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The Value and Significance of Life Span and Scheduled Termination Data in Long-term Toxicity and Carcinogenesis Studies*

HENK A. SOLLEVELD AND ERNEST E. MCCONNELL

National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

ABSTRACT

Consideration is given to the age association of lesions, the duration of long-term toxicity and carcinogenesis studies, and to the value and significance of including scheduled termination in such long-term studies. There is now enough evidence that age and cancer are associated. It is argued that the increase in incidence of lesions with age has such disadvantages that extension of the duration of the long-term toxicity and carcinogenicity study beyond 2 years is not warranted in most cases. Incorporation of scheduled termination in long-term studies gives more insight into the biologic behavior of toxic lesions and cancer and may enable one to make a distinction between "incidental" and "fatal" lesions. This distinction may be important for the statistical evaluation of data from chemical carcinogenesis studies.

INTRODUCTION

A dichotomy of opinion exists regarding the relevance of long-term toxicity and carcinogenesis studies for human hazard assessment. The expressed opinions vary from: "The bioassay can no longer be accepted as a definitive indication of potential human hazard" (15) to "It being the cornerstone in the assessment of human risk" (7). Without discussing all the pros and cons of the carcinogenesis study and realizing that it is only a part of the data used for human hazard assessment, we believe that the ultimate value of these carcinogenesis studies in the area of preventive oncology remains as the best predictor for potential effects in humans and thus at this stage in the science of chemical mutagenesis and carcinogenesis continues to be our best source of information. This judgment is based on the fact that nearly all human carcinogens show similar effects in animals. However, since the carcinogenesis study is actually an insensitive method for detecting chemical carcinogens, refinement of the test system continues to be pursued. As stated by Falk (3) attempts must be made to update our knowledge, to re-evaluate our procedures, and to change species, concepts, or protocol when that appears advantageous in spite of any attempt to fix protocols "in cement" for easier supervision, comparison, or legal action.

It is in this light that the present report discussing the value and significance of life span and scheduled termination data for the evaluation and interpretation of long-term toxicity and carcinogenesis studies should be viewed.

METHODS

Previously published life span data were used for the demonstration of an age-related increase in incidence of neoplasms in the F344 rat and for calculating the incidence of

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neoplasms in dead animals and in the population at risk at certain age periods (11). The data were based on 529 untreated control male and female F344 rats.

Scheduled termination data were obtained from studies performed at three laboratories under contract to the National Toxicology Program, i.e., Litton Bionetics, Inc., Kensington, MD, Mason Research Institute, Worcester, MA, and Southern Research Institute, Birmingham, AL. Untreated control male and female F344 rats were killed at 38, 47, 56, and 84 weeks, respectively. The respective numbers of animals of either sex at these time points were 50, 100, 50, and 100. Historic control data from 870 untreated control male and 1070 untreated control female F344 rats were used for the 110-week time point. These data were derived from animals that were killed at the end of 2-year studies at the three laboratories.

Results and Discussion

Age Association of Neoplasms. Numerous studies have demonstrated that the incidence of neoplasms increases with age in the human as well as in various animal populations, particularly in rodents. As summarized by Anisimov and Turusov (1) the age-associated increase in tumor incidence is generally attributed to the age-related accumulation of a total effective dose of carcinogenic agents and/or time of exposure, or is regarded as a consequence of disturbances of the hormono-metabolic pattern and the decline in immune function with age. It has been reported that in rats the age-associated increase in spontaneous tumor incidence manifests itself mainly in the development of neoplasms of the mammary and endocrine glands in post-reproductive periods (1) suggesting that at least in rats disturbances of the hormonal status play an important role in the development of cancer. A recent lifetime study in F344 rats, an inbred rat strain widely used in carcinogenicity testing programs in the United States and Japan, revealed similar findings, although high incidences (>10%) were not restricted to mammary gland neoplasms and neoplasms of the endocrine organ system (11). Neoplasms of the skin and subcutis and mononuclear cell leukemia were also found frequently indicating that factors other than hormono-metabolic changes play a role as well.

As an example of the age association of lesions, the age-specific prevalence rates of neoplasms, exceeding 10% in male and female F344 rats are given in Tables I and II, respectively. It appears from these data that the most common neoplasms were first recognized between 59 and 84 weeks of age and that the incidence of most neoplasms increased with age. It must be noted, however, that the reported age-specific prevalence rates are based mainly on findings in animals that died or were killed when moribund during the specified age intervals (11). This does not mean that these rates reflect the true incidence of neoplasms present in the living population of F344 rats at these periods (2), an aspect that will be discussed later.

Duration of Long-term Toxicity and Carcinogenesis Studies. The duration of a long-term toxicity and carcinogenesis study is generally 24 months for both mice and rats, although a study duration of 18 months is not uncommon for mice. In informal discussions on the subject, there are those who advocate extending the duration of these studies. However, little definitive information is available concerning the advantages and disadvantages of conducting toxicity and carcinogenesis studies beyond 24 months. Before an extension can be adopted one has to be knowledgeable about the survival characteristics and the natural history of nonneoplastic and neoplastic lesions of the species, strain, and / or stock used. The usual age reached in a 2-year study is 110 weeks (study started at 6 weeks of age). When this age is not significantly different from the maximum age, extension of the duration of the study is meaningless. On the other hand, when a significant difference exists between 110 weeks of age and the maximum life span, extension might be meaningful, particularly when the variety of lesions increases with age, meaning that other (additional) lesions occur after 110 weeks of age.

When life span data of the F344 rat are taken as an example it appears that the survival rates in a 2-year study are around 70% for both males and females (Table III). If the study duration is extended to the 10% survival rate, 30 to 40 more weeks are added to the study (Table III). During this period, the most common neoplasms occurring in each sex showed an upward trend in incidence during that period (Tables I and II). The same phenomenon was seen for neoplasms occurring with a lower frequency (11). However,

TABLE I—Age-specific Prevalence Rates of Neoplasms, Exceeding 10%, in 529 Male F344 Rats[a]

Tumor Type	Percentage of Lesions at Various Age Periods						
	0–58 wk ($n = 4$)	59–84 wk ($n = 24$)	85–97 wk ($n = 38$)	98–110 wk ($n = 77$)	111–123 wk ($n = 143$)	124–136 wk ($n = 148$)	>137 wk ($n = 95$)
Subcutaneous mesenchymal neoplasms	0	8	21	17	26	28	22
Mammary gland neoplasms	0	4	0	4	11	20	25
Mononuclear cell leukemia	0	8	21	40	38	40	39
Pituitary gland neoplasms	0	0	14	15	16	25	23
Thyroid C-cell neoplasms	0	8	13	14	20	30	40
Pancreatic islet cell neoplasms	0	0	6	7	7	17	17
Pheochromocytoma	0	4	13	16	23	40	51
Testicular interstitial cell neoplasms	0	75	92	92	95	99	100

[a] Modified from Solleveld et al (11).

TABLE II—Age-specific Prevalence Rates of Neoplasms, Exceeding 10%, in 529 Female F344 Rats[a]

Tumor Type	Percentage of Lesions at Various Age Periods						
	0–58 wk ($n = 4$)	59–84 wk ($n = 24$)	85–97 wk ($n = 55$)	98–110 wk ($n = 81$)	111–123 wk ($n = 120$)	124–136 wk ($n = 118$)	>137 wk ($n = 131$)
Mammary gland neoplasms	0	41	24	34	59	76	100
Mononuclear cell leukemia	0	32	18	27	39	47	47
Pituitary gland neoplasms	0	36	33	43	43	58	58
Thyroid C-cell neoplasms	0	5	4	11	17	23	36
Pheochromocytoma	0	5	5	10	12	14	27
Endometrial stromal neoplasms	25	5	13	15	15	15	23

[a] Modified from Solleveld et al (11).

TABLE III—Survival Data of 529 Male and 529 Female F344 Rats

Survival Rates	Age	
	Males	Females
%	*wk*	
90	94	92
80	105	103
70	111	110
60	117	116
50	121	122
40	126	127
30	130	133
20	135	139
10	140	146

the spectrum of neoplasms did not increase during this period (11). This means that the primary effect of extending the study duration in F344 rats beyond 2 years is a higher incidence of background lesions.

The important conclusion from this observation is that it may be more difficult to detect a toxic lesion in life span studies than in 2-year studies because lesions normally associated with aging may obliterate or at least mask an induced lesion (8). It also means that the older the animals, the heavier the tumor burden per animal will be. Such tumors may alter the normal function of organs, organ systems, and even of the body as a whole and may result in unpredictable and uninterpretable study results. In addition, higher spontaneous tumor rates increase the chance of obtaining false-positive statistical test results (4, 6) or might (if the spontaneous incidence approaches 100%) preclude observing a statistically significant evidence that a test compound is carcinogenic. Furthermore, an extension of the study duration by 30 to 40 weeks increases considerably the costs of a study. In our opinion these disadvantages outweigh the advantages of a longer exposure in most cases.

Another important consideration in the determination of study duration is the time required for carcinogens to increase signifi-

cantly tumor incidence. A recent review of 179 chemicals showed that evidence of chemical carcinogenicity was already apparent or suspect before 24 months in all instances (5). This further strengthens the evidence that a 2-year study duration is sufficient in most cases.

At present, even less data have been collected from the also commonly used B6C3F1 hybrid mouse than from the F344 rat. The survival characteristics and the natural history of nonneoplastic and neoplastic lesions are only partly known (14). The survival rates obtained from studies performed under the auspices of the National Toxicology Program are around 75% for both sexes at 110 weeks of age (unpublished data), which is even higher than the survival rates for the F344 rat at that time period. Since evidence is lacking that the B6C3F1 hybrid mouse is more sensitive in carcinogenicity testing programs than the F344 rat, a study duration of 18 months for the B6C3F1 hybrid mouse is not justified. An appropriate discussion on a shorter study duration is hampered by the fact that not enough data on spontaneously occurring and induced lesions in the F344 rat and B6C3F1 hybrid mouse are available. The recent incorporation of scheduled terminations in long-term toxicity and carcinogenesis studies by the National Toxicology Program may be helpful in collecting these data (8).

The Value of Scheduled Termination Data. The principle reasons for including scheduled termination in the design of long-term toxicity and carcinogenesis studies are 2-fold. First, scheduled terminations permit assessment of any toxic effects and aid in defining their pathogenesis (5, 8). Second, scheduled terminations yield information on the time of appearance and progression of lesions and may serve as early indicators of a potential carcinogenic response. In addition, more insight into the biologic behavior of lesions may enable one to make a distinction between "incidental" and "fatal" lesions (10). This distinction may be important for the statistical evaluation of data from chemical carcinogenesis studies. As reported by Burek (2) and Zurcher et al (16, 17), the incidence of lesions found in dead animals can be expressed in two ways, i.e., as a percentage of those which died in a specified interval or as a percentage of those which were alive at the start of each interval (= incidence of the population at risk). For the first method to be of predictive value it must be assumed that all animals still alive had the same incidence of a particular lesion as did those which died. In contrast when the second method is predictive it must be assumed that no lesion is present in any of the animals that are still alive at the end of the interval (16, 17). Both assumptions represent extremes and the incidence of most lesions may be expected to be intermediate (16, 17). Nonetheless, lesions which develop slowly and are not related to impending death may be near to the incidence found in dead animals ("incidental" lesions) and lesions that develop rapidly and are life-threatening may be more near to the incidence of the population at risk ("fatal" lesions) (16, 17). On the other hand lesions that develop rapidly but that are not life-threatening will be closest to the incidence found in dead animals. Burek (2) compared both ways of expression with scheduled termination data for a few nonneoplastic and neoplastic lesions occurring in the WAG/Rij and BN/BiRij rat strains. He concluded that neither the incidence of a lesion in animals that died or were killed when moribund nor the incidence of the population at risk predicts the incidence of all types of lesions in the living population at a given age.

We compared both ways of expression with scheduled termination data for a few common lesions in the F344 rat (Fig. 1). The incidence of testicular interstitial cell tumors in scheduled terminated males was comparable with the incidence found in dead animals. This finding supports the assumption made by Zurcher et al (16, 17) since it is well-documented that this type of tumor arises early and develops slowly (13). For mammary gland fibroadenomas in females different patterns with time were observed. The incidence in the killed animals was closest to the incidence of the population at risk in the age period of 59 to 84 weeks, while it was nearest to the incidence found in dead animals in the age period of 85 to 110 weeks. An explanation for this variable pattern is not available.

For mononuclear cell leukemia different patterns were found in males and females. The killed males showed an intermediate pattern, while the incidence in the killed females was closest to the incidence of the population at risk. The pattern in males was expected to be similar as in females since this tumor type is thought to be a rapidly growing neoplasm and the major cause of death in the

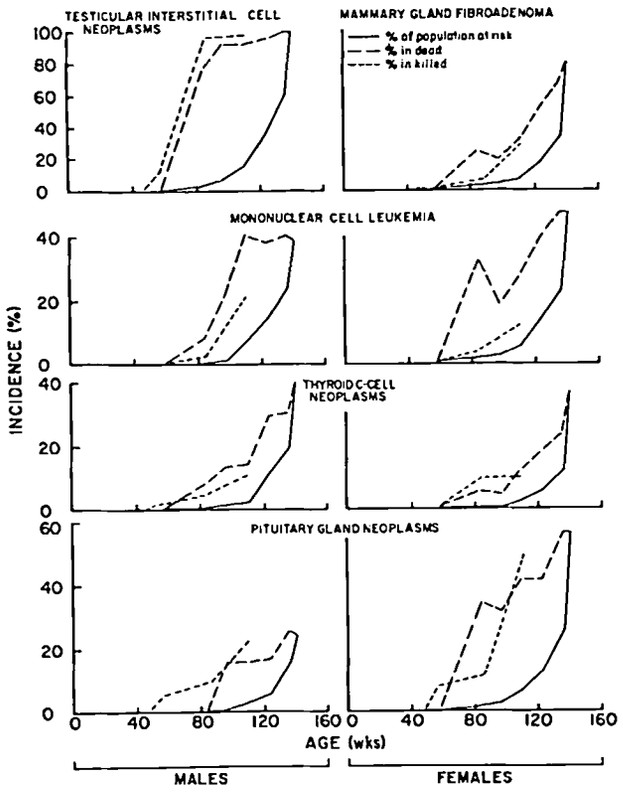

FIG. 1—The incidence with age of neoplasms in dead rats, the incidence of the population at risk, and the incidence in rats killed at certain ages for male and female F344 rats.

F344 rat (12). Others also found a discrepancy between early deaths and scheduled terminations for mononuclear cell leukemia, although they did not find significant differences in disease pattern between males and females (9). Early deaths appeared to be a well-defined group always in the end stages of the disease in their study, while the scheduled terminations were a highly variable group consisting of both animals in the very early state of disease as well as those with advanced lesions yet not moribund. Their explanation was that the observed differences in lesions were probably related to a progression of disease over a 2- to 5-week period.

For thyroid C-cell and pituitary gland neoplasms, male and female F344 rats showed incidences closest to the incidences found in dead animals. The incidence patterns for thyroid C-cell neoplasms in males and females indicate that this tumor type arises early and develops slowly. Although less clear in males than females, pituitary gland neoplasms showed a different incidence pattern. These tumors seem to develop rapidly after 80 weeks of age without being life-threatening. Two different patterns were found for thyroid C-cell neoplasms in female WAG/Rij and BN/BiRij rats (2). The incidence in scheduled killed female WAG/Rij rats was closest to the incidence in dead animals, while in BN/BiRij females the incidence was closest to the incidence of the population at risk. No explanation for this difference was given (2). For pituitary gland neoplasms, Burek (2) found in females of both strains an incidence closest to the incidence of the population at risk, while the opposite was observed in F344 rats. These observations suggest that the majority of pituitary gland neoplasms in WAG/Rij and BN/BiRij rats are slow-growing lethal tumors, while in F344 rats they are fast-growing nonlethal tumors. It is clear that further investigations with various species and strains of animals in specially designed studies are needed to establish the true tumor incidences and patterns in a living population in that particular species or strain.

In summary, based on available life span data from the F344 rat, extension of the duration of long-term toxicity and carcinogenesis studies beyond 2 years have more disadvantages than advantages. Incorporation of scheduled terminations in long-term toxicity and carcinogenicity studies should be regarded as a refinement of the test system. Scheduled killings may provide more insight into the biologic behavior of spontaneous and induced lesions and may serve as early indicators of a potential carcinogenic response.

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Discussion of the Paper

DR. NEWBERNE: Thank you very much, Dr. Solleveld. We have time for a question or two.

DR. IATROPOULOS: This is really an impressive presentation which is really long overdue and very helpful. However, I would like to add to this presentation. There is another factor, mentioned yesterday mainly by Dr. Koestner, about the morphologic characterization. The use of lethality as the end point is, of course, helpful if there is a one to one extrapolation between animals and humans. What I think is perhaps going to be more useful is a morphologic characterization, such as the cell kinetics of different tumors, because I think this will ultimately yield the answer. That can also be amplified by opening a window into the genome at various time intervals by, for example, deriving material from *in vivo* studies. Any kind of material, even bone marrow, could be used to do cytogenetic analysis, for example. How would you respond to that?

DR. SOLLEVELD: I would be quite pleased if that type of cytology program could be performed on animals from a study. From scheduled killing, one can get an impression of which is occurring in the living population, and consequently, the protocol could be adjusted or changed, based on the findings of experiments such as the type you have in mind. I think that that's a step toward a refinement of the system.

DR. NEWBERNE: We have time for another question.

DR. WEINBERGER: I agree with you. I think the points that you made regarding the disadvantages of lifetime studies were very cogent, and I have never been very high on lifetime studies for those reasons. I'd like to add another reason which I think, as a practicing pathologist, is extremely important, and that is the fact that the mortality level greatly accelerates after 2 years. The animals start to die very suddenly, and the possibility of intercepting the animals in order to examine them when they are moribund and the tissues are not yet autolyzed, becomes smaller. I think that's an extremely important point and one that should be added to those that you've mentioned.

DR. SOLLEVELD: I agree with you.

DR. WEINBERGER: One other point—what about using for the numerator/denominator the number of animals with tumors (tumor-bearing animals) over the number that are looked at histopathologically rather than the number at risk?

DR. SOLLEVELD: I don't think that is completely correct. As mentioned before, the incidence of lesion found in dead animals can be expressed in two ways, i.e., as a percentage of those which died during a specified time interval, or as a percentage of those alive at the start of that interval (population at risk). In using the first method, the number of the tumor-bearing animals is the numerator and the number of dead animals (examined histopathologically) is the denominator.

DR. WEINBERGER: I mean, you don't know those that are cannibalized or discarded.

DR. SOLLEVELD: You can discard these and subtract them.

DR. NEWBERNE: One more please.

DR. BHANDARI: I agree with the value of interim killing as well as the value of not exceeding 2 years for a chronic study. In fact, we have routinely used interim killings at 12 and 18 months, and even at 15 months in one of our later studies. From these studies, progression of lesions is quite convincing and a carcinogenic effect is clear, whether it's drug- or dose-related or not. One other point that may clarify this particular issue even more is the dose. We have had circumstances in which certain tumors only begin to appear in the high dose group at the 2-year termination. You do not see them in any of the dead animals, nor in the lower dose groups, but only with a low incidence in the high dose group. My question is: could we have gone further, and have we seen a drug-related effect of this compound? If we can increase the high dose a little more (even when it becomes a toxic dose) we may even terminate the study at 18 months. If, on the other hand, there is a carcinogenic potential, we start seeing these tumors earlier, even at the 1-year killing, with a clear time-related effect. Then, at 2 years we expect to find a higher incidence so that the carcinogenic effect becomes quite convincing. Again, I would like to propose that we should think about getting over the so-called maximum tolerated dose to clarify this gray area, at the same time recognizing that we have exceeded the maximum tolerated dose.

DR. SOLLEVELD: I know that the maximum tolerated dose is an issue which has been discussed for years, and a uniform agreement has still not been reached. I do not regard myself as being qualified to comment on this issue any further.