

The Scientific and Methodological Bases of Experimental Studies for Detecting and Quantifying Carcinogenic Risks

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ABSTRACT: This paper outlines the aims and potential scope of experimental research for risk identification and assessment in industrial carcinogenesis (environmental and occupational). It then reviews the basic, general, and specific requisites of a rigorously scientific nature that are required to render experiments to be more appropriate and better geared to the information they seek. A range of experimental approaches to risk assessment are illustrated by results achieved in the Cancer Research Centre of the Ramazzini Foundation (CRC/RF). The paper ends with a call for closer relations and integration among experimental, epidemiologic, and biostatistical studies.

INTRODUCTION TO THE ROLE OF EXPERIMENTAL CARCINOGENICITY STUDIES (BIOASSAYS)

The risks of cancer can be identified, characterized, and quantified by means of two basic methodologies, epidemiologic research on man and experimental research on animals.

Epidemiology studies would theoretically be the most direct method. However, this method grows less and less viable, in that it presupposes prolonged exposure of a human population. It is also complicated by a series of factors: (1) the need for extremely long observation periods, according to the length of latency time; (2) the large number of confounding situations; (3) the ensuing need to find sufficiently broad and relatively homogeneous exposed populations as well as proper control groups; (4) the complex web of political interference. Such factors explain why far too few epidemiology studies are being performed, why they often give ambiguous or borderline results, and why, if they are to give any useful result, their duration tends to outstrip that of the industrial compounds they refer to and the market for these compounds.

Experimental studies on animals, if conducted in keeping with certain binding scientific requirements, may provide information that leads not only to identification of cancer risk factors, but also to the quantification of those cancer risks according to the dose (dose-response ratio), duration, and chronology of exposure. This applies both to characterization of specific risks (i.e., the type of tumor produced), and to

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correlating the biological risk factors with exposed individual/population characteristics. Such information may be extrapolated to man and form a basis for preventive norms and strategies. The experimental trial run by our laboratories on vinyl chloride, the largest trial ever published on a single chemical agent, stands as a paradigm.¹ To date, bioassays have been performed on some 200 industrial agents^{2,3} by the CRC/RF. Many of the agents studied have involved trials that are comparable with that for vinyl chloride. Such studies concern, for example, gamma radiation, vitamins, and aldehydes. Many of these studies have been published, some are being written up, some are still in progress. It has been claimed that experimental research is unduly expensive, especially when run on adequate groups and using a sufficiently large number of animals with the trials protracted throughout their life. Such a fallacy cannot be too forcibly rebutted. It is without substance, serving as an excuse to justify limiting the number of adequate bioassays conducted on environmental carcinogens, especially of an industrial kind. The real cost of such studies, as with our mega-experiment on vinyl chloride, is meager in comparison with the economic impact of the agents being studied, and damage to health and environment that they may cause. Accordingly, we should persevere with the experimental studies. They can: (1) make a decisive contribution to risk assessment, which forms the kernel of primary tumor prevention in the case of agents that are already at large in the environment; and (2) offer viable prospects for predicting the existence of potential cancer risks from agents that result from mass production and commercialization.

To date, attention has been limited, almost exclusively, to cancer risks from exposure to individual agents. However, humans are exposed, at the same or at different times, to a whole range of carcinogens, whereas a body of data in the literature suggests that exposure to multiple carcinogens may give rise to additional/multiplicative effects (syncarcinogenesis). The time has come to promote syncarcinogenesis research and to deal with multiple carcinogen exposure and mixtures of various agents. The data and arguments that follow concern both single and multiple exposures.

BASIC KNOWLEDGE OF CARCINOGENESIS TO BE CONSIDERED IN EXPERIMENTAL CARCINOGENICITY BIOASSAYS

Physical or chemical carcinogens occur with a range of carcinogenetic power. In general highly potent agents are found to give rise to a high incidence of tumors, with a relatively short latency time (high potency carcinogen). Others have a low tumor incidence and a long latency (low potency carcinogen). Between these extremes lie agents with various degrees of carcinogenetic power. Chemical carcinogens may act directly on the tissues (direct carcinogens), or may achieve their effect by metabolic biotransformation in the organism (procarcinogens).

The type of tumor produced partly depends on the physicochemical properties of the agents involved, how they spread and are metabolized by the organisms exposed, how they are administered (since this may affect both absorption and diffusion/metabolism), and how toxic they are. The latter may in turn affect the weight and survival of the animal. Such factors are known to play an important part in the neoplastic response. A wide range of data show the connection between exposure

level (dose/agent concentration multiplied by duration of treatment) and neoplastic response.

The type of animal experiment is all important. According to the species, strain and sex of the animals, large differences are to be found in their tendency for spontaneous general or specific tumor formation (basic tumorigram). Under the effect of carcinogens, various experimental animal types tend to preferentially develop, with a greater incidence and/or shorter latency time, those tumors that they are prone to generate spontaneously. There are, therefore, both qualitative and quantitative parallels between the basic tumorigram and the type of neoplastic response to be expected from exposure to carcinogens. This emerges clearly in the case of agents that, by virtue of their properties, are able to reach the various anatomical regions of an organism.⁴ The choice of animal, therefore, has a decisive influence on the results of experimental carcinogenicity trials, both in assessing the general carcinogenicity of an agent, and in defining the precise oncological action for the site and type of tumor produced. The age of animals at the start of exposure also affects the neoplastic response. Biological targets may react differently at various ages.

Many carcinogens have been shown to be multipotent; that is, to cause various kinds of tumor in various tissues and organs of various kinds of animals. This effect depends on the ability of the agent to reach targets in various organs and tissues, and on the specific tissue/organ responsiveness of the animals studied. From the evidence we possess and basic assumptions from biology, we may conclude that, if appropriately tested, all carcinogens are presumably multipotent. The possibility that an agent may cause various kinds of tumor leads to a sort of tumor competition. More specifically, depending on the experimental conditions, animals treated with a carcinogen die mainly of high-incidence/short-latency tumors, thus curtailing the chance for other neoplasias to develop.

The neoplastic response depends not only on the kind of agent, its physicochemical and toxicologic properties, the mode of exposure, and the type of animal, but also to a great extent, on the length of the biophase in relation to the latency time of the tumor being caused, which varies and may be very long. The experimental findings concur that the latent neoplastic potential for causing a tumor increases with the length of the biophase (i.e., observation time or age). That is why we are convinced that experimental carcinogenicity trials should continue until spontaneous animal death and not be cut short before. Cutting short an experiment after two years of biophase may mask a possible carcinogenic response. Beginning exposure in the embryo or neonate may have a positive effect on the neoplastic response, not only through the greater responsiveness of some organs at that age, but also because it prolongs the experimental biophase. For experiments to be planned correctly these basic notions of carcinogenesis must on no account be ignored.

GENERAL PLANNING AND METHODOLOGY PREREQUISITES FOR OPTIMIZING EXPERIMENTAL CARCINOGENICITY BIOASSAYS

There are several prerequisites that must be fulfilled if experimental carcinogenicity trials are to be optimized. Some of them are general in nature, others concern specific points of information that the bioassays are designed to provide.

The following general prerequisites are imperative, in our view, for protecting this branch of research from the amateur or anecdotal approach:

1. Use of animal species and strains whose basic tumorigram and kind of response to cancer stimuli is not too remote from the human counterpart. For example, one should avoid strains of mouse or rat that are peculiarly prone to certain kinds of tumor that may shorten their life-span, compete with other potential neoplastic latencies and, hence, cramp the onset of other kinds of tumor, and may potentially giving rise to metabolic alterations in the organism that confuse the picture of neoplastic response.

2. Continuing bioassays until the end of the life of an animal. Truncation of experiments is an artificial departure from the human model for the principal reason that, in humans, tumors tend to appear mainly in later life. According to the data of the Nominal Mortality Registry, for all causes of death, especially tumors, in Bologna Province, more than 85% of all deaths from cancer occur after age 60. Sacrificing mice or rats after about two years is like carrying out epidemiologic studies on man excluding the “third age” that is only taking subjects younger than 45–55 (in reference to the lifespan of the rodents most often used in bioassays).

3. Following the rules of Good Laboratory Practice as a minimum standard in experiment management. Those practices may of course themselves be improved.

4. Choosing precise parameters to assess neoplastic response. In our opinion, such parameters are: total number and percentage of animals carrying benign and malignant tumors and the various kinds of tumor; total number of benign and malignant tumors, and number of the various kinds per 100 animals (in view of the fact that one and the same animal may develop multiple tumors of various kinds at various sites); latency time for all specific benign and malignant tumors; and incidence of malignancy precursors.

5. Standardizing the experimental conditions for conducting experiments, parameter assessment, and data presentation. Thus, the results of various experiments may be compared and used, say, in assessing the relative cancer risk of various agents — an important factor in industrial decisions and prevention strategy.

All too frequently the failure to adopt such minimal standards leads, on one hand, to a spate of inadequate data and, on the other, to spawning nonintegrable, usually discordant, information that no amount of systematic revision will ever bring into line (and that obviously brings discredit on the whole experimental approach).

Planning experimental bioassays and setting the specific methodological prerequisites depends on the type and extent of the information the research is required to provide. The bioassay plan must, therefore, make it quite clear just what kind of information is desired.

WHAT INFORMATION MAY BE OBTAINED FROM BIOASSAYS

Potentially, bioassays may provide a whole range of scientific information. This may consist of:

1. Exposure of the carcinogenic potential of an agent in general terms, whatever the experimental conditions used, but always observing the rules outlined in the previous section.

2. Information on the effect of exposure routes and chronology with special regard to those features that link with human scenarios.
3. Information on the neoplastic response at various doses of the risk agents under test; that is, information on dose-response.
4. Indicating the organs that form the main targets, the types of tumor and precursors that are found with the agent under study.
5. Detection of any correlated or cancer-associated pathologies.
6. Information on the relative carcinogenic potency among agents.
7. Exposing weak/diffuse cancer risks, including those due to multiple exposure or mixtures of agents.

As previously mentioned, in planning a proper carcinogenicity bioassay one must clearly establish what information one wishes to know about, so that a targeted experiment protocol may be drawn up. As a general rule, it is not professional to make assumptions, or ask questions, about a bioassay beyond the scope for which it was specifically designed.

PLANNING BIOASSAYS IN RELATION TO THE INFORMATION FOR WHICH THEY ARE BEING PERFORMED

It follows from the foregoing argument that bioassays must take account of the list of general requisites common to all bioassays, as well as specific requisites that vary from experiment to experiment, and yet are absolutely essential to the rationale of the study in question.

Identifying Potential Carcinogens

In this case any type of responsive animal may be used (except for those overly prone to developing certain kinds of tumor), and any exposure route (even widely divergent from the routes encountered by humans), employing high doses, although not so high as to markedly shorten the life-span through toxic effects. Such experiments are obviously most limited. In particular, should the data prove negative, one cannot be sure that by varying the dose, exposure route, and, hence, the target organ one might not find them positive. In any case, the result is always to be interpreted within the context of the test conditions.

Information on the Effect of the Administration Route and the Exposure Chronology

The route of administration may affect distribution of the test agents and hence the tissue/organ dose of the agent or its biotransformation products, thereby conditioning the neoplastic response in qualitative or quantitative terms. The tissue or organ dose can likewise be affected by varying the exposure schedule. The administration route may again influence the neoplastic response with direct or topical action agents, depending on the responsiveness of the tissues they are brought into contact with. Hence, if one is seeking information on the effects of a dose and administration schedule that may be extrapolated to man, the experiment needs to test various forms of administration and various schedules, with close reference to the human scenario and its characteristics.

Information on the Dose and its Effect

It has long been known that the carcinogenic effect, however expressed, increases as the dose increases. Quantifying the risk in relation to the dose is of vital importance and is a *sine qua non* in deciding the compatibility or otherwise of an agent in the environment and *socially acceptable* exposure standards (although let it be stressed, once and for all, there are no biologically safe dose levels in carcinogenesis). The doses tested must, at least, include the highest tolerable dose, a dose of the order of those that humans are exposed to, and a midlevel dose. Naturally, the higher the number of doses studied, the better the information for quantitative assessment. In our bioassay project on vinyl chloride, 14 concentrations were tested by inhalatory exposure. Having those data available accelerated the implementation of international norms to establish an acceptable exposure level in the workplace.^{1,2}

Information on the Site and Type of Tumors and Their Precursors

In experimental conditions, various kinds of animal are prone to develop tumors and their precursors of various kinds. Thus, there is a range of neoplastic responses to carcinogen exposure in qualitative and quantitative terms. If we wish to acquire knowledge leading to a forecast for which human organs will be targeted by exposure to a given carcinogen, we must choose experimental animals with the closest possible tumorigram to that of man (or, at least, one that is not too dissimilar).

Information on Pathologies in some way Related to Carcinogens

As well as tumors and their precursors, carcinogens may produce pathological alterations of a phlogistic and degenerative kind in the main tumor site organs and tissues, or at other sites, and these may relate in some way to, or be associated with, the neoplastic process. In any case, they may act as short- or medium-term markers, or may throw light on the cancer mechanisms specifically triggered by the agent in question. To acquire information on such lesions, the clinical, necropsy, histopathology, and laboratory investigations must include systematic observation of all lesion types.

Information on the Relative Cancer Potential of Various Agents

Such information bears heavily on production and marketing decisions, as well as on setting priorities for preventive action. It may be obtained by comparing the data from carcinogenicity tests on a range of agents, provided the tests have been carried out under comparable experimental conditions.

Weak or Diffuse Cancer Risk Exposure

One of the main problems with industrial carcinogenesis today is the weak cancer risk connected with exposure to single, multiple, or mixed cancer agents, often involving broad segments of the population, and, at times, the whole human race. These risk situations are due to low- or extremely-low doses of high- or medium-power carcinogens, or to weak or very-weak carcinogens at various doses (even small doses) and combinations of these factors.

By their very nature, traditional epidemiologic investigations are unsuited to detecting the tiny variations in cancer effects produced by such kinds of exposure.

Experimental trials offer a clear advantage in that they are conducted in strictly controlled conditions. For bioassays to be effective, their protocols must step up their power to reveal the effects of risks. In this case, not only must one make sure all the previously described general and specific requisites are met, but we must also have available a large animal population so as to reduce chance fluctuations and, as far as is possible, prolong the observation times, anticipating exposure to the prenatal period, and thus managing to cause a sufficient number of pathological events. The CRC/RF has wide experience here⁵ and has reported on it in another paper in this volume.⁶

EXAMPLES OF VARIOUS TYPES OF EXPERIMENT GEARED TO PROVIDING CLEARLY DEFINED PLANNED INFORMATION

Paradigm examples of data that provide information for risk identification and assessment are reported here. All derive from lifespan experiments performed at the CRC/RF laboratories.

Experiments to Identify Potential Carcinogens

Such experiments may be rather simple, the number of animals being limited and the exposure route easy. Subcutaneous injection or insertion is a speedy way of treatment for agents predicted to act topically or directly (direct carcinogen).

A clear example of this type of bioassay is provided by a series of experiments performed on the carcinogenicity of inorganic pigments. The test compounds were injected *one-off* in the subcutaneous tissues of 8–13-week-old, male and female Sprague-Dawley rats (a type of animal known to respond to this type of testing). The animals were kept under observation until spontaneous death. The carcinogenicity of these compounds was evaluated by the onset of sarcomas at the point of injection. The experimental plan and carcinogenicity results are presented in TABLE 1.

Experiments to Provide Information on the Effects of the Route and Site of Administration of Carcinogens

Experiments on the carcinogenic effects (production of local mesotheliomas) of crocidolite and erionite, by injection in the pleural and peritoneal cavities of male and female Sprague-Dawley rats held under observation until spontaneous death, have shown clear differences in carcinogenic potency depending on the site of injection. Furthermore, such experiments have shown that the capacity of crocidolite to produce mesothelioma is higher when the fibers are injected in the peritoneum, the opposite being true for erionite (see TABLE 2).

Experiments to Provide Information on the Effects of the Carcinogen Dose Administered (Dose-Response Relationship)

An example is provided by the carcinogenicity of ceramic fibers. The test compound was delivered one-off at various doses by intraperitoneal injection to male and female Sprague-Dawley rats kept under observation until spontaneous death. The onset of topical peritoneal mesotheliomas directly parallel the dose delivered (see TABLE 3).

TABLE 1. Carcinogenicity bioassays on inorganic pigments indicative of the carcinogenic potential (see Refs. 7 and 8)

Test compound ^a	Animals N			Animals with local sarcomas					
	M	F	M + F	M		F		M + F	
				N	%	N	%	N	%
Chromium yellow (lead chromate)	20	20	40	10	50.0	16	80.0	26	65.0
Chromium orange (basic lead chromate)	20	20	40	14	70.0	13	65.0	27	67.5
Chromium red (lead chromate, sulphate, molybdate)	20	20	40	19	95.0	17	85.0	36	90.0
Zinc chromate (CrO ₃ : 20%)	20	20	40	3	15.0	3	15.0	6	15.0
Zinc chromate (CrO ₃ : 40%)	20	20	40	9	45.0	8	40.0	17	42.5
Silica coated chromium-yellow	20	20	40	10	50.0	15	75.0	25	62.5
Cadmium yellow (cadmium sulphide)	20	20	40	9	45.0	7	35.0	16	40.0
Iron Yellow (iron oxide)	20	20	40	0	-	0	-	0	-
Iron Red (iron oxide)	20	20	40	1	5.0	0	-	1	2.5
Titanium oxide	60	60	120	0	-	0	-	0	-
None	20	20	40	0	-	0	-	0	-

^aAdministered by subcutaneous injection of 30 mg, one-off, to 8-13-week-old male (M) and female (F) Sprague-Dawley rats (Exp. BO 12 and BT 2007).

TABLE 2. Effect of route/site of exposure from carcinogenicity bioassays of crocidolite and erionite (see Ref. 9)

Test compound ^a	Site of injection	Animals N.			Animals with local mesotheliomas					
		M	F	M + F	M		F		M + F	
		N	%	N	%	N	%	N	%	N
Crocidolite	Peritoneum	20	20	40	19	95.0	20	100.0	39	97.5
	Pleura	20	20	40	13	65.0	5	25.0	18	45.0
Erionite	Peritoneum	20	20	40	9	45.0	11	55.0	20	50.0
	Pleura	20	20	40	18	90.0	17	85.0	35	87.5
None	Peritoneum	20	20	40	0	-	0	-	0	-
	Pleura	20	20	40	0	-	0	-	0	-

^aAdministered by intraperitoneal and intrapleural injection of 25 mg, one-off, to eight week-old male (M) and female (F) Sprague-Dawley rats (Exp. BT 2101 and BT 2103).

TABLE 3. Effect of exposure doses from carcinogenicity bioassays on ceramic fibers (see Ref. 10)

Dose of ceramic fibers (mg) ^a	Animals <i>N</i> .			Animals with peritoneal mesotheliomas					
	M	F	M + F	M		F		M + F	
	<i>N</i>	%	<i>N</i>	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%
10	20	20	40	7	35.0	6	30.0	13	32.5
5	20	20	40	1	5.0	3	15.0	4	10.0
1	20	20	40	1	5.0	0	—	1	2.5
0	50	50	100	0	—	0	—	0	—

^aAdministered by intraperitoneal injection one-off, to eight week-old male (M) and female (F) Sprague-Dawley rats (Exp. BT 2111).

TABLE 4. Effects of the concentration/dose on carcinogenicity bioassays for vinyl chloride (VC) (see Refs. I and II)

Dose of VC (ppm) ^a	Animals <i>N</i> .		Animals bearing liver angiosarcomas (%)				Total number of malignant tumors per 100 animals (%)					
			M		F		M + F		M		F	
	M	F	M	F	M	F	M	F	M	F	M	F
30,000	30	30	16.6	43.3	30.0	76.7	123.3	100.0				
10,000	30	30	10.0	13.3	11.7	80.0	83.3	81.7				
6,000	30	30	10.3	33.3	22.0	46.7	73.3	60.0				
2,500	30	30	20.0	23.3	21.7	53.3	73.3	63.3				
500	30	30	—	20.0	10.0	23.3	80.0	51.7				
250	30	30	3.4	6.7	5.1	23.3	36.7	30.0				
50	30	30	3.3	—	1.7	6.7	23.3	15.0				
0	30	30	—	—	—	—	26.7	13.3				

^aAdministered by inhalation, four hours daily, five days weekly, for 52 weeks, to 13–17 week-old male (M) and female (F) Sprague-Dawley rats (Exp. BT1 and BT6).

Another clear example of this type of bioassay is provided by experiments performed on the carcinogenicity of vinyl chloride (VC). The test compound was delivered by repeated exposure through inhalation, at different concentrations, to male and female Sprague-Dawley rats, kept under observation until spontaneous death. Since VC is a multipotential carcinogen and, therefore, there may be competition for the onset of various different tumors, the dose-response relationship was more consistently revealed by plotting the total malignant tumors per 100 animals, rather than the percentage of animals bearing a specific tumor, even when the tumor type acts as a *sentinel* event, as does liver angiosarcoma in the case of VC (see TABLE 4).

Experiments to Provide Information on the Site and Type of Tumors (and Possibly Their Precursors)

The type of neoplastic response is greatly affected by the type of the animal tested. Thus, in order to assess the extent of multipotential carcinogenic effects of the test agent it is necessary to use animals that are prone to respond with a variety of tumors, and to use various types of animals with differing kinds of responsiveness. Under these experimental conditions we were able to show that VC and benzene are typical multipotential carcinogens, producing a large spectrum of tumors of different types or at different sites (see TABLES 5 and 6). Had bioassays been conducted on a more limited number of animal types, important information would have been missed.

Experiments to Provide Information on Pathologies Related to Carcinogenesis

Such information may help throw light on risk assessment and carcinogenesis mechanisms. A classic case of this type is the lesions (necrosis) to the renal tubules of rodents exposed to vinylidene chloride. The incidence and intensity of these in Sprague-Dawley rats and male and female Swiss mice parallel the induction of renal tumors (see TABLE 7).

Experiments to Provide Information on the Relative Cancer Risk of Various Agents

Two sets of experiments show paradigmatically that experimental bioassays may contribute to relative quantitative risk assessment: the first deals with different types of asbestos (see TABLE 8), and the other deals with benzene and several related compounds (see TABLE 9).

For instance, the data on various different types of asbestos provide support for the assumption that there are no major differences in the carcinogenic potency of the different type of asbestos, when the results are evaluated as incidence of mesotheliomas.

Experiments to Assess Weak or Diffuse Cancer Risks

This is a highly important field in our opinion.^{5,6} For years the CRC/RF has been engaged in this type of experiment, the role of which continues to be mysteriously underestimated. Five mega-experiments have been, or are being, conducted or planned in our laboratories. Initial results point to their importance and also show

TABLE 6. Effects of the type of test animals on tumor response from carcinogenicity bioassays on benzene^a (see Ref. 12)

Species	Animal Type		Tumor response ^b
	Species	Strain	
Rat	Sprague-Dawley		Tumors of the lung
	Wistar		Hemolymphoreticular neoplasias
Mouse	Swiss		Angiosarcomas of the liver
	RFJ		Hepatomas
			Carcinomas of the mammary gland
			Carcinomas of the forestomach
		Carcinomas of the skin	
		Carcinomas of nasal cavity	
		Carcinomas of oral cavity	
		Zymbal gland (sebaceous carcinoma)	

^aAdministered by ingestion and by inhalation (Exp. BT 901, BT 902, BT 907, BT 908, BT 909, and BT 4004).^b+, clear evidence; (+), borderline evidence.

TABLE 7. The correlation between tubular kidney necrosis and onset of kidney adenocarcinomas in male (M) and female (F) Sprague-Dawley rats and Swiss mice, exposed to vinylidene chloride (see Ref. 13)^a

Animal type	Sex	Kidney tubular necrosis	Kidney carcinogenesis (incidence of adenocarcinomas)
Sprague-Dawley rats	M	(+)	–
	F	–	–
Swiss mice	M	+++	+++
	F	(+)	(+)

^aAdministered by inhalation (Exp. BT 401, BT 402, BT 403, and BT 404).

that they are the most suitable instrument nowadays to assess such risks. The plans of the five projects and preliminary data are presented in this volume.⁵

CONCLUSION

When planned for precise purposes with attention to the key, general, and specific requisites, and when conducted by standardized methods (throughout the biophase, in processing and examining the pathological specimens, and in data elaboration and presentation), experimental studies form an important instrument capable of providing adequate information and reducing the uncertainties of risk assessment. The need for adequacy in these data cannot be overlooked: such data cannot be upstaged by other kinds of information, or by sophisticated biostatistical analysis on improper

TABLE 8. Relative quantitative risk assessment from carcinogenicity bioassays of several types of asbestos (see Ref. 14)

Test compound ^a	Animals with peritoneal mesotheliomas		
	N.	%	Average latency time (weeks)
Crocidolite (UICC)	39	97.5	59.5
Amosite (UICC)	36	90.0	66.7
Anthophyllite (UICC)	35	82.5	73.3
Chrysotile (Canada, UICC)	32	80.0	92.2
Chrysotile (Rhodesia, UICC)	33	82.5	89.7
Chrysotile (California)	29	72.5	85.3
None	0	–	–

^aInjected at the dose of 25 mg, one-off, in the peritoneal cavity, to groups of 40 eight-week-old Sprague-Dawley rats (20 males and 20 females) (Exp. BT 2101).

Table 9. Relative quantitative risk assessment from carcinogenicity bioassays on benzene, toluene, xylenes, and ethylbenzene (see Ref. 15)

Test compound ^a	Number of total malignant tumors per 100 animals
Benzene	161
Toluene	69
Xylenes	56
Ethylbenzene	40
Olive oil	24

^aAdministered by ingestion, at a daily dose of 500 mg/kg b.w., 4–5 days weekly for 104 weeks, to 80 seven-week-old Sprague-Dawley rats (40 male and 40 female) (Exp. BT 902, BT 903, BT 904 and BT 905).

data. On the other hand, in planning and performing their experiments, researchers must strive to produce consistent data, fit for statistical analysis.

It is high time for a get together on risk assessment, concerted group action linking experimental researchers, epidemiologists, and biostatisticians, in order to decide how their efforts can best be integrated. The present forum certainly stands as an attempt, let us hope successful, to pursue this line.

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