A Database for a Meta-Analysis of Cancer Risk in Animals at Low Doses and Dose Rates of Ionising Radiation

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1. Introduction

The hypothesis according to which the risk of radiogenic cancer is linear with dose and without threshold is subject to intense debate. This issue is difficult to resolve because, in the vast majority of epidemiological studies and animal experiments the risk of cancer at low dose levels is affected by uncertainties such that the validity of the LNT hypothesis, or that of any other biologically plausible dose-effect relationship cannot be excluded.

The uncertainty about whether or not there is an increased risk of cancer at exposure conditions comparable to non-accidental occupational or environmental is the rationale for prudent and conservative regulatory dose limits and practices for the protection of the public and of the workers. Such regulatory and protective measures have the advantage of erring on the side of safety but their beneficial impacts in terms of occupational and public health are virtually impossible to demonstrate. On the other hand, the cost of their hypothetical health benefits may be extremely high compared to other public and occupational health issues. Moreover, the official endorsement of the LNT hypothesis as an established fact, although there is continued debate about this hypothesis.

It seems therefore useful to reduce the uncertainties surrounding the risk of radiogenic cancer at low doses. To this end, data from as many as possible animal experiments have been assembled and are being analysed, with a view to understanding better the relationships between dose and cancer risk at low exposure levels. This work is still in progress.

2. Material and methods

Database description

The literature was reviewed extensively to identify the experiments on low dose effects in animals. A comprehensive source of information in this domain is the International Radiobiology Archives of Long-Term Animal Studies (IRA) (1), in which experiments conducted by research institutions in North America, Europe, and Asia are succinctly described. Data were collected from the open literature (peer-review journals, annual reports of research institutions, conference proceedings). Without exception, all the experiments in which animals were exposed to radiation doses lower than 1 Gy are taken into account and included in the database. In experiments in which the animals suffered a significant life reduction toward the high end of the dose range, only the data concerning dose levels at which there was no significant life reduction (typically less than 10% of that in controls) were taken into account to assess the relationship between dose and cancer risk. Furthermore, data from studies in which no increase in cancer incidence or no reduction in duration of life were observed in exposed animals, whatever the dose, were considered “low dose” data. The application of these criteria made it possible to include data obtained at dose levels well in excess of 1 Gy.

Data from each relevant study were assembled in Microsoft EXCEL® tables. To the extent possible, the tables are designed to make possible the inter-comparison of data from different studies. The data being collected include

- Animal species
- Type or source of radiation
- Mode of exposure (internal, external)
- Exposure regime (acute, fractionated, chronic)
- Dose
- Dose rate (when available)
- Target organ
- Number and type of cancer cases at each dose level
- Mean or median survival time for controls and exposed animals (when available)

To date, dosimetry data such as radionuclide build-up and clearance rates and dose rate variation over time have not been included.

From the above information, the relative risk of cancer and its corresponding 95% confidence interval was calculated for each end point and for each dose level taken into consideration. When a reasonable linear no-threshold fit was possible, the difference between observed and predicted numbers of cases was determined for each dose level.

The collection and entry of data is on going. A maximum of about 170 studies of potential interest were identified in the International Radiobiology Archives of Long-Term Animal Studies, not all of interest for the research of low dose effects. Studies that meet the criteria for survival time, dose, and absence of excess cancers published since the publication of the International Radiobiology Archives are being included in the database. At present, the data collected cover 106 experiments, conducted at doses ranging from a few mGy for neutron exposures to over 5 Gy for exposures to beta emitters. Data concerning about 72,000 animals (52,000 exposed and 20,000 controls) have been examined. (A study described in the IRA or in a paper may concern more than one experiment. For example, a paper may report dose-effect relationships for different species, strains, sexes, radionuclides, dose rates, etc, each being considered as a specific experiment). A summary of the information collected is given in Tables 1, 2, 3, and 4.

Limitations and potential of the database

The types of analyses that can be conducted on the data collected are limited by the type of information given by the authors of the different animal studies. For example, the types of malignancies observed in the various studies are not always described using a uniform terminology and standard disease classification codes are not used in all publications. Furthermore, the number of tumour-bearing animals in control and exposed animals – perhaps the most important measure of the overall influence of radiation exposure in the induction of radiogenic cancer (2) is not given in some studies.

Conversely, in spite of these limitations, the database allows for a direct estimation of the influence of all the parameters on the risk of cancer at low doses of radiation, for the end points reported in each study.

2.1 Comparison of observed and predicted cancer incidence for internally deposited alpha emitters

2.1.1 Materials

The difference between the number of cancer cases observed in exposed animals and the numbers predicted by the LNT hypothesis was calculated, at each dose level, in experiments in which rats had inhaled $^{239}Pu0_2$ (2); beagle dogs were injected ^{226}Ra (3, 4); or mice were injected ^{226}Ra, ^{239}Pu, ^{241}Am, ^{249}Cf, and ^{252}Cf (5).

2.1.2 Method

In each experiment, the best linear fit between dose and cancer incidence was determined, with the intercept at the cancer rate axis set at the cancer rate in control animals. The best linear fit equation is of the form

$$I_{th} = m + \alpha D$$

where I_{th} is the theoretical incidence rate of cancer at dose D, m is the incidence rate of cancer in controls, α is the slope of the dose-effect relationship, and D is the dose.

In each study and for each dose point the theoretical number of cancer cases (predicted by the LNT hypothesis) was calculated using

$$a_{th,D} = (m + \alpha D)\, n(D)$$

where $a_{th,D}$ is the number of cancer cases predicted by the LNT hypothesis in n animals exposed to dose D.

In each experiment, and for all experiments together, the differences between observed and predicted numbers of cancer cases at each dose level were added together. The odds ratios for the observed ($\mathbf{OR_{obs}}$) and predicted ($\mathbf{OR_{th}}$) numbers of cancer cases were calculated. $\mathbf{OR_{th}}$ were calculated using the total number of predicted cancer cases rounded to the nearest integer. $\mathbf{OR_{th}}$ is a measure of the theoretical radiation risk based on the cancer rate predicted by the Linear No-Threshold hypothesis. Furthermore, the compatibility between $\mathbf{OR_{obs}}$ and $\mathbf{OR_{th}}$ was tested by means of a Compatibility Ratio (**CR**), similar to an odds ratio and defined as

$$\mathbf{CR} = \mathbf{a_{th}}.\mathbf{b_{obs}} / \mathbf{a_{obs}}.\mathbf{b_{th}}$$

where $\mathbf{a_{th}}$ is the predicted number of cancer cases in exposed animals, $\mathbf{b_{th}}$ the predicted number of exposed animals without cancer, $\mathbf{a_{obs}}$ the observed number of cancers cases in exposed animals, and $\mathbf{b_{obs}}$ the observed number of exposed animals without cancer.

The robustness of the LNT hypothesis can be tested by comparing $\mathbf{OR_{obs}}$ with $\mathbf{OR_{th}}$ and by examining **CR**. The LNT hypothesis is deemed to hold if the $\mathbf{OR_{Obs}}$ and $\mathbf{OR_{th}}$ values are close and if their respective confidence intervals overlap. Similarly, the LNT hypothesis is also deemed to hold if **CR** is close to one, with a confidence interval that includes one.

2.2 Modelling dose-response in animals exposed to beta, gamma, neutron radiation, and X-rays.

2.2.1 Materials

In the International Radiobiology Archives (IRA) (1) fifteen studies in which animals were exposed to X rays, gamma, beta, neutron radiation at doses typically less than 1 Gy, and for which is was possible to determine the slope of a dose-response function over several consecutive dose levels. An additional criterion was that the life span of the animals was significantly reduced over the considered dose range. Seven additional studies (6, 7, 8, 9, 10, 11, 12) that met the criteria for dose and life shortening but were published after the completion of the IRA Report were added to the database. Only the studies not mentioned in the IRA are listed in the references.

From these studies, 436 data sets were assembled into one Microsoft EXCEL® database. For the purpose of this work, a data set is the ensemble of information concerning the incidence of a particular endpoint at each relevant dose level, in a specific species and strain, under particular exposure conditions. For example, in a specific experiment, the incidence of myeloid leukemia in CBA/H mice exposed to Co-60 radiation at each dose level and at the same dose rate constitutes a data set.

The maximum of the considered dose ranges varied from 0.02 to 6.7 Gy, and 71.6% of the data sets have maximum dose less than or equal to 1 Gy. The studies analysed concern 14 different animal species or strains, and a total of 25 different organ sites. A summary of the descriptive statistics is given in Tables 1, 2, 3, and 4. Generally, the cancers were classified according to four classes, carcinomas, sarcomas, lymphomas, and leukemias.

2.2.2 Method

The generalized linear models (13), which include simple linear regression, probit regression and logistics regression were applied to the estimation of dose-response in cancer incidence. In general, the estimated slops for dose response from different models were similar. The distribution of these slopes from each model was further examined using normal probability-probability (P-P) plots. In the P-P plot, the observed cumulative distribution function (the proportion of non-missing values) is plotted against a theoretical cumulative distribution function in order to assess the fit of the theoretical distribution to the observed data. If all points in this plot fall onto a diagonal line (with intercept 0 and slope 1), then one concludes that the theoretical cumulative distribution adequately approximates the observed distribution. If the data points do not all fall on the diagonal line, then one can use this plot to visually assess where the data do and do not follow the distribution.

3. Results and discussion

3.1 Comparison of observed and predicted numbers of cancer cases in animals exposed to internally deposited alpha radiation

In the 78 dose groups exposed to alpha radiation (Table 5), 49 cancer cases were observed in 3041 exposed animals, against 83.4 predicted by the LNT hypothesis. In 1655 control animals, 8 cancer cases were observed. Owing to the overlap between OR_{Obs} and OR_{th} confidence intervals, the deficit in observed cases was not quite significant (OR_{Obs} = 3.3, C.I. = 1.6 - 7.0, P = 0.001; OR_{th} = 5.8, C.I. = 2.8 - 12.0; P ≤ 0.0001). However, this deficit becomes significant when one considers the compatibility ratio, whose confidence interval does not include one (CR = 0.6, C.I. = 0.4 - 0.8, P = 0.004). Although a strong correlation is often found between the LNT hypothesis and the data observed cancer rates in animals exposed to internally deposited alpha and beta emitters, the sum of the differences between observed and predicted numbers of cancer cases in several studies seems to be significant. The Compatibility Ratio suggests the possibility of a non-linear relationship between dose and cancer risk.

3.2 Modelling dose-response in animals exposed to beta, gamma, neutron radiation, and X-rays.

The distributions of slopes from fitting the three models (logistic, probit and linear regression) to the data from 54 experiments with leukemia and 153 experiments with carcinoma and sarcoma are examined in Figure 1. While for leukemia the P-P plots do not suggest an excess number of experiments with negative slopes, for carcinoma and sarcoma logistic and probit regression seems to indicate a surplus of negative slopes in the dose-response fits, which remain to be confirmed and quantified.

4. Conclusions

Data from a large number of animal experiments are being assembled in one database and analysed with a view to understanding better the relationship between exposure conditions and the risk of cancer. A series of preliminary analyses suggests possible departures from linearity between dose and risk. With the systematic modelling of dose-effect, by means of logistic, probit, and linear regression models, it seems at this time that no excess of negative slopes (apparent protective effect) is detectable for the risk of leukemia. However, logistic and probit modelling seems to reveal an excess of negative slopes for the induction of sarcomas and carcinomas. New data sets are being added to the existing database and finer analyses are underway to verify these initial observations and to determine the influence of parameters such as dose rate, species and strain, and cancer incidence in controls on cancer risk. The collection and analysis of all accessible data from low-dose animal experiments is continuing, and the relationships between radiation dose, dose rate and cancer risk described here may evolve as data analysis progresses.

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SPECIES	Strain	Frequency
Mouse	B6CF1	10
	BABLB/c/AnNBdf	7
	BALB/c	42
	BC3F1	72
	C3H/He	1
	C57BL	58
	CBA/Cne	52
	CBA/H	11
	RF/Un	55
	RFMf/Un	30
Rat	Sprague-Dawley	51
	WAG/Rij rats	9
	BN/Bi	3
Dog	Beagle	31

Table 1. Species and strains of animals

Source of radiation		Frequency
Gamma	Cs-137, Co-60	161
Neutrons	Energy range 0.4 to 36 Mev	202
Beta	HTO, Sr-90	22
X rays		51

Table 2. Sources of radiation

Organs			
Adrenal gland	Haematopoietic tissues	Ovaries	Thymus
Bladder	Harderian gland	Pancreas	Thyroid
Blood vessel	Kidney	Pituitary gland	Urinary system
Brain	Liver	Salivary gland	Uterus
Connective tissue	Lung	Skeleton	
G.I. Tract	Lymphatic system	Skin	
Genital organs	Mammary glands	Testis	

Table 3. Target organs

Type of radiation	Alpha	Beta	Gamma	X-rays	Neutrons
No. of experiments	Under construction	4	40	9	53
No. of controls	"	2257	10467	3067	5106
No. of cancers in controls	"	655	5747	1295	2543
No. of exposed Animals	"	5774	26239	4252	15673
No. of cancers in exposed	"	4417	17499	2287	9555

Total number of controls: 20 897
Total number of cancers in controls: 10 240
Total number of exposed: 51 938
Total number of cancers in exposed: 33 758

Table 4. Summary of information contained in the database

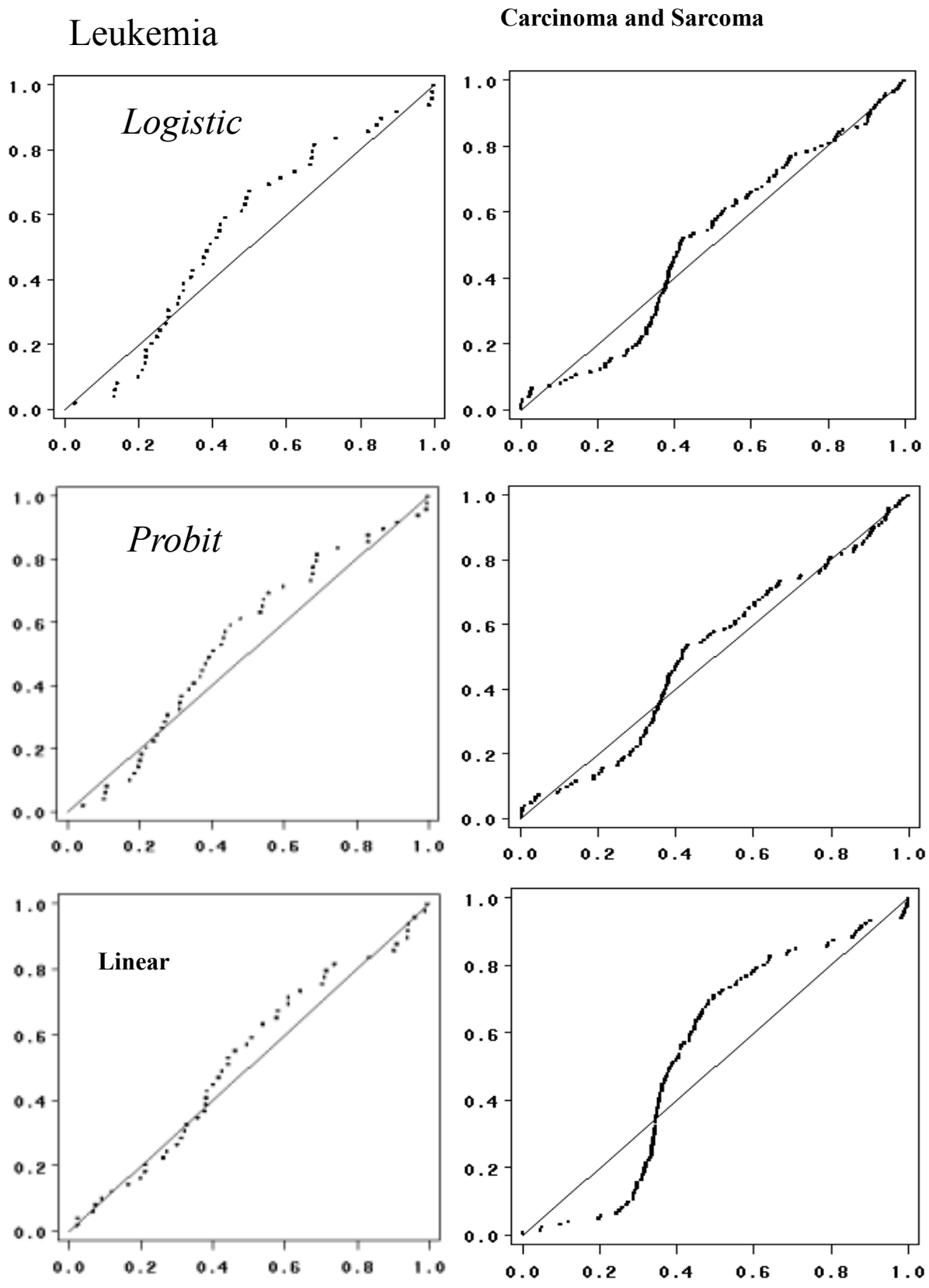

Figure 1. Probability-Probability plots for a slope distributions for leukemia (left panels) and carcinoma and sarcoma (right panels). Y-axis represents cumulative empirical distribution of slopes, X-axis represents cumulative standard normal distribution.