Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Laboratory Models in Carcinogenesis Based on Life-span Long-term Bioassays

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ABSTRACT

At present, long-term carcinogenicity bioassay is the best available tool for identifying environmental carcinogens, including weak and slow-acting ones, for revealing the effects of low doses, and for comparing carcinogenic potency of different agents.

From basic studies on carcinogenesis it is known that (1) under a given carcinogenic treatment the same and different species and strain of animals may develop different types of tumours with different latency periods, (2) the period of latency of tumours produced by weak and slow-acting carcinogens may be very long, and (3) the lower the dose of the carcinogen, the lower is the incidence and the longer the latency period of tumours associated with the treatment.

Therefore, life-long bioassays seem to be the most appropriate way to reveal the full impact of a carcinogenic treatment and to provide a standardized protocol, allowing comparative evaluation of carcinogenicity of different agents.

1 INTRODUCTION

At the present time, long-term carcinogenicity bioassay is the most valid laboratory tool for identifying and quantifying chemical carcinogens that may be present in the occupational or general environment. To be adequate, such bioassays must be able to:

(1) produce results which may be used to predict the carcinogenicity of chemicals for human subjects regarding:
   (a) carcinogenic capacity in general;
   (b) carcinogenic potency;
   (c) and, possibly, the target tissue(s);
(2) identify slow-acting carcinogens;
(3) reveal the carcinogenic effects of low doses of already known carcinogens.

2 EXTRAPOLATION OF ANIMAL RESULTS TO HUMANS (PREDICTION)

2.1 Prerequisites

It is known that carcinogenic response depends on a series of biological and experimental factors, and on laboratory procedures.

2.1.1 Biological Factors

The magnitude and the type of neoplastic response (specific tumours) are largely determined by the basic 'spontaneous' tumorigram and by the enzymatic profile of the animal. Both factors may vary greatly depending on the species, strain, sex and age of experimental animals used.

Therefore, for long-term carcinogenicity bioassays, it would be proper to use animals whose tumorigram and metabolic profile are similar to the average human organism.

2.1.2 Experimental Factors

The neoplastic response depends on the physico-chemical characteristics of the agent (affecting its distribution in the body); the dose (dose–response relationship); the number and/or duration of treatments; often on route of administration, and, when they are used, on carriers.

An important experimental factor is the time of observation of animals. The likelihood of neoplastic response increases with the age of animals and the time interval since the beginning of the treatment. Therefore, the life-long duration of the experiment is the basic condition for exploring the full carcinogenic potential of the agent, apart from the consideration that, in theoretical terms, this condition should be a mandatory prerequisite for experiments aiming at providing data for cancer risk assessment in humans.

The experiments may be designed in such a way as:

—to reproduce, to the extent possible, the human situation;
—to expose, to the extent possible, the full carcinogenic potential of the agents studied, by testing at the maximum tolerated dose (concentration and length of treatment) and keeping the animals under observation as long as possible;
—to quantify the carcinogenic response by testing at different doses, including the ones in the range of human exposures (which are often low);
—to compare the relative carcinogenicity and general toxicity of different agents, a goal which may be pursued by keeping all the biological and experimental factors highly standardized and by maintaining animals alive until spontaneous death, thus avoiding variability due to arbitrary choice of the time of observation;
—to use a sufficient number of animals for statistical analysis.

2.1.3 Laboratory Methods

Laboratory methods have been specified in recommendations on good laboratory practice (for example, FDA, 1982) and in guidelines (see OECD, 1981).

The adequacy of long-term bioassays strongly depends on:

(1) the maintenance of animals under physiological conditions (housing, feeding, cleaning, handling), which also affect survival;
(2) the adherence to accepted experimental protocols; and
(3) the quality of monitoring of pathological effect, by behavioural and clinical observation and by laboratory and pathological examinations.

It is my conviction that in general, with few exceptions, the prerequisites for adequate testing (protocol design, conduct of experiments, evaluation and interpretation of results) in laboratories involved in long-term carcinogenicity bioassays throughout the world are not infrequently below scientific needs and regulatory requirements.

2.2 Examples

2.2.1 Prediction of the Carcinogenic Potential

Experiments performed in our laboratory on vinyl chloride have clearly shown that long-term carcinogenicity bioassays can predict carcinogenicity of an agent in humans (Table 1) (Maltoni and Lefemine, 1975; Maltoni, 1977; Maltoni *et al.*, 1981).

2.2.2 Quantification of Carcinogenic Risk of a Given Agent

Experiments performed in our laboratory, again on vinyl chloride, have demonstrated that long-term carcinogenicity bioassays are of great value for the quantification of risk, with particular reference to the level of exposure (dose–response relationship) (Table 2) (Maltoni *et al.*, 1980a, 1981).

2.2.3 Qualitative and Quantitative Comparison of the Effects of Different Agents

Through experiments performed in our laboratory it has been demonstrated that long-term carcinogenic bioassays are a valid tool for comparing the effects of the different tested compounds (Table 3) (Maltoni, 1977; Maltoni *et al.*, 1977, 1980a, b, 1981).

Table 1 Tumours associated with exposure to vinyl chloride of experimental rodents and humans. (Maltoni, 1977; Maltoni *et al.*, 1981) (1)

Species	Angio-sarcomas of liver	Tumours of brain	Tumours of lung	Lympho-mas and leukaemias	Hepa-tomas	Mam-mary car-cinomas	Angio-sarcomas and an-giomas of other sites	Nephro-blasto-mas	Seba-ceous cuta-neous carci-nomas	Other cuta-neous epithe-lial tumours	Forestom-ach pap-illomas and acan-thomas	Mela-nomas
Rat	+	+			+	+	+	+	+	(+)	+	
Mouse	+		+			+	+			(+)	(+)	
Hamster	+			(+)			(+)			(+)	+	(+)
Humans	+	+	(+)	(+)	(+)	(+)						

(1) + : conclusive evidence
(+): strong, but not conclusive, evidence

Table 2 Incidence of total malignant tumours in Sprague-Dawley rats, in relation to concentration of vinyl chloride administered by inhalation 4 hours daily, 5 days weekly, for 52 weeks

Concentration (ppm)	Malignant tumours/100 animals		
	Males	Females	Total
10,000	80.0	83.3	81.7
6,000	46.7	73.3	60.0
2,500	53.3	73.3	63.3
500	23.3	80.0	51.7
250	23.3	36.7	30.0
50	6.7	23.3	15.0
0	0	26.7	13.3

The animals were kept under observation until spontaneous death (Maltoni *et al.*, 1980a, 1981).

Incidentally, results shown in Table 3 demonstrate that small changes in the molecules may significantly modify the carcinogenicity of a chemical.

3 IDENTIFICATION OF SLOW-ACTING CARCINOGENS

3.1 Prerequisites

It is known that there are carcinogens which produce tumours with a long latency time, even at the maximum tolerated doses. Those carcinogens have generally been classified as weak carcinogens, a definition which, in my opinion, is not correct. Weak carcinogens are agents which produce a low incidence of tumours with a long latency time, usually in the most responsive biological systems. Slow-acting carcinogens include not only weak carcinogens, but also agents which may produce a high incidence of tumours, not necessarily in particularly responsive organs, but with a very long latency time.

In order to identify slow-acting carcinogens it is, therefore, necessary to keep animals under observation as long as possible, i.e., until spontaneous death.

3.2 Examples

Among the many available examples, the results of long-term experimental bioassays performed in our laboratory for styrene oxide clearly support the need to extend the observation to complete life-span in order to detect carcinogenic effects of slow-acting carcinogens, given the long latency time of the tumours associated with treatment (Table 4) (Maltoni *et al.*, 1979; Maltoni, 1983).

Table 3 Comparative effects of three related compounds—vinyl chloride (VC), vinylidene chloride (VDC) and ethylene dichloride (EDC) tested by inhalation in the same animal systems, according to a comparable experimental protocol

Compound	Species	Angiosarcomas of liver	Tumours of brain	Tumours of lung	Hepatomas	Angiosarcomas and angiomas of other sites	Tumours of kidney: Nephroblastomas	Tumours of kidney: Adenocarcinomas	Sebaceous cutaneous carcinomas	Other cutaneous epithelial tumours	Mammary carcinomas	Forestomach papillomas and acanthomas
VC (1)	Rat (Sprague-Dawley)	+	+		+	+	+		+	(+)	+	+
	Mouse (Swiss)	+		+		+				(+)	+	(+)
VDC (1)	Rat (Sprague-Dawley)											
	Mouse (Swiss)			(+)				+				
EDC (1)	Rat (Sprague-Dawley)											
	Mouse (Swiss)											

(1) For protocols see:
VC : Maltoni, 1977; Maltoni *et al.*, 1980a, 1981.
VDC: Maltoni *et al.*, 1977.
EDC: Maltoni *et al.*, 1980b.

Table 4 Incidence and latency time of forestomach carcinomas in Sprague-Dawley rats, following exposure by ingestion (stomach tube) to styrene oxide, in olive oil, at 250 and 50 mg/kg, once daily, 4–5 days weekly, for 52 weeks

		Forestomach squamocellular carcinomas	
Concentration (mg/kg)	Sex	% of tumour bearing animals	Average latency time (weeks)
250	M	41.0	110.9
	F	52.6	105.3
	M + F	46.7	107.8
50	M	23.1	104.9
	F	18.9	121.1
	M + F	20.0	112.0
0 (olive oil alone)	M	0	—
	F	0	—
	M + F	0	—

The animals were kept under observation until spontaneous death (the last animal died after 156 weeks from the start of the experiment) (Maltoni *et al.*, 1979; Maltoni, 1983).

4 REVEALING THE EFFECTS OF LOW DOSES OF CARCINOGENS

4.1 Prerequisites

On the basis of a multitude of experiments using different carcinogens, tested in different ways and on various animal systems, it has been shown that, all other factors being standardized, there is a direct relationship between dose and neoplastic response. In general terms, the higher the dose of a carcinogen, the higher is the incidence of tumours (number of animals bearing tumours and number of tumours per tumour-bearing animal) and the shorter the latency period. By lowering the doses the incidence decreases and the latency period is prolonged.

Studying, by experimental bioassays, very low doses (which are often in the range of human exposure), it must be expected that the neoplastic response may manifest itself in a very low incidence of tumours with long latency period, i.e., at a very late age.

To reveal the carcinogenic effects of low doses of carcinogens it is, therefore, necessary:

—to use young animals and to keep them under observation until spontaneous death, in order to provide as much time as possible for a carcinogen to manifest its potential;

—to use groups of animals large enough to guarantee the survival of a sufficient number of animals at old age.

4.2 Examples

The effects of a large range of doses of vinyl chloride, administered by inhalation and ingestion, have been studied in our laboratory in Sprague-Dawley rats. The incidence and latency time of liver angiosarcomas in relation to the dose of the monomer are shown in Table 5.

The results indicate that:

—the incidence of tumours decreases with decreasing dose;
—the latency period tends to increase;
—the latency period may be very long at low doses. Historically, the first liver angiosarcoma reported in rodents following exposure to 50 ppm of vinyl chloride by inhalation was observed with experiment BT1, in a rat that died after 135 weeks from the beginning of the experiment (148 weeks old). This observation provided the basis for the 1974 US Occupational Safety and Health Administration standard which lowered the permissible exposure limit for vinyl chloride at the workplace to 1 ppm. The experimental result was then confirmed with experiment BT9, for which a larger number of animals was used;
—liver angiosarcoma may arise in untreated animals although with a low incidence and at an old age.

These observations were largely made possible by keeping the animals under observation until spontaneous death.

5 THE SUPERIORITY OF LIFE-SPAN VERSUS TRUNCATED LONG-TERM BIOASSAYS

In conclusion, life-long bioassays appear to be more adequate than truncated ones for the following reasons:

—they represent an experimental model equivalent to the human situation;
—they make it possible for a carcinogen to express all its neoplastic potential, and the degree of its carcinogenic potency, in different organs and tissues;
—they are a more suitable model for identifying slow-acting carcinogens;
—they permit a better monitoring of low dose effects of the carcinogenic agents;
—they provide a model for comparing relative carcinogenicity of different, related and unrelated, agents;
—and, last but not least, they provide useful information on the mechanism of carcinogenesis, and particularly on what we believe to be a crucial point, namely: do carcinogens act predominantly or exclusively by producing tumours *ex novo*, or enhancing and anticipating the 'expected' tumours in animals used, which often manifest themselves with a low incidence and at a late age?

Table 5 Incidence and average latency time of liver angiosarcomas in relation to vinyl chloride doses, in Sprague-Dawley rats, treated by inhalation (4 hours daily, 5 days weekly, for 52 weeks) and by ingestion (once daily, 5 days weekly, for 52–59 weeks)

Inhalation						Ingestion (by gavage in olive oil)					
	Liver angiosarcomas (2)						Liver angisarcomas (2)				
Experiment	Concentration	No. of animals	%	Average latency time (weeks) (1)		Experiment	Daily dose (in mg)	No. of animals	%	Average latency time (weeks) (1)	
BT 6	30,000	60	30.0	53.7	(70.7)	BT 11	50.00	80	21.0	78.7	(91.7)
BT 1	10,000	60	11.7	66.4	(79.4)	BT 11	16.65	80	12.0	79.3	(92.3)
BT 1	6,000	60	22.0	74.0	(87.0)	BT 11	33.33	80	0	—	
BT 1	2,500	60	21.7	77.4	(90.4)	BT 27	1.00	150	2.0	107.3	(117.3)
BT 1	500	60	10.0	84.7	(97.7)	BT 27	0.30	150	0.7	101.0	(111.0)
BT 1	250	60	5.1	76.0	(89.0)	BT 27	0.03	150	0	—	
BT 2	200	120	10.0	93.4	(106.4)						
BT 2	150	120	5.0	89.5	(102.5)						
BT 2	100	120	0.8	85.0	(98.0)						
BT 1	50	60	1.7	135.0	(148.0)						
BT 9	50	300	4.8	95.0	(108.0)						
BT 15	25	120	4.2	89.0	(102.0)						
BT 15	10	120	0.8	79.0	(92.0)						
BT 15	5	120	0	—							
BT 15	1	120	0	—							
Controls	0	465	0	—		Controls (olive oil)	0	230	0	—	
Historical controls	0	4,200	0.09	114.5	(124.2)						

The animals were kept under observation until spontaneous death. (Maltoni *et al.*, 1981)

(1) Out of parentheses the period from the start of the experiment; between parentheses the age of bearing animals.

(2) There are few mild discrepancies in dose–response relationships and also in two groups treated with the same dose of the monomer. This effect may be partially due to the differences in the number of treated animals/group and/or to the interference of other vinyl chloride related tumours, with various latency time (since vinyl chloride is a multipotential carcinogen).

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