

# Mega-experiments to Identify and Assess Diffuse Carcinogenic Risks

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**ABSTRACT:** Diffuse carcinogenic risks, that is, those of low potency involving large areas of population and sometimes all mankind, pose a serious public health problem. Controlling these risks might help to reduce the incidence of, and mortality from, cancer. Because of their low expected carcinogenic potential, these risks are difficult to expose or assess. Epidemiologic investigation is of limited use in this field and yields its data too late to be useful. Experimental studies offer the only possible approach for assessing such risks. To increase experimental sensitivity and consistency of results, mega-experiments must be designed. That is, experiments that use a large number of animals with a well-known basic tumorigram, that extend the exposure and the biophase for as long as possible, that carefully observe the effects, and that are performed with suitable standardized methods. In the last 15 years the Ramazzini Foundation, in its Cancer Research Center at Bentivoglio, has conducted or planned five mega-experiments. Initial results indicate the great potential of these methods for identifying and assessing diffuse risks.

## EXOGENOUS CARCINOGENIC AGENTS POSE A MAJOR HEALTH PROBLEM WITH A NEED FOR PRIMARY PREVENTION

It is a fact that the majority of tumors are caused by exogenous carcinogenic agents of natural or industrial origin that are present in the general and/or working environment, and/or that are linked to human life-styles. It is also a fact that industrial (man-made) carcinogens have increased during the last few decades as a result of the expansion of industry and life-styles linked to industrial development. Furthermore, environmental carcinogens, since they are exogenous and largely man-made, could in principle be removed. Thus, controlling these carcinogens (primary prevention) offers a means to contain them and, hence, lower the incidence of, and mortality from cancer. It must be stressed, however, that this preventive strategy is at present pursued far less than is needed.

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## IDENTIFICATION AND ASSESSMENT OF EXOGENOUS RISKS AS A NECESSARY PREREQUISITE TO PRIMARY PREVENTION

Primary prevention calls for the identification of carcinogenic risks as a necessary prerequisite. The identification of exogenous carcinogenic agents and the assessment of the risks they represent is, therefore, a crucial area of research aimed at controlling cancer. Studies and research in this area have evolved historically, and now they must be prepared to face new challenges.

### *The First Three Eras*

*First Era.* Carcinogenic agents were discovered by skilful observation of a self-emerging increase in certain tumors among population groups heavily exposed to strong carcinogens. Classic examples are: carcinoma of the scrotum in chimney-sweeps exposed to coal combustion products,<sup>1</sup> carcinoma of the lung in uranium miners exposed to radon,<sup>2,3</sup> carcinoma of the skin in sailors overexposed to sunlight,<sup>4</sup> carcinoma of the bladder in dyestuff industry workers exposed to aromatic amines,<sup>5</sup> and carcinoma of the skin among radiologists.<sup>6,7</sup> Some of these observations were subsequently confirmed by laboratory experiments in important, though limited, animal studies. For example, local treatment with soot extracts was found to cause skin carcinomas in mice<sup>8</sup> and administration of the aromatic amine,  $\beta$ -naphthylamine, was shown to induce bladder carcinomas in dogs.<sup>9</sup>

*Second Era.* The carcinogenic agents were identified by means of planned epidemiologic investigations (usually commenced as a result of medical observation of an unusual frequency of specific tumors) and also by studies on experimental animals. The epidemiologic investigations studied essentially high risk situations and did not attempt quantitative risk assessment as their primary goal. Experimental studies were continued, more with a view to confirming the positive epidemiology results, than to discover new factors or agents of risk. Only a few experimental studies provided quantitative data that could be used in public health regulations. During this era experiments were carried out on small numbers of animals, the biophase period was arbitrary, and the conduct of experiments was not codified or controlled. Paradigms for studies in this period are furnished by: (1) the epidemiologic investigations on leukemia among radiologists,<sup>10,11</sup> carcinoma of the nasal and paranasal cavities in workers exposed to nickel,<sup>12</sup> carcinoma of the bladder in workers exposed to aromatic amines,<sup>13-15</sup> and carcinoma of the lung in workers exposed to chromium<sup>16</sup>; and (2) experimental studies that demonstrated the carcinogenicity of benzidine,<sup>17</sup> nickel,<sup>18,19</sup> and chromium<sup>20</sup> in animals.

In both the first and second eras, the studies allowed only for the detection of major carcinogenic effects. In several cases, because of the nature of the carcinogens studied (such as nondiffusible chemical compounds that tended to concentrate in certain anatomical regions), these studies generated results suggesting that, for the various carcinogenic agents, there were specific target organs that varied from agent to agent.

*Third Era.* This era is characterized by important new facts and ideas. Epidemiology research became more adequate and was statistically based. It was geared to identifying the tumorigenic effects of exogenous carcinogens, not for a single specific tissue or organ, but for various anatomical sites of the human body. It also

aimed at quantifying the risk in terms of the dose-response relationship. Classic examples are found in the epidemiologic investigations of insulators in New York State exposed to asbestos,<sup>21</sup> and of the survivors of atom bombs in Nagasaki and Hiroshima.<sup>22</sup>

During the same period several studies demonstrated the potential of experimental bioassays as a tool for: (1) predicting the carcinogenicity of exogenous agents, particularly those of industrial origin; (2) providing information on the potential carcinogenic effects at various anatomical sites; and (3) providing quantitative risk assessments as a basis for regulatory action. The carcinogenicity of vinyl chloride provides a classic example. In this case experimental research led not only to prediction of the carcinogenicity of the compound and identification of its various target tissues and organs, but also provided quantitative data on carcinogenicity. This helped in making regulatory decisions on the permitted exposure levels.<sup>23,24</sup> These prerequisites form a basis for the two major projects employing carcinogenicity bioassays that started about 30 years ago and are still in progress: the project of the National Toxicology Program (NTP)<sup>25,26</sup>, and that of the European Ramazzini Foundation of Oncology and Environmental Sciences, at its Cancer Research Center (CRC) in the Castle of Bentivoglio/BT (Bologna).<sup>27,28</sup>

Epidemiologic and experimental studies conducted in this period have also indicated that, in humans and in animals, different carcinogenic agents may exert synergistic effects, such as is the case with the association of tobacco smoke and asbestos in humans,<sup>29</sup> and with various chemical agents in rodents.<sup>30</sup>

#### *The Present Fourth Era and its New Challenges*

The *fourth era* is just commencing. It is the result of new scientific knowledge and of newly emerging problems. Previous eras broadly evaluated the carcinogenicity of single agents, often known to be toxic and frequently shown to be strong carcinogens, by means of exposure to high doses. Apart from some pioneer research of the third era, studies generally measured carcinogenic effects on the basis of an increase in specific tumors. Both the scenario and the objective have now changed.

Basic and applied carcinogenic research has shown that: (1) Most carcinogenic agents are multipotential, that is, they may induce tumors of different types, in different tissues and organs. The types of induced tumors and their relative ratio may vary according to the type of exposure and host biological factors. (2) The most immediate and important (public health) parameter to employ in assessing carcinogenic effect is, therefore, represented by the total number of observed malignant tumors, whatever their type. (3) Carcinogenic agents with different natures, when delivered individually or in mixtures to the same organism in various sequences, may exert cumulative and multiplicative effects (syncarcinogenesis). (4) Because of the type of agent or use of a low dose, it is difficult to detect weak carcinogenic risks, due either to exposure to a single agent or to multiple agents or mixtures. This may only be possible by increasing the size of the human and animal populations studied and the duration of observation (epidemiologic follow up and experimental biophase, respectively). At the same time, new public health and social targets have become well-defined.

It is clear that, nowadays, risks must always be evaluated, not in terms of a single causal agent or a single tumor, but in terms of a multiplicity of agents that, singly or

together, may determine multiple tumors (total risk–total burden). It is increasingly considered necessary: (1) to define the levels of risk in terms of total burden following marked exposure to strong carcinogenic agent(s), involving limited groups of individuals; (2) to define the effects at different exposure doses to single or multiple agents or mixtures of agents suspected to be carcinogenic, thereby arriving at a quantitative risk assessment; and (3) to deeply reconsider the potential carcinogenic risk of some forms of historical exposure hitherto considered innocuous in the absence of any specific contrary study or evidence. In our opinion, the new and most important problems of environmental and industrial carcinogenesis are expansion, identification, assessment, and control of diffuse carcinogenic risks.

#### **DEFINITION AND DIMENSION OF THE PROBLEM AND METHODOLOGICAL APPROACHES FOR RISK IDENTIFICATION AND ASSESSMENT OF DIFFUSE CARCINOGENIC RISKS**

Diffuse carcinogenic risk is defined as the exposure to single or multiple agents or mixtures that are expected to have limited carcinogenic potential because of the agent type (weak carcinogen) and/or dose/concentration (low), but that involve large groups of the population—in some cases, all of mankind. Probably, this type of exposure in quantitative terms contributes more to the worldwide increase in incidence of tumors than do strong carcinogenic risks involving limited categories of the population.

Diffuse carcinogenic risks are difficult to identify and assess, let alone control. At present this problem is underestimated, or even ignored. To identify and assess diffuse carcinogenic risks, medical science falls back on epidemiology and experimental tools. Faced with this new challenge, epidemiologic research must be made as *powerful* as possible by adjusting its programs, dimensions, and methodologies. However, one must take into account that, when dealing with weak carcinogenic potential and multiple confounding factors, epidemiology has only a limited capacity for identifying and quantifying diffuse risks as defined here. Moreover, epidemiology provides delayed results, and by the time they are available, preventive strategy is long overdue.

To expose low carcinogenic risks, the experiments envisaged must possess the following characteristics: (1) as far as possible they must reproduce the various conditions of human exposure; (2) they must include large groups of animals in order to express variations in the effects more sharply; (3) they must be protracted for the lifespan of the animals, to allow for maximum emergence of all latent neoplastic potentialities; and (4) they must likewise evaluate all the neoplastic and non-neoplastic pathologies, since the latter may be complementary or may interfere with the incidence of the former. Furthermore, these experiments must be conducted on animals with a spontaneous neoplastic pathology that is as similar as possible to the human equivalent (and, therefore, not be characterized by an *unreasonable* incidence of particular types of tumor). They must also be performed under highly standardized conditions. One important, perhaps decisive, aspect is the availability of historical data on spontaneous pathology in the animal systems employed, collected under the same standardized conditions as for the proposed experimental studies, in order to distin-

guish induced pathology from that expected. Experiments of this kind, in particular concerning the size of experimental groups, are called *mega-experiments*.

Positive results from these studies serve to identify the risk and to measure the level of that risk. Negative results do not necessarily mean no risk, but they do serve to determine the existence of a *safeguard* limit.

In the face of a proposal to activate mega-experiments of this type for evaluating diffuse risks (a task of great social importance), objections of an economic nature have been raised by various parties including, strangely enough, scientists working in the biomedical field. To these objections one may answer that the cost is considerable if compared with the economic resources allocated to biomedical studies in general, and to the research on cancer prevention in particular (which are unfortunately minimal and in no way compare with the budget for technological research). The cost, by contrast, becomes insignificant when compared with: (1) the consequences of pathology (even in economic terms) that may derive from ongoing particular risk situations; (2) the cost of health programmes of limited efficacy, which in several cases are promoted for clinical control; and (3) more specifically, the enormous business turnover and income, in the case of many industrial technologies and products.

The age of mega-experiments has already started, with the experimental projects of the Cancer Research Center of the Ramazzini Foundation (CRC/RF).

#### **GENERAL DESCRIPTION, PLANS, AND SOME EARLY RESULTS FROM MEGA-EXPERIMENTS AT THE RAMAZZINI FOUNDATION CANCER RESEARCH CENTRE TO IDENTIFY DIFFUSE CARCINOGENIC RISKS**

A wide-ranging program of experimental mega-projects (in some instances including several mega-experiments) to evaluate the effects of diffuse carcinogenic risks was started in 1985 by the CRC/RF. To date five projects have been undertaken, or are planned, that aim to study: (1) the carcinogenicity of vitamins A, C, and E; (2) the carcinogenicity of compounds that potentially migrate into mineral water from PVC bottles; (3) the carcinogenic effects of various doses of ionizing  $\gamma$ -radiation, with particular regard to low doses; (4) the carcinogenicity of extremely low frequency electromagnetic fields, sinusoidal-50 Hz magnetic fields (S-50 Hz MF); and (5) the carcinogenic effects of radiofrequency and microwave electromagnetic fields, 1.8 GHz-GSM microwave electromagnetic emissions (1.8 GHz-Mw). Because of the nature of the agents and/or because of the low doses tested, all such exposures may be expected to represent low risks and yet a large part of the population are nowadays exposed to these agents.

##### ***Basic Methodology of the CRC/RF Mega-Experiments***

The routes of treatment reproduce the human exposure scenario. The doses tested include those to which humans may be exposed. All of these mega-experiments are being performed on Sprague-Dawley rats from the same colony used for more than 20 years. Data are available on about 15,000 historical controls kept under control for their life-span. The data include individual pedigree and behavioral, clinical, and pathologic observations. The mega-experiments are extended over the lifespan of the

**TABLE 1 (Part I). Plan of first experimental project to evaluate the carcinogenic effects of various levels of vitamin A (retinol palmitate and acetate) administered in the diet, to male (M) and female (F) Sprague-Dawley rats**

Vitamin	Experiment		Vitamin levels in the feed			Duration of diet regimen		Animals			
	N	Identification	A (iu) <sup>a</sup>	C (mg) <sup>a</sup>	E (mg) <sup>a</sup>	Age at start	Groups	M	F	M + F	
A	1	BT 8002	150,000	30	75	LS <sup>b</sup>	12 day embryos <sup>c</sup>	I	110	110	220
			75,000	30	75	LS		II	110	110	220
			16,900	30	75	LS		III	110	110	220
			3,900	30	75	LS		IV	110	110	220
			3,900	5	35	LS		V	110	110	220
	2	BT 8004	150,000	30	75	13 weeks <sup>d</sup>	12 day embryos <sup>c</sup>	I	100	100	200
			75,000	30	75	13 weeks <sup>d</sup>		II	100	100	200
			16,900	30	75	LS		III	110	110	220
			3,900	30	75	13 weeks <sup>d</sup>		IV	100	100	200
			3,900	5	35	13 weeks <sup>d</sup>		V	100	100	200
	3	BT 8001	150,000	30	75	LS	6 weeks	I	210	210	420
			75,000	30	75	LS		II	210	210	420
			16,900	30	75	LS		III	210	210	420
			3,900	30	75	LS		IV	210	210	420
			3,900	5	35	LS		V	210	210	420
4	BT 8003	150,000	30	75	LS	52 weeks	I	100	100	200	
		75,000	30	75	LS		II	100	100	200	
		16,900	30	75	LS		III	100	100	200	
		3,900	30	75	LS		IV	100	100	200	
		3,900	5	35	LS		V	100	100	200	

<sup>a</sup>Per Kg of feed.<sup>b</sup>LS, lifespan, from the commencement of the experiment until spontaneous death.<sup>c</sup>Administered to pregnant mothers.<sup>d</sup>The regimen is then followed by diet with historical vitamin levels, until spontaneous death.

**TABLE I (Part II). Plan of first experimental project to evaluate the carcinogenic effects of various levels of vitamin C administered in the diet, to male (M) and female (F) Sprague-Dawley rats**

Vitamin	Experiment		Vitamin levels in the feed			Duration of diet regimen	Age at start	Animals			
	N	Identification	A (iu) <sup>a</sup>	C (mg) <sup>a</sup>	E (mg) <sup>a</sup>			Groups	M	F	M + F
C	5	BT 8102	16,900	2,000	75	LS <sup>b</sup>	12 day embryos <sup>c</sup>	I	110	110	220
			16,900	30	75	LS		II	110	110	220
			16,900	5	75	LS		III	110	110	220
			3,900	5	35	LS		IV	110	110	220
	6	BT 8104	16,900	2,000	75	13 weeks <sup>d</sup>	12 day embryos <sup>c</sup>	I	100	100	200
			16,900	30	75	LS		II	110	110	220
			16,900	5	75	13 weeks <sup>d</sup>		III	100	100	200
			3,900	5	35	13 weeks <sup>d</sup>		IV	100	100	200
	7	BT 8101	16,900	2,000	75	LS	6 weeks	I	110	110	220
			16,900	30	75	LS		II	210	210	420
			16,900	5	75	LS		III	110	110	220
			3,900	5	35	LS		IV	210	210	420
	8	BT 8103	16,900	2,000	75	LS	52 weeks	I	100	100	200
			16,900	30	75	LS		II	100	100	200
			16,900	5	75	LS		III	100	100	200
			3,900	5	35	LS		IV	100	100	200

<sup>a</sup>Per Kg of feed.

<sup>b</sup>LS, lifespan, from the commencement of the experiment until spontaneous death.

<sup>c</sup>Administered to pregnant mothers.

<sup>d</sup>The regimen is then followed by diet with historical vitamin levels, until spontaneous death.

**TABLE 1 (Part III). Plan of first experimental project to evaluate the carcinogenic effects of various levels of vitamin E administered in the diet, to male (M) and female (F) Sprague-Dawley rats**

Vitamin	Experiment		Vitamin levels in the feed			Duration of diet regimen	Age at start	Animals			
	N	Identification	A (iu) <sup>a</sup>	C (mg) <sup>a</sup>	E (mg) <sup>a</sup>			Groups	M	F	M + F
E	9	BT 8202	16,900	30	2,000	LS <sup>b</sup>	12 day embryos <sup>c</sup>	I	110	110	220
			16,900	30	500	LS		II	110	110	220
			16,900	30	75	LS		III	110	110	220
			3,900	5	35	LS		IV	110	110	220
	10	BT 8204	16,900	30	2,000	13 weeks <sup>d</sup>	12 day embryos <sup>c</sup>	I	100	100	200
			16,900	30	500	13 weeks <sup>d</sup>		II	100	100	200
			16,900	30	75	LS		III	110	110	220
			3,900	5	35	13 weeks <sup>d</sup>		IV	100	100	200
	11	BT 8201	16,900	30	2,000	LS	6 weeks	I	110	110	220
			16,900	30	500	LS		II	110	110	220
			16,900	30	75	LS		III	210	210	420
			3,900	5	35	LS		IV	210	210	420
	12	BT 8203	16,900	30	2,000	LS	52 weeks	I	100	100	200
			16,900	30	500	LS		II	100	100	200
			16,900	30	75	LS		III	100	100	200
			3,900	5	35	LS		IV	100	100	200

<sup>a</sup>Per Kg of feed.<sup>b</sup>LS, lifespan, from the commencement of the experiment until spontaneous death.<sup>c</sup>Administered to pregnant mothers.<sup>d</sup>The regimen is then followed by diet with historical vitamin levels, until spontaneous death.

animals. The treatment is started very early in life, thus allowing for observation of the treated animal for as long as possible. In some experiments the animals are exposed from embryos (by treating pregnant breeders). In one project (the  $\gamma$ -radiation project) the effect of the exposure of male breeders before mating has also been studied. The mega-experiments are being conducted by following Good Laboratory Practices (GLP) with highly standardized intra- and inter-experimental procedures. The animals are submitted to periodic controls on behavior, clinical status, and grossly detectable pathologic changes throughout the biophase, and then, at spontaneous death, to complete necropsy followed by systematic histopathology.

### *The Projects*

#### *First Experimental Project*

##### *Evaluating the Carcinogenic Effects of Vitamins A, C, and E*

The aim of this project is to test the carcinogenic effects of three vitamins, A, C, and E, that are essential to the human organism. They are introduced into the body with the diet to a variable extent and they enjoy wide scale pharmacologic use and marketing (nowadays this includes their use for tumor intervention and prevention purposes). However, they have never been subjected to adequate, targeted carcinogenicity studies, either in general terms or in relation to dose. The vitamins studied at different dose-levels were supplied with the feed. The project plan is presented in TABLE 1. The biophase of this project has been completed and the pathology material is now under scrutiny. Preliminary data indicate that, when administered for the lifespan starting at six weeks of age, vitamin A causes an increase in the incidence of mammary cancer in females (see TABLE 2).

**TABLE 2. Evaluation of the carcinogenic effects of various levels of vitamin A from first experimental project. Incidence of malignant mammary tumors in female Sprague-Dawley rats, six weeks old at the start of the experiment**

Group	Vitamin A levels (iu/Kg in the feed) <sup>a</sup>	Animals <i>N</i>	Malignant mammary tumors			
			Animals with tumors		Tumors	
			<i>N</i>	%	<i>N</i> <sup>b</sup>	per 100 animals
I	150,000	200	30	15.0	37 <sup>c</sup>	18.5
II	75,000	200	26	13.0	32 <sup>c,d</sup>	16.0
III	16,900	200	27	13.5	32 <sup>c</sup>	16.0
IV	3,900	200	11	5.5	15 <sup>c,d</sup>	7.5

<sup>a</sup>For the life span.

<sup>b</sup>One animal can bear more than one carcinoma.

<sup>c</sup>Adenocarcinomas.

<sup>d</sup>One with sarcomatous component.

*Second Experimental Project*  
*Evaluating the Carcinogenic Effects of Compounds*  
*that Migrate into Mineral Waters from PVC Bottles*

This project was conducted to evaluate the carcinogenic effects of mixtures of chemical agents (in particular, vinyl chloride) that potentially migrate at low doses into drinking (mineral) water from the walls of PVC bottles.<sup>31</sup> At present bottles for beverages and other containers for food, formed from various plastic materials, are widely used. Apart from our studies on PVC bottles, other plastics have never been subjected to adequate long-term carcinogenicity studies.

The treatment consisted in supplying the experimental animals with drinking water with or without CO<sub>2</sub> addition, contained (stocked) in PVC bottles or in glass bottles for at least 30–60 days before use. The potential migration of chemicals from PVC was increased by treating the water contained in plastic bottles with granules of polymer (Benvic, 3–4 mm in diameter), in sufficient quantity to obtain a threefold increase in the contact surface with PVC. The granules were added at least 24 hours before the water was drunk, and filtered out before daily filling of the animal drinking bottles. The plan of the project is presented in TABLE 3. The experiment has ended and the most important results have been published.<sup>31</sup> The results did not show the onset of any unexpected specific tumors, nor variation in the relative incidence of different tumor types, within groups. No increase in the total number of malignant tumors was found in the animals drinking water from PVC bottles (see TABLE 4). On comparing the two groups drinking both types of water, with or without CO<sub>2</sub>, contained either in glass or in PVC bottles, or likewise the two groups drinking the same water contained in both glass and PVC bottles, one finds practically no difference in the onset of total malignant tumors (see TABLE 5). These results do not exclude the fact that drinking water contained in PVC bottles may contain carcinogenic micropollutants but, because of the high detection potential of the experiment and because of the consistent stability of the data, they constitute a good safeguard level for public health.

*Third Experimental Project*  
*Evaluating the Carcinogenic Effects of Various Doses of  $\gamma$ -Radiation*

This project was conducted to evaluate the carcinogenic effects of  $\gamma$ -radiation, in relation to dose (low doses in particular), schedule of treatment, and age of animals at the time of exposure. The project tested for any effect, on the tumorigenesis of descendants, of exposing male breeders before conception and females when pregnant, and the effect of direct exposure on six-week-old animals. One part of the project set out to assess the carcinogenic risks of food sterilized with high doses of  $\gamma$ -radiation. In the various project experiments the animals were randomized within the various groups by breeders, thus also affording information on the role of familial predisposition in  $\gamma$ -radiation carcinogenesis. The entire experimental population was composed of litters born within the same week. The plan of the experiment is presented in TABLE 6. The biophase of the experiment has ended and the pathology data is now under scrutiny.

Preliminary data from Experiment 3 show that  $\gamma$ -radiation delivered one-off to six–eight-week-old female Sprague-Dawley rats at doses of 300, 100, and 10 rads are carcinogenic for the mammary gland. In fact,  $\gamma$ -radiation treatment causes: (1) a

**TABLE 3. Plan of the second experimental project to evaluate the carcinogenic effects of compounds that migrate into drinking water from PVC bottles on male (M) and female (F) Sprague-Dawley rats**

Experiment	Treatment (drinking water)				Animals			
	Identification	Type	Duration	Start	Groups	M	F	M + F
I	BT 9001	Mineral water without CO <sub>2</sub> addition, contained in glass bottles	LS <sup>a</sup>	Since breeder matching	I	250	250	500
		Mineral water without CO <sub>2</sub> addition, contained in PVC bottles	LS	Since breeder matching	II	250	250	500
		Mineral water with CO <sub>2</sub> addition, contained in glass bottles	LS	Since breeder matching	III	250	250	500
		Mineral water with CO <sub>2</sub> addition, contained in PVC bottles	LS	Since breeder matching	IV	250	250	500

<sup>a</sup>LS, lifespan from the commencement of the experiment until spontaneous death.

**TABLE 4. Evaluation of the carcinogenic effects of compounds migrating into drinking water from PVC bottles from the second experimental project. Total incidence of malignant tumors in male (M) and female (F) Sprague-Dawley rats**

Group	Treatment (drinking water)	Animals		Animals bearing tumors			Malignant tumors	
		Sex	N	N	%	N	Tumors	
							per 100 animals	
I	Mineral water without CO <sub>2</sub> addition, contained in glass bottles	M	250	100	40.0	118	47.2	
		F	250	89	35.6	110	44.0	
		M + F	500	189	37.8	228	45.6	
II	Mineral water without CO <sub>2</sub> addition, contained in PVC bottles	M	250	92	36.8	103	41.2	
		F	250	67	26.8	89	35.6	
		M + F	500	159	31.8	192	38.4	
III	Mineral water with CO <sub>2</sub> addition, contained in glass bottles	M	250	91	36.4	108	43.2	
		F	250	72	28.8	86	34.4	
		M + F	500	163	32.6	194	38.8	
IV	Mineral water with CO <sub>2</sub> addition, contained in PVC bottles	M	250	88	35.2	106	42.4	
		F	250	79	31.6	96	38.4	
		M + F	500	167	33.4	202	40.4	

**TABLE 5. Evaluation of the carcinogenic effects of compounds migrating into drinking water from PVC bottles from the second experimental project. Total incidence of malignant tumors aggregated by type of water and by type of bottle**

Group	Treatment (drinking water)	Animals		Malignant tumors			
		Sex	N	Animals bearing tumors		Tumors per 100 animals	
				N	%		N
I	Mineral water with and without CO <sub>2</sub> addition, contained in glass bottles	M	500	191	38.2	226	42.5
		F	500	161	32.2	196	39.2
		M + F	1000	352	35.2	422	42.2
II	Mineral water with and without CO <sub>2</sub> addition, contained in PVC bottles	M	500	180	36.0	209	41.8
		F	500	146	29.2	185	37.0
		M + F	1000	326	32.6	394	39.4
III	Mineral water without CO <sub>2</sub> addition, contained in glass and PVC bottles	M	500	192	38.4	221	44.2
		F	500	156	31.2	199	39.8
		M + F	1000	348	34.8	420	42.0
IV	Mineral water with CO <sub>2</sub> addition, contained in glass and PVC bottles	M	500	179	35.8	214	42.8
		F	500	151	30.2	182	36.4
		M + F	1000	330	33.0	396	39.6
V	Both types of water in both types of containers	M	1000	371	37.1	435	43.5
		F	1000	307	30.7	381	38.1
		M + F	2000	678	33.9	816	40.8

TABLE 6. Plan of the third experimental project to evaluate carcinogenic effects from various doses of ionizing radiations

Experiment N	Identification	Type	Treatment ( $\gamma$ -rays)		Schedule	Animals		
			Dose (rads)	Groups		M	F	M + F
1	BT 3R	Irradiation of male breeders before mating	300	I	One off	154	167	321
				II	One off	401	398	799
				III	One off	743	694	1,437
				IV <sup>a</sup>		514	537	1,051
2	BT 2R	Irradiation of pregnant females at 12th day of pregnancy	100	I	One off	286	289	575
				II	One off	363	365	728
				III	One off	737	759	1,496
				IV <sup>a</sup>		514	537	1,051
3	BT 1R	Direct irradiation of 6–8 weeks old male and female rats	300	I	One off	211	205	416
				II	Fractionated <sup>b</sup>	83	107	190
				III	One off	318	301	619
				IV	Fractionated <sup>c</sup>	126	133	259
4	BT 4R	Irradiated feed since 12th day of embryonal life <sup>f</sup>	10	V	One off	524	522	1,046
				VI	Fractionated <sup>d</sup>	220	215	435
				VII <sup>a</sup>		514	537	1,051
				I	LS <sup>e</sup>	272	258	530
			1,000,000	II	LS <sup>e</sup>	292	317	609
			0	III <sup>a</sup>		514	537	1,051

<sup>a</sup>Control group common for four experiments.<sup>b</sup>10 doses of 30 rads at four week intervals.<sup>c</sup>10 doses of 10 rads at four week intervals.<sup>d</sup>10 doses of 1 rad at four week intervals.<sup>e</sup>LS, lifespan from the commencement of the experiment until spontaneous death.<sup>f</sup>Administered to pregnant breeders.

**TABLE 7. Evaluation of carcinogenic effects of various doses of ionizing radiations from the third experimental project. Incidence of fibroadenomas following one-off exposure of female Sprague-Dawley rats to  $\gamma$ -rays from  $\text{Co}^{60}$**

Group	N of /dose litters animals (rad)	Fibroadenomas (FA)/fibroadenomas with glandular hyperplasia (FA+)																		
		FA						FA+						Total FA, FA+						
		Bearing litters		Bearing animals		Tumors per 100 animals		Bearing litters		Bearing animals		Tumors per 100 animals		Bearing litters		Bearing animals		Tumors per 100 animals		
N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
I	40	39	97.5	145	70.3	326	159.0	34	85.0	80	39.0	130	63.4	40	100.0	165	84.5	456	222.4	(300)
II	58	54	93.1	162	53.8	266	88.4	48	82.7	107	35.5	165	54.8	58	100.0	217	72.1	441	146.5	(100)
III	99	85	85.9	222	42.5	367	70.3	57	57.6	105	20.1	127	24.3	92	92.9	269	51.5	494	94.6	(10)
IV	100	88	88.0	227	42.3	307	57.2	69	69.0	147	27.4	206	32.4	92	92.0	304	56.6	513	95.5	(0)



**TABLE 9. Evaluation of carcinogenic effects of various doses of ionizing radiations from the third experimental project. Incidence of mammary adenocarcinomas following one-off exposure of female Sprague-Dawley rats to  $\gamma$ -rays from  $C_{60}^{60}$**

Group <i>N</i> of /dose litters animals (rad)	Adenocarcinomas (ADCA)/anaplastic adenocarcinomas (AADCA)																			
	ADCA				AADCA				Total ADCA, AADCA											
	Bearing litters	Bearing animals	Tumors per 100 animals	Tumors per 100 animals	Bearing litters	Bearing animals	Tumors per 100 animals	Tumors per 100 animals	Bearing litters	Bearing animals	Tumors per 100 animals	Tumors per 100 animals								
I (300)	40	205	36	90.0	80	39.0	108	52.7	4	10.0	4	1.9	5	2.4	37	92.5	82	40.0	113	55.1
II (100)	58	301	46	79.3	77	25.6	107	35.5	1	1.7	1	0.3	1	0.3	47	81.0	78	25.9	108	35.9
III (10)	99	522	50	50.5	73	13.9	96	18.4	1	1.0	2	0.4	3	0.6	50	50.5	74	14.2	99	19.0
IV (0)	100	537	42	42.0	68	12.6	86	16.0	0	—	0	—	0	—	42	42.0	68	12.6	86	16.0

**TABLE 10.** Evaluation of carcinogenic effects of various doses of ionizing radiations from the third experimental project. Number of aggregated mammary adenocarcinomas and their precursors (dysplasias) per 100 animals resulting from one-off exposure to  $\gamma$ -rays from  $\text{Co}^{60}$

Group/ dose (rad)	Mammary adenocarcinomas plus their precursors <i>N</i> per 100 animals
I (300)	66.8
II (100)	55.8
III (10)	47.5
IV (0)	23.4

dose-related increase in mammary fibroadenomas at 300 and 100 rads (see TABLE 7); (2) a non dose-related increase in mammary gland dysplasia at 300, 100, and 10 rads (see TABLE 8), probably because a higher percent of such lesions evolve to carcinomas in the groups exposed to the higher dose levels; (3) a dose-related increase in mammary carcinomas at 300, 100, and 10 rads, as a percentage of bearing litters and of bearing animals, and number of adenocarcinomas per 100 animals (see TABLE 9). When the number per 100 animals of mammary adenocarcinomas and their precursors (dysplasias) are aggregated, a clear cut dose-response relationship can be observed. The carcinogenic risk of the lowest tested dose (10 rads) is shown in TABLE 10.

*Fourth Experimental Project*  
*Evaluating the Carcinogenicity of Extremely*  
*Low Frequency Electromagnetic Fields*

This project aims to study the carcinogenic potential of extremely low frequency, sinusoidal, 50 Hz magnetic fields (S-50 Hz MF) at different doses, as well as their carcinogenic potential in association with known carcinogenic exposure. In all the experiments exposure to S-50 Hz MF will start from the 12th day of pregnancy of the breeder and continue throughout the lifespan of the offspring. In the project experiments, the animals are to be randomly placed into various groups by breeders, thus helping to assess the role of family factor(s) in the response. The plan of the project is shown in TABLE 11. This project is on the verge of commencing.

*Fifth Experimental Project*  
*Evaluating the Carcinogenicity of*  
*Radiofrequency/Microwave Electromagnetic Fields*

The fifth experimental project is intended to test the carcinogenicity of 1.8 GHz-GSM microwave electromagnetic emissions (1.8 GHz-Mw) at different doses. Ex-

**TABLE 11. Plan of the fourth experimental project to evaluate carcinogenic effects of extremely low-frequency electromagnetic fields on male (M) and female (F) Sprague-Dawley rats**

Experiment	Treatment				Animals		End points to be evaluated							
	N	Identification	Other exposures		Groups			N						
			Type	Dose										
1	BT 1CMS	1000 C	—	—	LS	I	M	250	F	250	500	carcinogenic effects		
		1000 O/O	—	—	LS	II		250		250	500	carcinogenic effects		
		100 C	—	—	LS	III		500		500	1000	carcinogenic effects		
		20 C	—	—	LS	IV		500		500	1000	carcinogenic effects		
		2 C	—	—	LS	V		500		500	1000	carcinogenic effects		
		0	—	—	LS	VI		500		500	1000	(control)		
		1000 C	• Low frequency (200 KHz), C <sup>b,c</sup>	10 μTesla	LS	VII		150		150	300	300	synergic carcinogenic effects	
			• 1.8 GHz-Mw <sup>c</sup>	400 mwatt/kg b.w.										
			• Low frequency (200 KHz), C <sup>b</sup>	10 μTesla	LS	VIII		250		250	500	500	500	synergic carcinogenic effects
			• γ-rays <sup>d</sup>	10 rads	LS	IX		100		100	200	200	200	synergic carcinogenic effects
2	BT 2CMS	1000 C	• γ-rays <sup>d</sup>	10 rads	LS	X		100		100	200	200	synergic carcinogenic effects	
		20 C	• Aflatoxin B1 <sup>e</sup>	70 μg/rat	IS <sup>f</sup>	I		100		100	200	200	increase in the incidence of hepatic preneoplastic foci induced by Aflatoxin B1	
		1000 C	• Aflatoxin B1 <sup>e</sup>	70 μg/rat	IS	II		100		100	200	200	200	200
		0	—	—	IS	III		100		100	200	200	200	
		0	—	—	IS	III		100		100	200	200	200	
		0	—	—	IS	III		100		100	200	200	200	

<sup>a</sup>The exposure is to be performed 20 hours daily, continuously (C) or intermittently (I) or 30 minutes on and 30 minutes off (O/O), 7 days weekly, starting during embryo life by irradiating the pregnant breeders from the 12th day of pregnancy and continuing on the offspring for their lifespan (LS).

<sup>b</sup>Continuous and concomitant exposure along with S-50 Hz MF exposure.

<sup>c</sup>Radiofrequency/microwave electromagnetic fields: 1.8 GHz-GSM microwave electromagnetic emissions (1.8 GHz-Mw).

<sup>d</sup>Administered one-off at the age of six weeks, as an initiating treatment.

<sup>e</sup>Administered by gavage nine times in two weeks, at age 6-7 weeks, as an initiating treatment.

<sup>f</sup>IS, interim sacrifices.

**TABLE 12. Plan of the fifth experimental project to evaluate carcinogenic effects of radiofrequency/microwave electromagnetic fields, 1.8 GHz-GSM microwave electromagnetic emissions (1.8 GHz-MW), on male (M) and female (F) Sprague-Dawley rats**

Experiment		Treatment			Animals		
<i>N</i>	Identification	Exposure to 1.8 GHz-Mw (mwatt/kg b.w.) <sup>a</sup>			<i>N</i>		
					<i>M</i>	<i>F</i>	<i>M + F</i>
1	BT 1 Mw	2000	LS	I	250	250	500
		400	LS	II	500	500	1000
		80	LS	III	500	500	1000
		4	LS	IV	500	500	1000
		0	LS	V	500	500	1000

<sup>a</sup>The exposure is to be performed 20 hours daily, seven days weekly, starting during embryo life by irradiating the pregnant breeders from the 12th day of pregnancy and continuing for the lifespan (LS) of the offspring.

posure to 1.8 GHz-Mw is to be performed starting from the 12th day of pregnancy of the breeder and continued through the lifespan of the offspring. In the experiments the animals are randomly placed into various groups by breeders so as to throw light on the role of family factor(s) in the responses. The plan of the project is shown in TABLE 12. This project is about to commence.

## CONCLUSIONS

Mega-experiments, as defined in this report, are the most adequate instruments at present available for identifying and assessing diffuse potentially carcinogenic exposure. In our experience they are feasible, they can detect low and extremely low risk situations, and they produce consistent results that may resolve uncertainties in risk assessment.

The problem with diffuse carcinogens is enormous and it is of increasing concern. It forms one of our main public health issues. This calls for an increase in the use of mega-experiments to investigate industrial and environmental carcinogenesis. The Ramazzini Foundation leads the way in this research field and will give it priority in years to come.

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