesenteric lymph nodes.

- h) *Fixation and trimming*: all specimens were fixed in 70% alcohol, except for bones and other tissues of a bone-like consistency which were fixed in 10% formalin. The trimming was performed according to the CRC/RF standard operating procedures (SOP). All pathological tissues were trimmed in order to maintain a border of normal tissue around the lesions. As regards normal tissue and organs: 1) the decalcified cranium was trimmed at 5 levels from nose to occipital bone, including oral and nasal cavities and internal ear ducts; 2) parenchymal organs were dissected through the hilus to expose the widest surface; and 3) hollow organs were sectioned across the greatest diameter(s).
- i) *Histopathology*: the trimmed specimens were processed according to CRC/RF SOP. They were embedded in paraffin blocks; then 3–5  $\mu$ -sections were sliced, and routinely stained with haematoxylin-eosin. Histopathological evaluation was performed by the same group of pathologists on all tissues and organs. The same supervisor revised all lesions of oncological interest as well as any open to dubious interpretation. In the pathological diagnosis, all the pathologists used the same evaluation criteria and the same classification, long adopted at the CRC. The diagnoses are reported in the experimental registries.
- l) *Reports*: arranging and examining several hundred thousand slides will obviously require a long time. It has thus been decided to publish interim reports as sets of data become ready.

#### First results on mammary carcinogenesis (Exp BT 1R)

This report deals with results on carcinogenesis of the mammary gland in female rats from experiment BT 1R, exposed at 6 weeks of age to three different dose levels of  $\gamma$ -radiation, delivered in one shot (Table 3).

#### Relevant scientific information on mammary carcinogenesis from ionizing radiation

Ionizing radiation of different types and sources has proved to be carcinogenic for the mammary gland in women and experimental animals.

An increased risk of breast cancer has been observed among women exposed to ionizing radiation in various situations: during atom bomb explosions (Tokunaga *et al.*, 1994); when checked by repeated chest fluoroscopies (MacKenzie, 1965; Miller *et al.*, 1989; Boice *et al.*, 1991; Howe and McLaughlin, 1996); when undergoing radiotherapy for post partum mastitis (Shore *et al.*, 1986); and possibly/probably when treated with radiotherapy for controlateral breast cancer (Boice *et al.*, 1992) and for Hodgkin's disease (Hancock, Tucker and Hoppe, 1993). It has been reported that the same risk of breast cancer may be associated with radiation exposure from annual mammography in women over the age of 35 (Mettler *et al.*, 1996). Ionizing radiation causes mammary tumours in mice (Furth, 1959; Law, 1960; Cottier, 1961; Upton, 1961) and rats (Shellabarger *et al.*, 1957; Durbin *et al.*, 1958; Law, 1960).

Rats are the most suitable animal species for studying mammary carcinogenesis by radiation since they are responsive and develop very similar tumours to their human counterpart. The responsiveness in developing mammary tumours depends on the strain: Sprague-Dawley rats are among the most susceptible.

For a given radiation dose, neutrons are more tumorigenic than X-rays or  $\gamma$ -rays (Haran-Ghera *et al.*, 1959; Upton *et al.*, 1960).

In female Sprague Dawley rats, the incidence of mammary tumours appearing within the first year varies as a linear function of X-ray dose over the range 25-400 rads (Bond *et al.*, 1960). Animals irradiated with fractionated doses of  $\gamma$ -radiation prove to have a linear quadratic dose-response (van Bekkum *et al.*, 1986).

From a number of studies it emerges that mammary carcinogenesis in women, and above all experimental animals, when due to ionizing radiation, depends on a whole gamut of factors, such as: dose, genetic susceptibility (rat strain) (Russo and Russo, 1996), age at irradiation (Bartstra *et al.*, 1998), and hormonal factors (Segaloff and Maxfield, 1971).

The effect of low doses of ionizing radiation on mammary carcinogenesis is a crucial item, which still remains largely unsolved. It has been reported that in female Sprague-Dawley rats neutrons at a single dose as low as 0.1 rad "produced a significant increase of the tumour rate that corresponded to a forward shift of roughly 35 days of the spontaneous incidence" (Shellabarger, Chmelysky and Kellerer, 1980). In ACI female rats, on which the effect of ionizing radiation appears to be synergistic with diethylstilbestrol (DES) treatment (Segaloff and Maxfield, 1971; Shellabarger, Stone and Holtzman, 1976; Stone, Holtzman and Shellabarger, 1980), Shellabarger *et al.* (1982) reported that in DES-treated rats definite findings were observed at neutron doses as low as 0.01 Gy (1 rad).

Because of the limited number of animals in such experimental groups and various other experimental limitations, animal data on the effect of low doses of ionizing radiation on mammary carcinogenesis can only be considered "indicative" at the present time. For mammary carcinogenesis the statement by Baruch Modan (1992) on low-dose radiation carcinogenesis in general is more than ever relevant: "the question of concrete carcinogenic risk estimates following low dose radiation exposure will stay with us for years to come: it's there, but how much of it?"

## Results

The various mammary oncological lesions (Table 12) have been histopathologically evaluated and plotted.

The results are given in Tables 13-18.

A dose-correlated increase in fibroadenomas (total, without and with marked cellular hyperplasia) has been detected at the two highest doses (Table 13).

An increase in dysplasias (dysplasia in mammary glands and dysplastic fibroadenomas) has been observed in all treated groups, with an incidence inversely proportional to dose (Table 14). It may be that many such lesions have evolved into adenocarcinomas in the two groups treated at the highest doses.

Exposures to  $\gamma$ -radiation caused an increase in adenocarcinomas in all treated groups, and this is dose-response correlated. Anaplastic adenocarcinomas are seen only in the exposed groups (Table 15). A slight, non dose-correlated increase in total sarcomas (all types) has been observed in the three treated groups (Table 16).

Table 17 shows a clear-cut dose-related increase in the number per 100 animals of aggregated adenocarcinomas and their precursor glandular dysplasias.

**Table 12** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Mammary oncological lesions which have been evaluated

### Fibroadenomas

FA: without marked glandular hyperplasia  
FA+: with marked glandular hyperplasia

### Dysplasias

DMG: in mammary gland  
DFA: in fibroadenomas

### Adenocarcinomas

ADCA: ductal and lobular adenocarcinomas  
AADCA: anaplastic adenocarcinomas

### Sarcoma

LISA: liposarcomas  
OSA: other types of sarcoma

**Table 13** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Fibroadenomas

Group/ dose (rads)	No. of litters	No. of animals	Fibroadenomas (FA)/Fibroadenomas with glandular hyperplasia (FA+)																	
			FA						FA+						Total FA, FA+					
			Bearing litters		Bearing animals		Tumours		Bearing litters		Bearing animals		Tumours		Bearing litters		Bearing animals		Tumours	
			No.	%	No.	%	No.	/100 animals	No.	%	No.	%	No.	/100 animals	No.	%	No.	%	No.	/100 animals
I (300)	40	205	39	97.5	145	70.3	326	<b>159.0</b>	34	85.0	80	39.0	130	<b>63.4</b>	40	100.0	165	84.5	456	<b>222.4</b>
II (100)	58	301	54	93.1	162	53.8	266	<b>88.4</b>	48	82.7	107	35.5	165	<b>54.8</b>	58	100.0	217	72.1	441	<b>146.5</b>
III (10)	99	522	85	85.9	222	42.5	367	<b>70.3</b>	57	57.6	105	20.1	127	24.3	92	92.9	269	51.5	494	94.6
IV (0)	100	537	88	88.0	227	42.3	307	<b>57.2</b>	69	69.0	147	27.4	206	<b>32.4</b>	92	92.0	304	56.6	513	<b>95.5</b>

**Table 14** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Dysplasias in mammary glands/dysplastic fibroadenomas

Group/ dose (rads)	No. of litters	No. of animals	Dysplasias in mammary glands (DMG)/Dysplastic fibroadenomas (DFA)																	
			DMG						DFA						Total DMG, DFA					
			Bearing litters		Bearing animals		No. /100 animals	Bearing litters		Bearing animals		No. /100 animals	Bearing litters		Bearing animals		No. /100 animals			
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%				
I (300)	40	205	3	7.5	4	1.9	4	<b>1.9</b>	13	32.5	19	9.3	20	<b>9.7</b>	14	35.0	23	11.2	24	<b>11.7</b>
II (100)	58	301	13	22.4	13	4.3	13	<b>4.3</b>	32	55.2	36	12.0	47	<b>15.6</b>	37	63.8	43	14.3	60	<b>19.9</b>
III (10)	99	522	34	34.3	48	9.2	69	<b>13.2</b>	47	47.5	68	13.0	80	<b>15.3</b>	59	59.6	96	8.4	149	<b>28.5</b>
IV (0)	100	537	5	5.0	5	0.9	5	<b>0.9</b>	25	25.0	30	5.6	35	<b>6.5</b>	29	29.0	35	6.5	40	<b>7.4</b>

**Table 15** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Adenocarcinomas

Group/ dose (rads)	No. of litters	No. of animals	Adenocarcinomas (ADCA)/Anaplastic adenocarcinomas (AADCA)																	
			ADCA						AADCA						Total ADCA, AADCA					
			Bearing litters		Bearing animals		Tumours /100 animals	Bearing litters		Bearing animals		Tumours /100 animals	Bearing litters		Bearing animals		Tumours /100 animals			
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%				
I (300)	40	205	36	90.0	80	39.0	108	<b>52.7</b>	4	10.0	4	1.9	5	<b>2.4</b>	37	92.5	82	40.0	113	<b>55.1</b>
II (100)	58	301	46	79.3	77	25.6	107	<b>35.5</b>	1	1.7	1	0.3	1	<b>0.3</b>	47	81.0	78	25.9	108	<b>35.9</b>
III (10)	99	522	50	50.5	73	13.9	96	<b>18.4</b>	1	1.0	2	0.4	3	<b>0.6</b>	50	50.5	74	14.2	99	<b>19.0</b>
IV (0)	100	537	42	42.0	68	12.6	86	<b>16.0</b>	0	-	0	-	0	-	42	42.0	68	12.6	86	<b>16.0</b>

**Table 16** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Sarcomas

Group/ dose (rads)	No. of litters	No. of animals	Liposarcomas (LISA)/Other sarcomas (OSA)																	
			LISA						OSA						Total LISA, OSA					
			Bearing litters		Bearing animals		Tumours /100 animals	Bearing litters		Bearing animals		Tumours /100 animals	Bearing litters		Bearing animals		Tumours /100 animals			
			No.	%	No.	%		No.	%	No.	%		No.	%	No.	%				
I (300)	40	205	2	5.0	3	1.5	3	1.5	1	2.5	1	0.5	1	0.5	2	5.0	3	1.5	4	<b>1.9</b>
II (100)	58	301	2	3.4	2	0.7	2	0.7	4	6.9	4	1.3	4	1.3	6	10.3	6	2.0	7	<b>2.0</b>
III (10)	99	522	6	6.1	6	1.1	7	1.3	8	8.0	8	1.5	9	1.7	13	13.1	13	2.5	16	<b>3.1</b>
IV (0)	100	537	5	5.0	5	0.9	5	0.9	2	2.0	2	0.4	2	0.4	7	7.0	7	1.3	7	<b>1.3</b>

Table 18 presents the number per 100 animals of the different oncological lesions observed.

### Discussion

Our data confirm that Sprague-Dawley rats are a suitable animal model for studying mammary carcinogenesis in general, with particular reference to ionizing radiation carcinogenesis and more specifically to  $\gamma$ -radiation; they show, furthermore, that mega-experiments produce clear-cut quantitative results.

They prove definitively that  $\gamma$ -radiation is carcinogenic for the mammary gland and that all three doses tested are effective. A sharp dose-response relationship emerges for mammary adenocarcinomas, and mammary adenocarcinomas aggregated with glandular dysplasias. The increase in mammary adenocarcinomas plus their precursor glandular dysplasias is very marked even at the lowest dose tested. These results call for reflection with reference to all manner of low radiation exposure scenarios.

Since the animals were randomized by breeders, and since all the families of the experiments reported can be identified, we are now evaluating the synergism between familial factors and  $\gamma$ -radiation exposure with a view to measuring the effect of  $\gamma$ -radiation above all at the lowest dose and on the most susceptible part of the population exposed.

**Table 17** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Number per 100 animals of adenocarcinomas and their precursors (DMG and DFA) aggregated

Group/dose (rads)	Per 100 animals
I (300)	<b>66.8</b>
II (100)	<b>55.8</b>
III (10)	<b>47.5</b>
IV (0)	<b>23.4</b>

**Table 18** - CRC/RF project on carcinogenicity of  $\gamma$ -radiation. Mega-experiment on the mammary carcinogenic effects of different doses, delivered in one shot, to female Sprague-Dawley rats (BT 1R). Number per 100 animals of fibroadenomas (FA and FA+), dysplasias (DMG and DFA), adenocarcinomas and sarcomas

Group dose (rads)	FA and FA+ per 100 animals	DMG and DFA per 100 animals	Adenocarcinomas per 100 animals	Adenocarcinomas and sarcomas per 100 animals
I (300)	<b>224.4</b>	<b>11.7</b>	<b>55.1</b>	<b>57.0</b>
II (100)	<b>146.5</b>	<b>19.9</b>	<b>35.9</b>	<b>39.2</b>
III (10)	94.6	<b>28.5</b>	<b>19.0</b>	<b>22.0</b>
IV (0)	<b>95.5</b>	<b>7.4</b>	<b>16.0</b>	<b>17.3</b>

### Conclusion

These first results on mammary carcinogenesis relating to the first of the 7 experiments performed already indicate the need for our project.

As and when results become available from the various parts of the whole project, they will be published.

In consideration of the relevance to public health of the results of the project, we wish it to be known that we are open to discussion of the data and to collaboration with scientifically interested parties.

### Acknowledgement

The Authors are indebted to Dr. Arthur Upton (USA), Dr. David Hoel (USA) and Dr. Vito Foà (Italy), members of the Quality Assurance Unit (QAU) for their input in planning and conducting our experimental project.

### References

- Bartstra R.W., Bentvelzen P.A.J., Zoetelief J., *et al.*: Induction of mammary tumors in rats by single-dose gamma irradiation at different ages. *Radiat. Res.*, **150**, 442-450, 1998.
- Boice J.D. Jr., Harvey E.B., Blettner M., *et al.*: Cancer in the contralateral breast after radiotherapy for breast cancer. *N. Engl. J. Med.*, **326**, 781-785, 1992.
- Boice J.D. Jr., Preston D., Davis F.G., *et al.*: Frequent chest X-ray fluoroscopy and breast cancer incidence among tuberculosis patients in Massachusetts. *Radiat. Res.*, **125**, 214-222, 1991.
- Bond V.P., Cronkite E.P., Lippincott S.W., *et al.*: Studies on radiation-induced mammary gland neoplasia in the rat. III. Relation of the neoplastic response to dose of total-body radiation. *Radiat. Res.*, **12**, 276-285, 1960.
- Clunet J.: Recherches expérimentales sur le tumeurs malignes. Steinheil, Paris, 1910.
- Cottier H.: Strahlenbedingte Lebensverkürzung. Springer, Berlin, 1961.
- Curtis R.E., Boice J.D. Jr., Stovall M., *et al.*: Risk of leukemia after chemotherapy and radiation treatment for breast cancer. *N. Engl. J. Med.*, **326**, 1741-1751, 1992.
- Durbin P.W., Willet Asling C., Johnston M.E., *et al.*: The induction of tumors in the rat by Astatine-211. *Radiat. Res.*, **9**, 378-397, 1958.
- Friebe A.: Demonstration eines Cancroids des rechten Handrückens, das sich nach langdauernder Einwirkung von Röntgenstrahlen entwickelt hatte. *Fortschr. Röntgenstr.*, **6**, 106, 1902.
- Furth J.: Radiation neoplasia and endocrine system. In: Radiation biology and cancer, 7-25. University of Texas Press, Austin, TX, 1959.

- Hancock S.L., Tucker M.A., and Hoppe R.T.: Breast cancer after treatment of Hodgkin's disease. *J. Natl. Cancer Inst.*, **85**, 25-31, 1993.
- Haran-Ghera N., Furth J., Buffett R.F., *et al.*: Studies on the pathogenesis of neoplasms by ionizing radiation. II. Neoplasms of endocrine organs. *Cancer Res.*, **19**, 1181-1187, 1959.
- Härting F.H., and Hesse W.: Lung cancer, the disease of miners in the Schneeberg mines. *Vierteljahreschrift für gerichtliche medizin*, **30**, 296-309, 1879.
- Howe G.R., and McLaughlin J.: Breast cancer mortality between 1950-1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study and a comparison with breast cancer mortality in the atomic bomb survivors study. *Radiat. Res.*, **145**, 694-707, 1996.
- Lacassagne A.: Les cancers produits par les rayonnements corpusculaires. Mécanisme présumable de la cancérisation par les rayons. Hermann et C., Paris, 1945a.
- Lacassagne A.: Cancer produit par les rayonnements electromagnetiques. Hermann et C., Paris, 1945b.
- Land C.E., Hayakawa N., Machado S., *et al.*: A case-control interview study of breast cancer among Japanese A-bomb survivors. I. Main effects. *Cancer Causes Control*, **5**, 157-165, 1994.
- Law L.W.: Radiation carcinogenesis. *Adv. Biol. Med. Physics*, **3**, 295-342, 1960.
- Lazarus-Barlow W.S.: An attempt at the experimental production of carcinoma by means of radium. *Proc. Roy. Soc. Med. (Sect. Pathol.)*, **11**, 1-17, 1918.
- MacKenzie I.: Breast cancer following multiple fluoroscopies. *Br. J. Cancer*, **19**, 1-8, 1965.
- MacMahon H.E., Murphy A.S., and Bates M.I.: Endothelial-cell sarcoma of liver following thorotrast injection. *Am. J. Pathol.*, **23**, 585-613, 1947.
- Maltoni C.: Il contributo della cancerogenesi sperimentale alla conoscenza degli agenti causali, della storia naturale e del controllo della crescita del carcinoma mammario. *Acta Oncol.*, **3**, 97-112, 1982.
- March H.C.: Leukemia in radiologists. *Radiology*, **4**, 275-278, 1944.
- Martland H.S.: The occurrence of malignancy in radioactive workers. *Am. J. Cancer*, **25**, 2435-2516, 1931.
- Martland H.S., and Humphries R.E.: Osteogenic sarcoma in dial painters using luminous paint. *Arch. Pathol.*, **7**, 406-417, 1929.
- Mettler F.A., Upon C.A., Kelsey C.A., *et al.*: Benefits versus risks from mammography. *Cancer*, **77**, 903-909, 1996.
- Miller A.B., Howe G.R., Sherman G.J., *et al.*: Mortality from breast cancer after irradiation during fluoroscopic examinations in patients being treated for tuberculosis. *N. Engl. J. Med.*, **321**, 1285-1289, 1989.
- Modan B.: Low-dose radiation carcinogenesis. *Eur. J. Cancer*, **28A**, 1010-1012, 1992.
- National Research Council, Committee on the Biological Effects of Ionizing Radiation: Health effects of exposure to radon (BEIR IV). National Academy Press, Washington, DC, 1998.
- Oberling C., et Guérin M.: Action du thorotrast sur le sarcome de Jensen du rat blanc. *Bull. Assoc. Franc. Etude Cancer*, **22**, 469-489, 1933.
- Rajewsky B.: Bericht über die Schneeberger Untersuchungen. *Z. Krebsforschung*, **49**, 315-340, 1939.
- Rajewsky B., Schraub A., und Kahlau G.: Experimentelle Geschwülsterzeugung durch Einatmung von Radiumemanation. *Naturwissenschaften*, **31**, 170-171, 1943.
- Ron E.: Ionizing radiation and cancer risk: evidence from epidemiology. *Radiat. Res.*, **150** (suppl.), S30-S41, 1998.
- Rostoski O., Saupe E., and Schmorl G.: Die Bergkrankheit der Erzbergleute in Schneeberg in Sachsen. *Z. Krebsforschung*, **23**, 360-384, 1926.
- Russo I.H., and Russo J.: Mammary gland neoplasia in long-term rodent studies. *Environ. Health Perspect.*, **104**, 938-967, 1996.
- Segaloff A., and Maxfield W.S.: The synergism between radiation and estrogen in the production of mammary cancer in the rat. *Cancer Res.*, **31**, 166-168, 1971.
- Shellabarger C.J., Chmelevsky D., and Kellerer A.M.: Induction of mammary neoplasms in the Sprague-Dawley rat by 430-keV neutrons and X-rays. *J. Natl. Cancer Inst.*, **64**, 821-833, 1980.
- Shellabarger C.J., Chmelevsky D., Kellerer A.M., *et al.*: Induction of mammary neoplasms in the ACI rat by 430-keV neutrons, X-rays, and diethylstilbestrol. *J. Natl. Cancer Inst.*, **69**, 1135-1146, 1982.
- Shellabarger C.J., Cronkite E.P., Bond V.P., *et al.*: The occurrence of mammary tumours in the rat after sublethal whole-body irradiation. *Radiat. Res.*, **6**, 501-511, 1957.
- Shellabarger C.J., Stone J.P., and Holtzman S.: Synergism between neutron radiation and diethylstilbestrol in the production of mammary adenocarcinomas in the rat. *Cancer Res.*, **36**, 1019-1022, 1976.
- Shore R.E., Hildreth N., Woodard E., *et al.*: Breast cancer among women given X-ray therapy for acute post-partum mastitis. *J. Natl. Cancer Inst.*, **77**, 689-696, 1986.
- Stewart A.M., Webb J., and Hewitt D.: A survey on childhood malignancies. *Br. Med. J.*, **i**, 1495-1508, 1958.
- Stone J.P., Holtzman S., and Shellabarger C.J.: Synergistic interactions of various doses of diethylstilbestrol and X-irradiation on mammary neoplasia in female ACI rats. *Cancer Res.*, **40**, 3966-3972, 1980.
- Thompson D.E., Mabuchi K., Ron E., *et al.*: Cancer incidence in atomic bomb survivors. Part II. Solid tumors, 1958-1987. *Radiat. Res.*, **137** (suppl.), S17-S67, 1994.
- Tokunaga M., Land C.E., Tokuoka S., *et al.*: Incidence of female breast cancer among atomic bomb survivors, 1950-1985. *Radiat. Res.*, **138**, 209-223, 1994.
- Upton A.C.: Radiation as an etiologic factor. In: *Proc. 4th Natl. Cancer Conf. Univ. of Minnesota, Minneapolis*, 63-69. Lippincot, Philadelphia, PA, 1961.
- Upton A.C.: Radiation carcinogenesis. In: H. Busch: *Methods in cancer research*, Vol. IV, 53-82. Academic Press, New York-London, 1968.
- Upton A.C., Albert R.E., Burns F., *et al.*: *Radiation carcinogenesis*. Elsevier, New York, 1986.
- Upton A.C., Kimball A.W., Furth J., *et al.*: Some delayed effects of atom-bomb radiation in mice. *Cancer Res.*, **20**, 1-92, 1960.
- van Bekkum D.W., Broerse J.J., Hennen L.A., *et al.*: The gene transfer-misrepair hypothesis of radiation carcinogenesis tested for induction of mammary tumors in rats. *Leuk. Res.*, **10**, 761-765, 1986.
- Wolbach S.R.: The pathological histology of chronic X-ray dermatitis and early X-ray carcinoma. *J. Med. Res.*, **21**, 415-449, 1909.