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3. CANCER PREVENTION: THE LESSON FROM THE LAB

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For several decades cancer has represented one of the principal problems facing public health in industrialized countries, not only because of its epidemiological dimension and related quality of life and economic issues, but also because of the causes behind its increase to today's epidemic proportions. In the United States, one out of two males and one out of three females is destined to become ill with cancer over the course of a lifetime. Although cancer mortality began to diminish beginning in the early 1990s, especially in males in 2002 cancer was responsible for over 500,000 deaths, equivalent to 22.8% of all US mortality (1). According to data from the period 1982-2002 from the Nominative Mortality Register of European Ramazzini Foundation, cancer deaths in Bologna and Province show a decline beginning in 1995, especially in males (Figure 1). Nevertheless, cancer deaths in the year 2002 still represented 35.60% and 25.88% of total mortality in the area for males and females respectively (2). Cancer strikes above all in the oldest segment of the population. In fact, according to the US National Cancer Institute, 80% of all tumors are diagnosed in patients over 55 years of age (1). In the Province of Bologna, 80% of cancer deaths occur after the age of 60-65 years. If one reasons that in 25 years the number of persons over 70 will have doubled, it is necessary to prepare for a dramatic increase in the number of tumors that will arise. *In the United States alone, estimates predict that the number of tumors will indeed double by 2050 (1).*

Although the scientific effort and economic resources dedicated to cancer have been perfuse over the last 35 years (directed especially toward the discovery of effective cancer drug therapies), in the US, the 5-year relative survival rates based on patient follow up from 1976-2000 have not substantially improved, with the exception of female breast cancer and prostate and colon-rectal cancers for which early diagnosis has certainly played an important role (Table I).

There are two new major factors at the origin of the increased incidence, and therefore increased mortality, of cancer over the last 50-60 years:

- the increase in life expectancy (about 10 years for males and 15 years for females);
- the increase in the diffusion of agents and situations presenting carcinogenic risks in both the occupational and general environment.

Figure 1. Cancer death rates, all sites combined, in the Province of Bologna. The death rate is age-adjusted to the 2001 Italian standard population and is expressed per 100,000 persons. Source: Registro di Mortalità Nominativa di Bologna e Provincia (2).

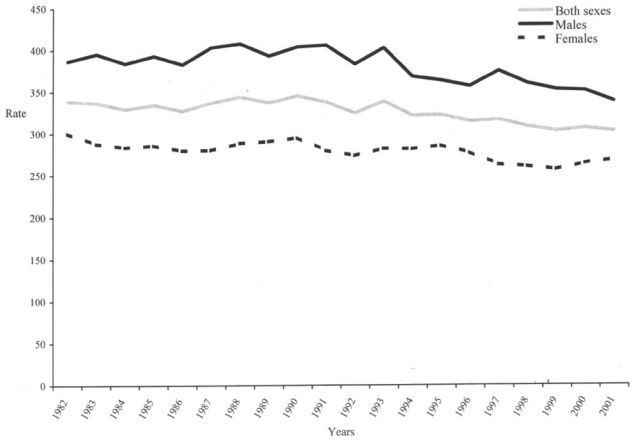


Table I. Relative survival[a] (%) during three time periods, by cancer site in the United States[b]

Site	1974-1976	1983-1985	1995-2000
All sites	50	53	64
Breast (female)	75	78	88
Colon	50	58	63
Leukemia	34	41	46
Lung and bronchus	13	14	15
Melanoma of the skin	80	85	91
Non-Hodgkin lymphoma	47	54	59
Ovary	37	41	44[c]
Pancreas	3	3	4
Prostate	67	75	99
Rectum	49	55	64
Urinary bladder	73	78	82

[a] 5-year relative survival rates based on follow up of patients through 2001
[b] Source: American Cancer Society 2005 (1)
[c] Recent changes in classification of ovarian cancer have affected 1995-2000 survival rates

A third factor which is also important in the carcinogenetic process is genetic predisposition, however, it is unlikely that this factor has changed significantly during the last decades.

As a whole, the process of carcinogenesis may be expressed, albeit simplistically, by the following formula:

C = f (P+E+A)

where cancer is a function (f) of predisposition (P), exposure (E) and age (A), the last of which when increasing, allows an environmental carcinogen to more fully express its effects, in particular those which are attributable to the sum/synergy of low and extremely low dose exposure to carcinogenic agents (3). Of the three factors which condition the carcinogenetic process, it is difficult to imagine changing predisposition (P) by modifying the genetic profile, even more so regarding age (A). The possibility to modify the trend in cancer incidence and mortality therefore depends on the capacity to identify agents and situations of carcinogenic risk in the environment related to occupation and/or lifestyle (E). Once identified, it follows that exposure to these risks should then be eliminated or at least reduced as much as possible.

ENVIRONMENTAL RISKS AND CANCER

There exists a great deal of evidence demonstrating the existence of a correlation between exposure to environmental carcinogenic risks and cancer. Examples include:

- the **elevated incidence of some types of tumors in some geographic areas** of the planet compared to others;
- **studies on immigrants** from countries with a low incidence of a certain type of tumor to countries with a higher incidence of the same tumor show that incidence becomes aligned with that of the new country of residence by just the second or third generation. A typical example is that of Japanese women (Japan = low incidence of breast cancer) moving to the United States (USA = higher incidence of breast cancer);
- the existence of ***occupational tumors.*** Typical examples include, but are not limited to: an increase in the risk of mesothelioma of the pleura and peritoneum and of lung and kidney cancers in workers exposed to asbestos, bladder cancer in workers in the coloring and synthetic rubber industries exposed to aromatic amines, liver angiosarcoma in workers exposed to vinyl chloride (the chemical compound used for the production of polyvinyl plastics) and the risk of nasal cancer and leukemia in workers exposed to formaldehyde;
- results of **long term carcinogenicity studies on rodents** (especially rats and mice) for the identification of potential carcinogenic effects of chemical agents, particularly those of industrial origin. In the two principal institutions in the world which conduct carcinogenicity bioassays, the National Toxicology Program of the US government (NTP) and the Cesare Maltoni Cancer Research Center of the European Ramazzini Foundation of Bologna (CMCRC/ERF), results have been published on the carcinogenicity of 500 and 100 agents respectively. Of these agents, almost 50% were shown to be carcinogenic in rodents (4);

• **transplacental carcinogenesis.** Cases of insurgence of clear cells adenocarcinoma of the vagina are well known in young women exposed during fetal life to diethystilbestrol, a synthetic estrogen used between 1940 and 1960 to prevent miscarriage and premature delivery (5);

• **carcinogenic risks due to behavior and lifestyle,** for example tobacco use. It has been known for decades that cigarette smoke is responsible for the increase in cancer, lung, but also of the pancreas and bladder. It is significant that mortality attributed to lung cancer is slowing decreasing in males (who have been smoking less), but remains stable in women (who have not yet modified their smoking habits).

If it is true that the carcinogenetic process depends in large part on the role of genetic predisposition, environment and age, and that in order to modify the present epidemiological dimension of cancer the most compatible solution is intervening to reduce exposure to agents and situations of environmental cancer risk, it is also true that our knowledge of these risks is still unfortunately extremely limited. At the beginning of the 1980s, the US National Academy of Sciences published the results of a survey designed to collect the available toxicological information on chemical compounds on the market in order to evaluate the associated risks (6). The results of that survey, including the categories, number of compounds in each category and the percent of compounds with complete or non-existent toxicological information are reported in Table II. Only about 2% of chemicals examined were found to have sufficient toxicological information. Although adequate scientific tools to identify and quantify these risks have been available for more than twenty years, the situation has not substantially changed.

Table II. Data availability for risk assessment for different categories of agents[a]

Category	**Agents**					
	Number	**Complete**			**None**	
		N.	**%**		**N.**	**%**
Pesticides and inert ingredients used in their formulations	3,350	335	10		1,273	38
Cosmetics	3,410	68	2		1,910	56
Drugs	1,815	327	18		454	25
Food Additives	8,627	431	5		3,968	46
Other chemicals in commerce	48,523	0	0		38,439	79
TOTAL	65,725	1,161	1.77		46,909	71.37

[a] Source: US National Academy of Sciences 1984 (6).

THE SCIENTIFIC TOOLS FOR IDENTIFYING ENVIRONMENTAL CANCER RISKS

The identification of carcinogenic agents may be pursued today using three types of research, all of which offer various possibilities and advantages, but each of which are limited in different ways.

1. Short and medium term studies
   Short and medium term studies include research on toxic effects (acute, subacute and subcronic), on mutagenic effects (in bacterial and animal cells) and on biomolecular mechanisms which may be at the origin of these effects (using also transgenic animal models). These types of research may be completed in a relatively short time frame, but with regard to carcinogenicity, these studies provide only indirect data. Since these data are not based on end points, they cannot reveal a specific final event such as the presence or absence of a tumor. *The utility of these studies therefore has a precise limit,* even if in some cases they can provide, in addition to an initial orientation, useful data about the mechanistic aspects of the action of agents on biological systems (related or unrelated to carcinogenesis).
2. Long term carcinogenesis bioassays on experimental animals
   When well planned and conducted using adequate animal models (as close as possible to the human equivalent) and reproducing as much as possible human exposure scenarios, *long term carcinogenesis bioassays may provide specific indications on carcinogenicity which may then be extrapolated to the human in both qualitative and quantitative terms.* Much has been done by interested parties to discredit these bioassays and associated scientists. However, there are three important considerations which support their use:
   • all agents demonstrated via epidemiological studies to be carcinogenic in humans also resulted carcinogenic in experimental animals. The carcinogenicity of one third of the agents recognized to be carcinogenic by the International Agency for Research of Cancer (IARC) (Table III) was first demonstrated in rodent studies and only subsequently in humans (7);
   • no agent demonstrated to be carcinogenic in animals has been found to be non-carcinogenic in humans when adequate epidemiological studies were performed;
   • the type of relationship between exposure to a carcinogenic agent and the neoplastic response and evolution of the carcinogenesis process are comparable in animals and humans.
3. Oncological epidemiological surveys
   Oncological epidemiological surveys, when conducted with adequate information on the exposure scenarios and correct methodology, produce clear results and represent the most direct proof of the carcinogenicity of an agent, factor or situation. Often however, these surveys have precise limitations:
   • their feasibility, in that the dimension of the exposed or follow-up populations may be insufficient and/or in that the data related to the level of exposure may be

limited due to the difficulty of retracing the universe of exposed persons or of a representative sample;

• the capacity to produce clear results due to confounding factors or, in the case of low or medium-low exposure, the difficulty of finding a large enough sample of exposed persons and adequate controls. Methodological inadequacies may also impede clear results;

• the inherent delay in epidemiological results due to the long period of tumor latency in humans (on average 10-30 years), during which time agents may continue to produce their grave effects.

Table III. Chemicals shown to cause cancer first in laboratory animals and then in humans[a]

1. Aflatoxins	14. Diethylstilbestrol
2. 4-Aminobiphenyl	15. Melphalan
3. Asbestos	16. 8-methoxypsoralen + UVA
4. Azothioprine	17. Mustard gas
5. Betel quid with tobacco	18. Myleran
6. 1,3-butadiene	19. Nonsteroidal estrogens
7. Chlorambucil	20. Silica, crystalline
8. Chloranaphazine	21. Solar radiation
9. Chloromethyl methyl ether (technical grade)	22. Steroidal estrogens
10. Ciclosporin	23. 2,3,7,8-TCDD
11. Coal-tar pitches	24. Thiotepa
12. Coal-tars	25. Trichloroethylene
13. Cyclophosphamide	26. Vinyl chloride

[a] Source: Huff 1999 (7).

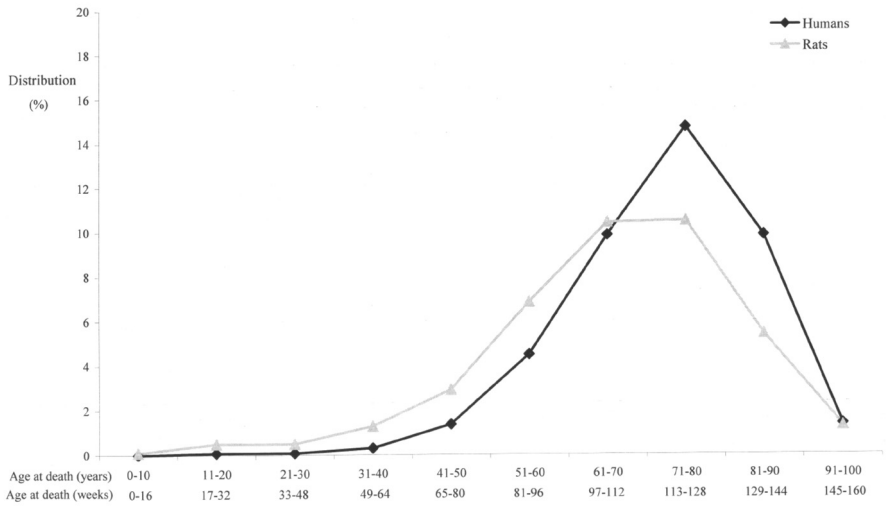
Since the period of latency is proportional to the average lifespan of an organism, latency is proportionally shorter in rodents commonly used in the laboratories for long term bioassays. An example is that of Sprague Dawley rats used in the laboratory of the Cesare Maltoni Cancer Research Center of the European Ramazzini Foundation. This strain of rat has a lifetime of 160 weeks which corresponds to 95-100 human years. A latency of one year is therefore equivalent to slightly more than 30 years of latency in humans (Figure 2). In other words, long term bioassays allow for a very rapid surrogate response, avoiding the frequent exposure of humans to unknown agents posing carcinogenic risk.

In conclusion, it may be affirmed that:

• the results of short and medium term studies, even in the best cases, provide only an orientation for further exploration and are best used for mechanistic studies. It is difficult to think that the results of short and medium term studies could ever substitute those of long term carcinogenesis bioassays;

• carcinogenesis bioassays, if adequately conducted, are relatively rapid and highly predictive of effects on humans (it is also true that studies of this type which are not correctly planned and conducted represent a factor of confusion);

Figure 2. Comparative distribution by age at death of 1,114 people with malignant tumors out of 2,650 autopsied men and women deceased at Trieste Hospital in 1989 (personal communication from Prof. Luigi Giarelli) and of 1,212 Sprague-Dawley rats with malignant tumors out of 3,051 necropsied male and female untreated rats, under observation until spontaneous death.



• epidemiological surveys are those which technically provide the most direct (albeit delayed), information, but are often impeded by obstacles to their feasibility and frequently produce non conclusive results due to methodological and non-methodological inadequacies.

Considering that long term bioassays provide a valid tool for the identification of carcinogenic agents, it is surprising that they are not more fully used to provide a timely evaluation of the carcinogenicity of agents which are ubiquitous in the human habitat. Historical examples of agents for which sufficient and timely attention was not given include: plastic monomers, halogenated solvents, artificial sweeteners, fibers (in particular asbestos), wastes, low doses of ionizing radiation, non ionizing electromagnetic fields, and last but not least, fuels for transportation, their constituents and additives and their combustion products.

Given the aforementioned examples, it is also difficult to explain why clear indications do not exist regarding who are the responsible parties that should promote these types of studies. Finally, even more inexplicable, is the circumstance in which self-initiated studies (such as the majority conducted in the laboratories of the European Ramazzini Foundation), which objectively reveal unsuspected health dangers, are cast in a negative light by those who have a primary responsibility to protect public heath. Often these critics have not conducted their own valid research and must therefore rely on unsubstantiated opinion or worse yet, on sponsored studies (for example by the producers of a product), which are not aimed at evaluating specific end point pathologies and are sometimes without the necessary scientific requisites.

THE EXTRAPOLABILITY OF EXPERIMENTAL CARCINOGENESIS RESULTS FROM RODENTS TO HUMANS

Throughout the history of medicine and biology, animal studies (in particular involving rats and mice) have facilitated the acquisition of important knowledge regarding the physiological, pathological and behavioral processes of humans. The results of experimental studies have permitted the evaluation of the efficacy and tolerability of pharmaceuticals (including cancer drugs), which have led to a fundamental improvement in the quality of life. Since Yamagiwa opened the doors of experimental chemical carcinogenesis in April 1915, inducing cutaneous tumors of the ear in rabbits following treatment with coal tar (8), numerous scientific contributions over the last 40 years have highlighted the great potential of carcinogenesis studies and above all, the vast biological similarities between various species of mammals and the concordance of the carcinogenic response between experimental animals and humans. An exhaustive review of the literature on this subject was recently published (4).

Carcinogenesis studies, if appropriately planned and conducted, provide information that allow not only for the qualitative identification of carcinogenic risks, but also the quantitative extrapolation of such risks to humans. In order to consent the extrapolation of experimental data to the human situation, the design and conduct of experiments must follow certain fundamental requirements. These requirements include: the animal model, the number of levels of dose/concentration/intensity of exposure, the number of animals per sex/per group, the route of exposure, the age of animals at the start of the experiment, the length of treatment, the length of the overall experiment, the experiment conduct, the accuracy and consistency in the evaluation of pathological lesions, and finally of paramount importance, the availability of adequate data regarding the spontaneous pathologies in a vast number of historical controls.

There exist two major programs in the world which conduct long term experimental carcinogenesis studies aimed at the identification and quantification of environmental carcinogenic risks, in particular those of industrial origin. The first is that of NTP, founded in 1980 thanks to the intuition and efforts of its first Director, David Rall. The number of agents studied by NTP and the results obtained are reported in Table IV. The second major program is that of the CMCRC/ERF.

Table IV. The ongoing program of long term carcinogenicity bioassays conducted by the US National Toxicology Program for the identification of carcinogenic risks

• Chemicals studied and published	503	
• Overall carcinogenicity results		
- Clear evidence	268	(53%)
- Equivocal evidence	68	(14%)
- No evidence	158	(31%)
- Inadequate bioassays	9	(2%)

THE LONG TERM EXPERIMENTAL CARCINOGENESIS PROGRAM OF THE CMCRC/ERF

The program of the CMCRC/ERF was begun in the laboratories of the Castle of Bentivoglio in the mid-1960's by Professor Cesare Maltoni, then director of the Oncological Institute "F. Addari" in Bologna, Italy. Maltoni's profound scientific and cultural knowledge of cancer led him to intuit important hypothesis, many of which ran contrary to the conventional wisdom of the time. Maltoni asserted that:

- cancer could be effectively combated if the agents and situations at its origin were known and then eliminated or reduced;
- long term carcinogenesis bioassays on rodents present the most congruent instrument to predict risks;
- such experiments must possess a high level of sensitivity and specificity such that the results can be utilized for legislation protecting public health;
- in order to achieve high levels of reliability, carcinogenesis bioassays must be planned and conducted according to standard and controllable procedures, anticipating the application of what at the end of the 1980s would come to be known as Good Laboratory Practices, later codified by law in the USA and other industrialized countries.

The majority of long term carcinogenesis bioassays executed at the CMCRC/ERF are designed and conducted following a **standard protocol** as reported in Table V. In some cases, experiments are planned and conducted according to a protocol which differs from the standard. This alternative protocol is used when evaluating the potential effects of so-called "diffused" carcinogenic risks.

Table V. Characteristics of the standard protocol of the long term carcinogenicity bioassays performed at the Cesare Maltoni Cancer Research Center (CMCRC) of the European Ramazzini Foundation (ERF)

1.	*Animal model*: the animal model used at the CMCRC/ERF is the Sprague-Dawley rat, from the colony used in the laboratory for more than 30 years, whose basic tumorigram is well known and whose cancer susceptibility is close to that of humans (Figure 2). In some cases, other species and strains (Wistar rats, Swiss mice, Golden hamsters) are used.
2.	*Experimental groups and group size*: the number of experimental groups are 2-3 or more, including control group (depending on the public health importance of the agent). Groups consist of a minimum of 50 animals per sex per group.
3.	*Route of exposure*: the routes used mimic human exposure. The routes most frequently used are: ingestion, injection, inhalation and external exposure (for physical agents, such as radiation).
4.	*Concentration/dose/intensity of agents tested*: at least three dose levels are tested, including the maximum tolerable level (MTL), a level comparable to the human exposure and an intermediate level. If MTL is not available from scientific literature, range-finding experiments are performed to assess it.
5.	*Start of the treatment*: treatment may start during embryonic life (12th day), perinatally, or at 6-8 weeks of age. In some exceptional cases, start of the treatment may also occur at other ages.
6.	*Duration of the treatment*: treatment typically lasts 104 weeks or for the entire life-span. Duration may sometimes differ.

7.	*Duration of the biophase*: the duration of the biophase is until spontaneous death, differently from typical long term bioassays in other facilities which terminate 104 weeks after the start of the treatment. This distinctive characteristic allows an agent to fully express its carcinogenic potential.
8.	*Conduct of the experiment*: during the experiment mean daily water and feed consumption are measured per cage and body weights individually, once a week for the first 13 weeks and every two weeks until 110 weeks of age. Body weights continue to be measured until the end of the experiment.
9.	*Pathology*: each animal undergoes complete necropsy and histopathological evaluation of all organs following natural death. During the necropsy the following organs are harvested: any organs or tissues with pathological lesions (with a portion of normal tissue), skin, mammary gland (4 sites), brain (3 sagittal sections), pituitary gland, Zymbal glands, salivary glands, Harderian glands, cranium (5 sections, with oral and nasal cavities, external and internal ear ducts), tongue, thyroid and parathyroid, pharynx and larynx, thymus and mediastinal lymph nodes, trachea, lung and mainstem bronchi, heart, diaphragm, liver (2 lobes), spleen, pancreas, kidneys, adrenal glands, esophagus, stomach (fore and glandular), intestine (duodenum, jejunum-ileum, colon, sigma-rectum), urinary bladder, prostate, gonads, interscapular brown fat pad, subcutaneous and mesenteric lymph nodes. All organs are preserved in ethanol 70%, except for bones which are fixed in 10% formalin and then decalcified with 10% formaldehyde and 20% formic acid in water solution. Normal specimens are trimmed following CMCRC/ERF standard operating procedures. Trimmed specimen are processed as paraffin blocks and 3-5 micron sections of every specimen are obtained.

Diffused carcinogenic risks are those of presumably low potency but to which almost the entire population of the planet may be exposed. These risks are represented by:

- agents which are slightly carcinogenic at any dose;
- low or extremely low doses of a carcinogenic agent of any kind;
- mixtures of small doses of carcinogenic agents of any kind.

We know definitively that these risks exist, but we do not know their dimension. Examples of potential diffused carcinogenic risks and the perceived degree of risk by the public are reported in Table VI.

Table VI. Diffuse carcinogenic risks: some examples

Type	Public perception
Extremely low frequencies electromagnetic fields (electric current)	+++
Radio frequency of electromagnetic fields (mobile phone)	++++
Particles (PM_{10}, $PM_{2,5}$, PM_1)	++++
Genetically modified organisms (GMOs)	++
Depleted Uranium	+

Given the influence of confounding factors, the effects of diffused carcinogenic risks are difficult to quantify using epidemiological or ordinary experiments. Instead, it is necessary to conduct studies which may be defined as "mega-experiments" having the following characteristics:

- they must be conducted using a vast number of animals (at least 300-1000 per experimental group);
- they must test several levels of exposure;

• exposure must occur in all phases of development (from fetal life to natural death).

In the case that a carcinogenic risk is revealed, mega-experiments are able to identify the type and entity of the tumoral response. In the event that a study does not show evidence of a carcinogenic effect, this result does not exclude the presence of a theoretic risk that could not be revealed by the applied experimental conditions. In any case, mega experiments provide a safety threshold which is proportional to the correctness of the methodology followed and the overall potential of the experiment.

In over 35 years of activity in the laboratories of the CMCRC/ERF, 205 agents have been studied using over 160,000 rodents. In total, 409 experiments have been conducted and the results of studies on 112 agents have been published to date (Table VII).

Table VII. The ongoing program of long term carcinogenicity bioassays conducted by the European Ramazzini Foundation for the identification of carcinogenic risks

• Chemicals/agents studied	205	
• Compounds/agents with available results	112	
• Overall carcinogenicity results		
- Clear evidence	49	(44%)
- Equivocal evidence	18	(16%)
- No evidence	45	(40%)

Compared with other research centers, two distinctive characteristics of the methodological approach followed by the CMCRC/ERF are that of protracting the length of a study until the natural death of the experimental animals and testing an elevated number of exposure levels. By contrast, arbitrarily truncating the length of an experiment after two-thirds of the lifespan of the animals and/or testing only three dose levels (as routinely practiced in other facilities), may lower the sensitivity and specificity levels of the experiment and therefore fail to reveal possible carcinogenic effects of the agent studied. The following are paradigmatic cases:

Ethyl Alcohol

Various epidemiological studies have shown a positive relationship between assumption of alcoholic beverages and the increase of cancer risks of the oral cavity, pharynx, larynx, esophagus and liver. Many experimental studies conducted on rats and mice exposed to various concentrations of ethyl alcohol administered in drinking water did not however show the same effects (9). In contrast with previous studies, in the test conditions of the CMCRC/ERF ethyl alcohol was indeed demonstrated to be carcinogenic for various organs and tissues, in particular inducing malignant tumors of oral cavity, tongue and lip, and esophagus, the same sites which were shown to be target organs in the aforementioned epidemiological studies (10).

Vinyl acetate

Vinyl acetate monomer (VAM) is an important compound used in the plastics industry. It is also used in the production of resin in chewing gum. The limited available epidemiological data on VAM do not allow for an evaluation of its potential carcinogenic risks in humans. Previous carcinogenicity studies on rats and mice have been in one way or another inadequate to evaluate the carcinogenic potential of VAM (11). In the 1980s, a series of experiments were conducted at the CMCRC/ERF using Sprague-Dawley rats, Wistar rats and Swiss mice simultaneously. In the tested conditions, VAM was demonstrated to be a multipotent carcinogenic agent inducing malignant tumors of the oral cavity, tongue, esophagus and forestomach in both strains of rats and mice. A slight increase of the incidence of adenomas/carcinomas of the lung and of malignant tumors of the uterus in mice was also observed (12-14).

Formaldehyde

During the 1990s, the worldwide production of formaldehyde totaled more than 12 million tons per year (15). The widest use of formaldehyde is in the production of resins used in adhesives and in impregnating resins used in the manufacture of particle board, furniture and other wood products. Formaldehyde itself is used as a preservative and disinfectant in many cosmetic products, hair treatments, deodorants, lotions, make-up, mouth washes and nail products. Formaldehyde has been shown to be genotoxic in both *in-vitro* and *in vivo* tests. Previous long term carcinogenicity bioassays on Fisher 344 rats and B6C3F1 mice who received whole-body exposure to formaldehyde vapor for up to 24 months showed an increased incidence of nasal cavity squamous cellular carcinoma (16). Overall epidemiological studies suggested a causal relationship between nasopharingeal cancer and exposure to formaldehyde (17). In a long term carcinogenicity bioassay performed at the CMCRC/ERF, formaldehyde was administered by ingestion in drinking water to 8 week-old male and female Sprague-Dawley rats until 111 weeks of age. In the tested conditions, formaldehyde was demonstrated to be a multipotent carcinogenic agent, inducing mammary cancers, gastrointestinal malignant tumors and lymphomas and leukemias (18,19). In a recent reevaluation, IARC considered formaldehyde to be a human carcinogen based only on the causal relationship with nasopharigeal cancer (20). Moreover, strong (though not considered sufficient) evidence of an association between formaldehyde exposure and leukemia in humans was cited.

Mancozeb

Mancozeb, an ethylene-bis-dithiocarbamate (EBDC), has been one of the most commonly used fungicides in commercial use for several decades. In Italy, particularly in the Emilia Romagna Region, mancozeb is one of the most widely used fungicides in agriculture. Although mancozeb has been commercially produced for almost 60 years, previous carcinogenicity studies of this fungicide produced inadequate results due to the limited number of animals used per sex and group and the duration of the experiments. Given these inadequacies and the vast chain of people exposed to the compound (work-

ers engaged in its production and use, citizens living in agricultural areas where it is sprayed and citizens consuming polluted products), a long-term carcinogenicity bioassay was conducted at the CMCRC/ERF in 1987 to evaluate its carcinogenic effects.. In the tested conditions mancozeb was demonstrated to be a multipotential carcinogenic agent, inducing an increase of the incidence of malignant tumors of the head and neck and of the thyroid gland in males and females and of lymphomas and leukemias in males (21).

Aspartame

Aspartame is an artificial sweetener consumed by hundreds of millions of people worldwide. It is used in over 6,000 diet products including soft drinks, chewing gum, candy, desserts and yogurt as well as in pharmaceuticals such as syrups and antibiotics for children. The average daily intake of aspartame is calculated to be about 2-3 mg/Kg of body weight, a figure which increases for children and women of childbearing age. Current daily intake allowed by regulatory bodies is 50 mg/Kg of body weight in the US and 40 mg/Kg of body weight the European Union. Prior to the commercialization of aspartame in the 1970s, the manufacturers of the compound conducted various experimental studies on rats and mice to test its carcinogenicity. When taken together, the results of these studies were considered negative with regard to the carcinogenicity of aspartame. Doubts were however raised by some in the scientific community about the conduct of the experiments and the fact that some cases of malignant brain tumors were found among animals treated with aspartame while none were found among the control group. Given the limitations of these studies due to the number of animals per sex and group and the duration of the experiment as well as the ever growing use of aspartame throughout the years, the CMCRC/ERF decided in the late 1990s to plan and perform an experiment that would, based on the total number of animals used (1800), the number of dose levels studied (7 including control) and the conduct of the experiment according to Good Laboratory Practices, provide an adequate evaluation of the potential carcinogenic effects of aspartame. The study demonstrates that aspartame administered at varying levels in feed causes a statistically significant increase of lymphomas-leukemias and malignant tumors of the renal pelvis in females and malignant tumors of peripheral nerves in males. These results demonstrate for the first time that aspartame is a carcinogenic agent, capable of inducing malignancies at dose levels lower than the acceptable daily intake for humans (22).

The aforementioned examples confirm that the sensitivity and specificity of long term carcinogenicity bioassays are proportionate to the design and conduct of the experimental studies.

CURRENT STUDIES AT CMCRC/ERF ON POTENTIAL ENVIORNMENTAL CARCINOGENIC AGENTS

Beginning in 2002, a large program of mega-experiments was undertaken at the CMCRC/ERF to evaluate the potential carcinogenic effects of:

- *non-ionizing radiation* (50 Hz magnetic fields from alternated electric current and radiofrequency electromagnetic fields of cellular telephones, in particular those generated from radio base station antennas);
- *artificial sweeteners,* beginning with further rat and mice studies on aspartame (useful for obtaining an improved risk assessment), followed by a long term study on sucrolose, a sweetener which is starting to be used instead of aspartame in many products.

The mega-experiment on magnetic fields generated by electric current was designed using 7,133 Sprague Dawley rats exposed to various intensities and exposure situations. At present, the biophase of the experiment has terminated and the processing of the experimental material and the elaboration of data are currently underway. Results of this experiment will be available in 2008. The mega-experiment on radiofrequency electromagnetic fields of cellular telephones was designed using 2,448 Sprague Dawley rats exposed to various intensities from fetal life to spontaneous death. The biophase began at the end of 2005 and will last until at least mid-2008. An additional year will be required to evaluate the data and publish the results. These studies on the possible carcinogenic effects of non-ionizing radiation are of great importance for public health as they may provide information to create a more adequate scientific basis for legislation in this sector.

CONCLUSIONS

Today cancer is one of the greatest heath problems facing industrialized countries, a problem which is destined to worsen if adequate provisions are not undertaken. This is true not only in industrialized parts of the world, but also in developing countries. The magnitude of the problem stems from its epidemiological dimension and associated social consequences and above all, from environmental factors and risks which have emerged from the development model employed in recent decades. In the previous century, the defeat of major epidemics was possible even prior to the introduction of vaccines and antibiotic therapies, thanks to environmental beautification and the introduction of hygiene legislation which combated environmental microorganisms. Likewise, it follows that the pursuit of an effective strategy for controlling tumors cannot disregard the role of prevention and in particular, the identification of agents and situations that present carcinogenic risks.

Long term carcinogenesis experiments are fundamental for the identification of carcinogenic agents, both for evaluating products which are already on the market and for studying those which have yet to be commercialized. The reliability of these studies is

such that their use is constantly growing, especially for the evaluation of the efficacy and tolerability of pharmaceuticals/active principles which could potentially be used for the chemoprevention of tumors. Also of great importance is their use for the identification and validation of biological markers and diagnostic devices.

What is the lesson from the lab? John Bailar (23) Professor Emeritus at the University of Chicago and founding Chair of its Department of Health Studies affirms that ‹‹decades of effort (and publicity) devoted to progress in treatment have not been reflected in the realities of clinical medicine. It is no longer clear that there are wonderful new cancer treatments just waiting to be discovered. Imagine where we might be today if we had put that effort into research on prevention, and perhaps to a lesser extent, screening. It is not too late to make the needed changes in our research priorities››.

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