462/B

PREDICTIVE VALUE OF CARCINOGENESIS BIOASSAYS

Cesare Maltoni

Institute of Oncology
and Tumour Centre
Bologna, Italy

It has been estimated that from 80 to 90% of human tumors are caused by factors present in the occupational and general environment. Therefore, cancer must be largely considered an ecologic disease.

On the other hand, the number and quantity of oncogenic agents in the occupational and general environment have progressively increased in the last few decades, due mainly to the following factors: the concentration of oncogenic agents already present in the Earth's surface, surfacing of oncogenic agents from beneath the Earth's surface, and production of new potentially oncogenic compounds by chemical and petrochemical industries.

The major problem is now represented by the multitude of products of synthetic chemical industries, especially the petrochemical industry, that are unknown to animal and human protoplasm and of whose effects we are therefore fully ignorant.

Factories represent the observatory: there, the compounds are planned, produced, and released into the general environment as consumer goods and pollution; there, the most exposed population works. In this situation, one should be therefore aware that tools are needed for assessing the risk of agents concentrated, or brought, or introduced *de novo* into the occupational and general human environment.

This need is strengthened by the following basic facts:

The potentially oncogenic agents in the human environment are progressively increasing.

Different oncogenic agents may have additive effects.

Changes produced by oncogenic agents are largely irreversible.

Oncogenic agents may exert their effects on different organs and tissues and widely affect the target organs.

A large part of the natural history of tumors occurs without any clinically, and sometimes otherwise, detectable pathologic changes.

Cancer is not a reversible disease.

The potential oncogenic risk for man by occupational and environmental agents has so far been identified when the incidence of a particular type of tumor in an exposed population has been especially high or on the basis of retrospective epidemiologic studies.

The gravity of the present situation, however, no longer permits the old policy of "let's wait and see." In other words, we must now predict oncogenic risks to avoid human exposure to such agents. It is a fact that the three most important cases of environmental and occupational tumors, discovered after 1970, were indirectly or directly predicted experimentally.

The first case was the adolescent clear-cell vaginal adenocarcinoma found in girls born from mothers treated during pregnancy with synthetic nonsteroid

estrogen therapy. It should be noted that, in 1938, Lacassagne [1] had already reported that mammary carcinomas arose in male mice treated with stilbestrol; several years later, it was shown, by several scientists, that the same hormone was producing a variety of tumors in hormone-dependent and -independent tissues among different experimental animal species.[2]

The second case was pulmonary carcinoma among workers exposed to bis(chloromethyl)ether. This new type of occupational tumor was discovered by experimental evidence: Van Duuren *et al.*[3] and Laskin *et al.*[4] showed, respectively, that, when injected subcutaneously into rats or applied to the skin of mice, the compound was producing subcutaneous fibrosarcomas and skin carcinomas, and that, when inhaled by rats, it induced squamous cell carcinomas of the lung.

The third example is the history of vinyl chloride cancerogenicity.[5-10]

In 1971, following our early observations on the increased incidence of atypical cells in the sputa of workers exposed to vinyl chloride and the results of Viola *et al.*,[11, 12] which showed that rats that experimentally inhaled 30,000 ppm of this monomer developed Zymbal gland carcinomas, we undertook a large program of experiments to study the effects of vinyl chloride, administered at different doses and by different routes, on animals of different species, strains, sexes, and ages.

In August 1972, we realized that this compound was producing angiosarcomas of the liver and of other sites and nephroblastomas other than Zymbal gland carcinomas.

These results were communicated by us, among others, to the factories that supported our studies, and to the Manufacturing Chemists Association, to enable them to adopt adequate preventive measures and to promote these epidemiologic investigations, which in December 1973 first identified an occupational tumor, a liver angiosarcoma in a worker of a United States factory (B. F. Goodrich in Louisville, Kentucky) that was producing vinyl chloride-polyvinyl chloride (VC-PVC).

No histologic difference could be found among the angiosarcomas in animals and in man exposed to vinyl chloride.

Updated results of some experiments on VC carcinogenicity are shown in TABLE 1–9.

The onset of several hepatomas among animals experimentally treated with VC (TABLE 10) is particularly relevant, in light of recent observations by Selikoff [13] and Popper [14] of a few cases of these tumors among workers of VC-PVC industries.

All types of tumors in man that have so far been found to be correlated in some way to VC exposure are included among those already observed in animals (TABLE 11).

Experiments are now underway in our laboratories on the effects on rats of inhalational exposure to 25, 10, 5, and 1 ppm of VC and of oral administration of 1, 0.3, and 0.03 mg/kg of body weight of the monomer, with the same schedule of experiments BT1 and BT11, respectively.

From the results presented on VC carcinogenicity in laboratory animals, it appears that experimental bioassays may predict carcinogenicity, give indications of target organs, give information on the risk level based on the dose-response relationship, and may be able to assess the risk in function of the route of exposure.

At present, much emphasis is focused on VC carcinogenicity. We hope that

TABLE 1

EXPERIMENTS BT1 AND BT6: EXPOSURE BY INHALATION TO VC IN AIR AT 30,000, 10,000, 6000, 2500, 500, 250 AND 50 PPM FOR 4 HR. DAILY, 5 DAYS PER WEEK FOR 52 WEEKS. RESULTS AFTER A 135-WEEK PERIOD

Groups and Treatment	Total No. of Animals (Sprague-Dawley rats) ♀ and ♂	No. of Animals with Tumors									
		Zymbal Gland Carcinomas	Nephroblastomas	Angiosarcomas		Subcutaneous Angiomas	Skin Carcinomas *	Hepatomas	Brain Neuroblastomas	Mammary Carcinomas	Pregastric Papillomas
				Liver	Other Sites						
I VC, 30,000 ppm	60	35	—	18	1	1	1	1	1	2	11
II VC, 10,000 ppm	69	16	5	9	3	4	3	1	7	3	—
III VC, 6000 ppm	72	7	4	13	3	3	1	1	3	—	—
IV VC, 2500 ppm	74	2	6	13	3	3	1	2	5	1	—
V VC, 500 ppm	67	4	4	7	2	1	1	3	—	1	—
VI VC, 250 ppm	67	—	6	4	2	—	4	—	—	1	—
VII VC, 50 ppm	64	—	1	1	1	1	1	—	—	2	—
VIII No treatment	68	—	—	—	—	—	—	—	—	—	—
Total	541	64	26	65	15	13	12	8	16	10	11

* Most of these tumors occurred in the sebaceous glands.

TABLE 2

EXPERIMENT BT2: EXPOSURE BY INHALATION TO VC IN AIR AT 200, 150, AND 100 PPM FOR 4 HR DAILY, 5 DAYS PER WEEK FOR 52 WEEKS. RESULTS AFTER 89 WEEKS

Groups and Treatment	Animals (Sprague-Dawley rats) ♀ and ♂		No. of Animals with Tumors			
			Zymbal Gland Carcino-mas	Nephro-blastomas	Angiosarcomas	
	Total	Survivors			Liver	Other Sites
I VC, 200 ppm	120	41	—	2	7	1
II VC, 150 ppm	120	45	—	4	3	1
III VC, 100 ppm	120	49	1	8	1	—
IV No treatment	185	76	1	—	—	1
Total	545	211	2	14	11	3

this emphasis will not mask the more general problems and the multitude of needs of occupational cancerogenesis.

In our Institute, other compounds have been or are being tested. Experimental bioassays have been performed on chromium compounds and other inorganic pigments by subcutaneous injection of rats (TABLE 12). These results point out the potential risk of some of the most widespread inorganic pigments.

Experiments have also been performed to assess the oncogenic potential of asbestos on peritoneal mesothelium, by injecting crocidolite into the abdominal cavity (TABLE 13). Our data are consistent with the well-known epidemiologic results that point out the high risk among asbestos workers of developing peritoneal mesotheliomas.

Carcinogenicity bioassays on several other compounds produced and distributed on a large scale are now underway; the compounds being tested include monomers used in plastic industries, such as styrene, acrylonitrile, and vinylidene chloride; polymers; estrogens and progestins; and petroleum proteins.

Let us hope that experimental bioassays will no longer be procrastinated due to skepticism of their validity, based on past results; fatalistic resignation, because of the huge number of newly produced compounds; and complications of elaborate testing procedures.

In regard to skepticism, I think that we should now critically review the methods and results of past experiments, which may now appear to have been poorly conducted.

With regard to the large number of compounds, it is true that there are already millions of newly produced chemicals; however, those that urgently need to be tested, mainly because of their widespread use, actually number only in the hundreds.

Concerning complications in performing elaborate testing procedures, it is true that the more experimental tests on animals reproduce the conditions of human exposure, the more relevant they are to man; it should be pointed out,

TABLE 3

EXPERIMENT BT3: EXPOSURE BY INHALATION TO VC IN AIR AT 10,000, 6000, 2500,500 250 AND 50 PPM 4 HR DAILY, 5 DAYS PER WEEK FOR 17 WEEKS. RESULTS AFTER 114 WEEKS*

Groups and Treatment	Animals (Sprague-Dawley) rats) ♀ and ♂		No. of Animals with Tumors				
	Total	Survivors	Zymbal Gland Carcinomas	Nephroblastomas	Angiosarcomas: Liver	Angiosarcomas: Other Sites	Brain Neuroblastomas
I VC, 10,000 ppm	60	1	6 (16)	1 (5)	— (9)	1 (3)	6 (7)
II VC, 6000 ppm	60	3	6 (7)	1 (4)	1 (13)	1 (3)	2 (3)
III VC, 2500 ppm	60	3	3 (2)	2 (6)	1 (13)	2 (3)	2 (5)
IV VC, 500 ppm	60	10	1 (4)	— (4)	1 (7)	1 (2)	—
V VC, 250 ppm	60	4	—	2 (6)	— (4)	— (2)	—
VI VC, 50 ppm	60	9	—	—	—	2	—
VII No treatment	190	35	—	—	—	1	—
Total	550	65	16 (29)	6 (25)	3 (46)	8 (13)	10 (15)

* In parentheses are recorded the number of tumors observed after 114 weeks among the Sprague-Dawley rats in experiment BT1, in which the animals were treated for 52 weeks.

TABLE 4

EXPERIMENT BT7: EXPOSURE BY INHALATION TO VC IN AIR AT 10,000, 6000, 2500, 500, 250 AND 50 PPM FOR 4 HR DAILY, 5 DAYS PER WEEK FOR 52 WEEKS. RESULTS AFTER 88 WEEKS *

Groups and Treatment	Animals (Wistar rats) ♂		No. of Animals with Tumors					
	Total	Survivors	Zymbal Gland Carcinomas	Nephroblastomas	Angiosarcomas: Liver	Angiosarcomas: Other Sites	Hepatomas	Brain Neuroblastomas
I VC, 10,000 ppm	30	1	1 (10)	1 (3)	8 (4)	— (2)	— (1)	1 (2)
II VC, 6000 ppm	30	4	— (3)	3 (3)	2 (3)	1 (1)	1 (1)	1 (2)
III VC, 2500 ppm	30	4	— (1)	— (4)	2 (4)	1 (2)	— (1)	— (2)
IV VC, 500 ppm	30	10	— (2)	— (2)	2	—	— (2)	—
V VC, 250 ppm	30	8	—	— (1)	1	1	—	—
VI VC, 50 ppm	30	11	—	—	—	—	—	—
VII No treatment	40	23	—	—	—	—	—	—
Total	220	61	1 (16)	4 (13)	15 (11)	3 (5)	1 (5)	2 (6)

* In parentheses are recorded the number of tumors observed after 88 weeks among a comparable group of male Sprague-Dawley rats treated in the same way.

TABLE 5

EXPERIMENT BT5: EXPOSURE OF BREEDERS BY INHALATION TO VC IN AIR AT 10,000 AND 6000 PPM 4 HR DAILY FOR 1 WLEK (FROM 12TH TO 18TH DAYS OF PREGNANCY). RESULTS AFTER 115 WEEKS

Groups and Treatment	Animals (Sprague-Dawley rats)		No. of Animals with Tumors			
	Total	Survivors	Zymbal Gland Carcinomas	Nephroblastomas	Angiosarcomas Liver	Angiosarcomas Other Sites
I VC, 10,000 ppm, breeders	30	—	1	—	—	1
II VC, 6000 ppm, breeders	30	—	—	—	—	—
III VC, 10,000 ppm, offspring	54	12	3	1	—	2
IV VC, 6000 ppm, offspring	32	8	1	—	—	2
Total	146	20	5	1	—	5

however, that tests of agents should follow a pattern that progresses in degree of precision and scrutiny so as to eventually filter out and expose the most dangerous compounds.

Finally, I should mention that we must place emphasis on both scrutiny of compounds already produced and in widespread use and on compounds yet to be produced, before economic, social, and political interests become so entrenched that solution of these problems will be difficult indeed.

TABLE 6

EXPERIMENT BT14: EXPOSURE OF NEWBORN RATS BY INHALATION TO VC IN AIR AT 10,000 AND 6000 PPM 4 HR DAILY, 5 DAYS WEEKLY, FOR 5 WEEKS (FROM 1 DAY TO 5 WEEKS OF AGE) RESULTS AFTER 48 WEEKS

Groups and Treatment	Animals (Sprague-Dawley rats) ♀ and ♂		No. of Animals with Tumors				
	Total	Survivors	Zymbal Gland Carcinomas	Nephroblastomas	Angiosarcomas Liver	Angiosarcomas Other Sites	Hepatomas
I VC, 10,000 ppm	46	42	—	—	1	—	1
II VC, 6000 ppm	43	41	—	—	1	—	2
Total	89	83	—	—	2	—	3

TABLE 7

EXPERIMENT BT4: EXPOSURE BY INHALATION TO VC IN AIR AT 10,000, 6000, 2500, 500, 250 AND 50 PPM FOR 4 HR DAILY, 5 DAYS WEEKLY, FOR 30 WEEKS. RESULTS AFTER 81 WEEKS (END OF THE EXPERIMENT)

Groups and Treatment	Animals (Swiss mice) ♀ and ♂		No. of Animals with Tumors					
	Total	Survivors	Pulmonary Tumors	Mammary Carcinomas *	Liver Angiosar-comas	Vascular Tumors of Other Types and/or Sites	Epithelial Tumors of the Skin †	Pregastric Papillomas
I VC, 10,000 ppm	60	—	35	13	8	9	3	1
II VC, 6000 ppm	60	—	38	8	5	9	6	1
III VC, 2500 ppm	60	—	30	9	11	12	3	1
IV VC, 500 ppm	60	—	38	7	11	18	1	—
V VC, 250 ppm	60	—	33	11	11	22	2	—
VI VC, 50 ppm	60	—	2	12	1	13	—	—
VII No treatment	150	—	8	—	—	1	—	—
Total	510	—	184	60	47	84	15	3

* Most included squamous metaplasia.
† Some occurred in sebaceous glands.

TABLE 8

TABLE 8

EXPERIMENT BT8: EXPOSURE BY INHALATION TO VC IN AIR AT 10,000, 6000, 2500, 500, 250 AND 50 PPM 4 HR DAILY, 5 DAYS WEEKLY, FOR 30 WEEKS. RESULTS AFTER 76 WEEKS

Groups and Treatment	Animals (Golden hamsters) ♂		No. of Animals with Tumors							
			Liver Tumors			Skin Trichoepitheliomas and Basaliomas	Melanomas	Lymphomas *	Pregastric Papillomas and Acanthomas	Others
	Total	Survivors	Angiosarcomas	Angiomas	Hepatomas					
I VC, 10,000 ppm	35	1	—	—	—	6	1	—	4	2 (1 subcutaneous angioma) (1 adenocarcinoma of gallbladder)
II VC, 6000 ppm	32	3	—	2	1	2	2	2	4	—
III VC, 2500 ppm	33	4	—	3	1	1	—	1	8	—
IV VC, 500 ppm	33	4	2	—	—	3	—	1	4	(1 subcutaneous angioma) (1 bronchial carcinoma)
V VC, 250 ppm	32	4	—	—	—	3	—	1	—	—
VI VC, 50 ppm	33	5	—	—	—	6	1	1	2	—
VII No treatment	70	14	—	—	—	2	—	2	—	—
Total	268	35	2	5	2	23	4	8	22	4

* Average latency times were 48 weeks in treated animals and 92 weeks in controls.

TABLE 9

EXPERIMENT BT11: EXPOSURE BY INGESTION (STOMACH TUBE) TO VC IN OLIVE OIL AT 50.00, 16.65, AND 3.33 MG/KG BODY WEIGHT, ONCE DAILY, 4–5 DAYS WEEKLY, FOR 52 WEEKS. RESULTS AFTER 55 WEEK.

Groups and Treatment	Animals (Sprague-Dawley rats)		No. of Animals with Tumors			
			Zymbal Gland Carci-		Angiosarcomas	
	Total	Survivors	nomas	Nephroblastomas	Liver	Other Sites
I VC, 50.00 mg/kg	80	57	—	—	1	1
II VC, 16.65 mg/kg	80	66	—	—	1	—
III VC, 3.33 mg/kg	80	62	—	—	—	—
IV Control: olive oil	80	68	—	—	—	—
Total	320	253	—	—	2	1

TABLE 10

HEPATOMAS OBSERVED SO FAR ON RATS AND HAMSTERS EXPOSED BY INHALATION TO VC IN AIR, 4 HR DAILY, 5 DAYS WEEKLY

Animals		Treatment	No. of Hepatomas
Species, Strain, Age	No.		
Sprague-Dawley rats 13 weeks old	60	30,000 ppm, 52 weeks	1
	69	10,000 ppm, 52 weeks	1
	72	6000 ppm, 52 weeks	1
	74	2500 ppm, 52 weeks	2
	67	500 ppm, 52 weeks	3
Sprague-Dawley rats, 1 day old	46	10,000 ppm, 5 weeks	1
	43	6000 ppm, 5 weeks	2
Wistar rats, 11 weeks old	30	6000 ppm, 52 weeks	1
Golden hamsters 11 weeks old	32	6000 ppm, 30 weeks	1
	33	2500 ppm, 30 weeks	1
Total			14

TABLE 11

TUMORS PRESENTLY CORRELATED TO VC EXPOSURE (BY INHALATION) ON EXPERIMENTAL RODENTS AND MAN

	Tumors										
Species	Angiosarcomas of Liver	Tumors of Brain	Tumors of Lung	Lymphomas and Leukemias	Angiosarcomas and Angiomas of Other Sites	Nephroblastomas	Sebaceous Cutaneous Carcinomas	Squamous Tumors of Epidermis	Mammary Carcinomas	Hepatomas	Pregastric Papillomas and Acanthomas
Rat	+	+			+	+	+	+	(+)	+	+
Mouse	+		+		+		+	+	+		+
Hamster	+		(+)	(+)	(+)			+		(+)	+
Man	+	(+)	(+)	(+)						(+)	

TABLE 12

EXPERIMENTS BO12 AND BT2001: TREATMENT BY ONE SUBCUTANEOUS INJECTION OF 30 MG OF THE COMPOUND IN 1 CC OF H_2O

Groups and Treatment	Animals (Sprague-Dawley rats)		Tumors (sarcomas at the site of injection)			Length of Experiments (weeks)
	Total	Survivors	No. of Animals with Tumors	Average Latency Time (weeks)	Histology Type	
I, Chromite	40	—	—	—	—	133
II, Neochromium	40	—	9	78	rhabdomyosarcomas and fibrosarcomas	149
III, Chromium allumen	40	—	8	72	rhabdomyosarcomas and fibrosarcomas	137
IV, Chromium yellow (lead chromate)	40	—	26	40	rhabdomyosarcomas and fibrosarcomas	150
V, Chromium orange (lead chromate)	40	—	27	47	rhabdomyosarcomas and fibrosarcomas	150
VI, Molybdenum orange (lead chromate, sulfate, and molybdate)	40	—	36	32	rhabdomyosarcomas and fibrosarcomas	117
VII, Cadmium yellow (cadmium sulfide)	40	—	16	86	rhabdomyosarcomas and fibrosarcomas	143
VIII, Iron yellow (iron oxide)	40	—	1	77	rhabdomyosarcomas and fibrosarcomas	125
IX, Iron red (iron oxide)	40	—	—	—	rhabdomyosarcomas and fibrosarcomas	134
X, Control	140	—	—	—	—	127

TABLE 13

EXPERIMENT BO8: TREATMENT BY ONE ENDOPERITONEAL INJECTION OF 25 MG OF CROCIDOLITE IN 1 CC OF SALINE

Groups and Treatment	Animals (Sprague-Dawley rats)			Animals with Peritoneal Mesotheliomas				
	Sex	No.	Corrected No.*	No.	%	Average Latency Time (weeks)	Animals with Metastases	
I, Crocidolite	♀	50	49	34	69.3	73	13	38.2
	♂	50	48	31	64.5	65	13	41.9
II, Saline (control)	♀	45	45	—	—	—	—	—
	♂	15	15	—	—	—	—	—

* Animals alive when the first peritoneal tumor arose.

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