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Refinement of Long-Term Toxicity and Carcinogenesis Studies[1]

GHANTA N. RAO[2] AND JAMES HUFF

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

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Refinement of Long-Term Toxicity and Carcinogenesis Studies. RAO, G. N., AND HUFF, J. (1990). *Fundam. Appl. Toxicol.* **15**, 33–43. The chance that alternatives will completely replace animals for toxicology research in the foreseeable future is nil. Continual refinement of animal toxicity and carcinogenesis studies, however, can be an effective means of reducing the numbers of animals used and conserving time and resources without compromising scientific quality. We must continue to strive to find species and strains that can metabolize chemicals similar to humans, are small enough to be housed in large numbers, and have low prevalence of spontaneous lesions with sufficient life span to express the toxic and carcinogenic potential of chemicals. Adequate care of animals with control of variables such as light, temperature, diet, bedding, diseases, and genetic characters of laboratory animals will decrease the variability. Humane considerations and euthanasia of animals with large masses and other conditions interfering with eating and drinking, major injuries and ulcers related to husbandry and treatment, and diseases indicating pain and suffering will help not only to alleviate further pain and distress but also to facilitate collection of tissues without secondary complications for detection of chemical treatment-related lesions. Limiting the duration of studies to decrease the variability due to age-associated changes will also refine long-term studies. Other considerations for refinement of carcinogenesis studies include selection of the most sensitive sex of one or more species for evaluation of selected chemicals in a class where toxic and carcinogenic potential of other representative chemicals are known. Genetically engineered animal models with known oncogenes may reduce the duration and increase the sensitivity of carcinogenesis studies with a reduction in the use of animals.

> The search for alternatives to the use of animals in research and testing remains a valid goal of researchers, but the chance that alternatives will completely replace animals in the foreseeable future is nil.
>
> (National Research Council, 1988).

We must use alternatives to whole animals such as bacterial systems and cell culture methods only when they are reliable predictors of toxicity and carcinogencity. However, we must evaluate potential alternative tests objectively, free of subjective attachment. In the field of toxicology alone there are hundreds (Goldberg, 1985; Tennant *et al.*, 1987; Mehlman, 1989) of *in vitro* assays and other alternative methods for evaluation of toxic and carcinogenic potential of drugs and chemicals. Many of these alternative tests with cell systems and subcellular components will aid in understanding effects and mechanisms at cellular and subcellular levels. But

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[2] To whom correspondence and reprint requests should be addressed at NIEHS/NTP, MD AO-01, P.O. Box 12233, Research Triangle Park, NC 27709.

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many of these tests do not predict the response of the whole animal (Tennant *et al.*, 1987). There are a number of reasons for this difference. In a living animal during the expression of physiologic, pharmacologic, and toxic responses to an agent, in addition to subcellular component interactions, there are other interactions. They include (a) cell to cell communication between cells of the same or different types in organs such as liver, and (b) organ to organ communication, interaction, and compensation between organs of a living animal such as liver and kidney, thymus and lymphoid tissue, pituitary and other endocrine organs, which we cannot predict by alternative assays. In addition the living animal as a whole by its complex feedback mechanisms between organs and tissues adjusts to varying chemical and environmental insults, compensates, and survives or overreacts and dies, which we cannot predict by any single or battery of *in vitro* and alternative tests. Notwithstanding these drawbacks, we must continue to strive for reliable *in vitro* or alternative methods to the use of whole animals. It is the most humane, ethical, and economical direction for us to follow. But diseases like cancer and acquired immunodeficiency syndrome (AIDS) humble us with regard to limitations in our understanding of complex biologic systems such as mammals. Alternatives are useful for mechanistic research and a limited number of alternative tests currently have some utility for assessing the toxic and carcinogenic potential of selected classes of chemicals (e.g., polycyclic aromatic hydrocarbons). However, the probability that alternatives will completely replace animals for toxicology research in the foreseeable future is nil.

If a study must be done using animals, then we are obligated to do it right the first time. In the long run this will save money and time, will decrease the number of animals used, and is one of the best refinements we can do. In recent years a number of chemicals were selected by the National Toxicology Program (NTP) for reevaluation, because the previous studies on these chemicals were considered to be incomplete or inadequate. Continual refinement and optimization of animal toxicity and carcinogenesis studies can be an effective means of reducing the numbers of animals used and of conserving time and resources without compromising scientific quality. Essential components for refinement of long-term studies include selection of proper species and strains, proper care of the animals, humane considerations during the course of a study, and adequate duration of the studies. Each of these essential components is discussed below.

SELECTION OF PROPER SPECIES, STRAINS, AND NUMBER OF ANIMALS

We should continue to strive to find species and strains of experimental animals (a) that metabolize or dispose a chemical in a manner similar to humans; (b) with (median) life spans long enough to adequately assess the carcinogenic potential of chemicals (mouse strains such as A and AKR with relatively short life spans may be useful for assessing the carcinogenic potential of potent carcinogens but will not provide adequate information on weaker carcinogens); (c) with low spontaneous lesions and tumors, especially in the target organs of the chemical, to enhance the specificity and sensitivity and to facilitate interpretation; and (d) small enough that they can be housed conveniently in large numbers, thus allowing the use of a greater number of animals to increase the sensitivity (statistical power) for detecting toxic responses, especially carcinogenic effects. The number of animals per group should be adequate to assess the toxic and carcinogenic potential. However, increasing the number of animals beyond 50 to 60 per group does not result in a proportional increase in sensitivity. As shown in Table 1, for animals having a background tumor rate of 5%, if the number per group is increased by 40 animals from 20 to

TABLE 1

RELATIONSHIP OF NUMBER OF ANIMALS PER GROUP WITH TUMOR RATES FOR A POSITIVE EFFECT AT 5 TO 30% BACKGROUND TUMOR PREVALENCES

Background tumor prevalences (%)	Number of animals per group									
	10	20	30	40	50	60	100	150	200	1000
	Tumor rates for carcinogenic effect (%)[a]									
5	69	46	37	31	27	24	19	16	14	8
10	76	55	45	39	36	33	27	23	21	14
20	87	69	59	53	49	46	40	36	33	26
30	94	80	70	65	61	57	51	47	45	36

Note. Courtesy of Dr. Joseph K. Haseman, National Institute of Environmental Health Sciences.
[a] $p < 0.05$ with 90% power. (Calculations based on Fisher exact test.)

60, there will be more than a doubling in sensitivity (after compensating for background rate); whereas the same increase of 40 animals from 60 to 100/group will result in an increase of sensitivity by only a third. Approximately 50 animals per group (Portier and Hoel, 1983; NTP, 1984) achieves a reasonable balance between the cost, animal use, and study sensitivity.

CARE OF ANIMALS ON STUDIES

Much of the variation between animals and between studies is a product of genetic and environmental factors. Contribution of genetic variability may be substantial when outbred stock (e.g., Sprague–Dawley rats, ICR–Swiss mice) are used. However, with genetically defined inbred and hybrid strains, most of the variability is due to environmental factors. The environmental variables could be physical or biological factors and some of these are listed in Table 2. The effects of these factors were discussed in detail elsewhere (Rao, 1986; Haseman *et al.*, 1989, and the references cited therein). Some recent findings on the effects of light, diet, and viral infections are discussed below.

Light. The duration, intensity, and quality of light will influence many physiologic responses and reactions. Light may cause eye lesions and influence tumor incidences (Greenman *et al.*, 1984; Wiskemann *et al.*, 1986). High light intensity may cause eye lesions in albino rodents, (Bellhorn, 1980; Greenman *et al.*, 1982). In some NTP studies, for example, Fischer 344 rats housed in the top row and side columns of racks where they were exposed to more light than the rats in other cages have been observed to develop opacity of the eyes and this appears to be due to inflammation of various structures of the eye. This lesion later progressed to generalized inflammation of the eye with retinal degeneration and cataract formation. Prevalence of eye lesions by cage position and light intensity in male F344 rats is illustrated by an example shown in Table 3. If the chemical under investigation has the potential to cause eye lesions, then the light intensity related eye lesions may complicate the interpretation of chemical effects. As shown in Table 4 (NTP, 1989), high doses of the chemical appear to have increased the incidence of eye lesions in male F344 rats. However, the contribution of light intensity for development of eye lesions in the treated groups is not known. The prevalence of light intensity-associated eye lesions in rodents could be markedly decreased by (a) reducing the light intensity in the cages to <15 foot-candles or the animal room light intensity to <50 foot-candles at 5 ft from the

TABLE 2

ENVIRONMENTAL VARIABLES INFLUENCING THE BIOLOGIC RESPONSES OF TEST ANIMALS[a]

Physical factors
Light
Intensity
Duration
Quality
Temperature
Relative humidity
Ventilation
Air quality
Air velocity
Atmospheric conditions
Barometric pressure
Altitude
Ion balance of the air
Noise
Diet
Nutrients
Contaminants
Bedding
Endogenous hydrocarbons
Contaminants
Biological factors
Group compatibility
Group composition
Infections
Diseases

[a] From Rao, 1986.

floor and (b) rotating the cages of each column of a rack from top to bottom when cages or racks are changed (NTP, unpublished data).

Diet. A casein-based purified diet (Newberne *et al.*, 1973) considered to be adequate for a multigeneration reproduction study in rats was found to be deficient in manganese, warranting invalidation of a 2-year *in utero* exposure chemical carcinogenesis study (Rao, 1988a). Optimal diets for rodents in long-term toxicity and carcinogenesis studies should be nutritionally adequate for growth and maintenance without excesses of high energy and growth enhancing nutrients. There should be established formulation with standards for ingredients, nutrient concentrations, and contaminant limits. Contaminant concentrations should be as low as practical. Each batch/lot of diet should be analyzed for macronutrients and selected micronutrients with complete micronutrient analyses on selected batches/lots (Rao, 1988a).

Viral infections. Sendai virus (SV), pneumonia virus of mice (PVM), and mouse hepatitis virus (MHV) are the most common viral infections of mice and SV, PVM, and rat corona virus/sialodacryoadenitis virus (RCV/SDAV) infections are most common in rats (Boorman *et al.*, 1986). Viral infections may complicate toxicology research (Collins, 1986). In a systematic comparison of control and chemically treated groups of B6C3F1 mice, viral infections did not cause consistent adverse effects on survival and tumor prevalences. However, SV infection in mice was associated with significantly ($p < 0.05$) higher survival and survival-associated increase in liver tumors (Rao *et al.*, 1989a). Viral infections did not cause consistent adverse effects on survival or tumor prevalences in control groups of F344 rats (Rao *et al.*, 1989b). However, viral infections were associated with nonneoplastic lesions in lungs, nasal cavity, liver, and other organs of rats and mice and may complicate the identification and interpretation of toxic effects of chemicals (NTP, unpublished data).

HUMANE CONSIDERATIONS

Animals used in toxicology or carcinogenesis studies should be monitored closely by experienced professional scientists, especially if the chemical is toxic or if there are increases in tumor-bearing animals. There should be specific criteria supplemented with professional judgment for euthanasia of moribund animals during the course of these long-term studies. The objective of these criteria is not only to relieve excessive pain and distress to animals but also to allow collection of tissues for pathologic assessment that are free of secondary complications. Major reasons for eu-

TABLE 3

PREVALENCE OF EYE LESIONS BY CAGE POSITION AND LIGHT INTENSITY IN MALE F344 RATS[a]

Row	Treatment	Column or cage				
		1	2	3	4	5
1 (top)	Light intensity	50[b]	37	30	37	50
	Eye lesions	4[c]/4[d]	5/5	4/4	5/5	5/5
2	Light intensity	40	28	20	28	40
	Eye lesions	3/4	1/5	0/3	0/3	4/5
3	Light intensity	30	21	15	21	30
	Eye lesions	1/3	1/4	0/4	1/4	1/5
4	Light intensity	20	15	11	15	20
	Eye lesions	1/4	0/4	0/4	1/4	1/5
5	Light intensity	16	11	6	11	16
	Eye lesions	0/4	0/5	0/5	0/5	0/5
6	Light intensity	8	5	3	5	8
	Eye lesions	0/5	0/5	0/5	0/5	1/5

[a] An example from the NTP unpublished data.
[b] Light intensity in foot-candles inside front of the cages under conditions where the cage rack is parallel to the ceiling light fixtures and the room light intensity is 120 fc at 5 feet from the floor.
[c] Number with lesions.
[d] Number surviving at the time of observation at 22nd month of a study.

thanasia of animals during the course of a study include: (a) large masses and other conditions interfering with eating and drinking, (b) major injuries and ulcers related to husbandry, fighting, or chemical exposure, and (c) diseases and conditions indicating pain and suffering as judged by an experienced laboratory animal specialist. For example, a large mammary tumor may be injured during the normal movement in the cage leading to ulceration and infection resulting in secondary lesions in the tumor and in the animal. Major injuries such as accidental fracture of a limb or wounds resulting from fighting of group-caged animals may lead to unrelieved pain and nonhealing lesions. For humane

TABLE 4

PREVALENCE OF EYE LESIONS BY CAGE POSITION AND CHEMICAL TREATMENT IN MALE F344 RATS[a]

Row	Treatment	Column or cage				
		1	2	3	4	5
1	Low dose	2[b]/5[c]	3/5	3/5	1/5	3/5
2	Low dose	3/5	2/5	3/5	1/5	2/5
3	High dose	4/5	2/5	5/5	4/5	5/5
4	High dose	4/5	4/5	4/5	4/5	2/5
5	Control	1/5	2/5	1/5	0/5	2/5
6	Control	1/5	1/5	2/5	1/5	0/5

[a] From the NTP Technical Report No. 368.
[b] Number with lesions.
[c] Number per cage.

reasons and to prevent complications of chemical-induced lesions by secondary changes, it is prudent to euthanatize and necropsy the animal. In studies such as dermal toxicity and carcinogenesis where treatment causes persistent or enlarging ulcers and fast growing tumors which are injured by the animal or components of the cage, it is appropriate to euthanatize and necropsy the animal not only to relieve pain and distress but also to prevent infections. Infections and inflammation may lead to secondary lesions which can complicate the interpretation of toxic effects and may decrease the sensitivity of a toxicology study. Other considerations for euthanasia of rodents in chronic studies are listed in Table 5; most of these conditions will change the normal physiology and may affect morbidity and mortality. Any one of these conditions may not lead to mortality in a short period, but a combination of these conditions will compromise the physiology and health of the animals. If these animals are allowed to continue in a study, secondary affects may mask or exacerbate the chemical effects, complicate the interpretation of toxic effects, and decrease the sensitivity and quality of a long-term study.

TABLE 5

ADDITIONAL CONSIDERATIONS FOR EUTHANASIA OF RODENTS IN LONG-TERM STUDIES

- Loss of 20 to 25% body wt in less than 1 week
- Gradual but continuous decline in body weight indicating partial and sustained anorexia
- Prolonged unhealthy appearance such as rough coat, hunched posture, and distended abdomen
- Prolonged diarrhea leading to emaciation
- Prolonged or intense diuresis leading to emaciation
- Persistent coughing, wheezing, and respiratory distress
- Paralysis and other nervous disorders leading to anorexia and continuous decline in body weight
- Bleeding from natural orifices not due to minor injuries
- Persistent self-induced trauma complicating minor injuries
- Microbial infections interfering with toxic and carcinogenic responses

DURATION OF TOXICOLOGY AND CARCINOGENESIS STUDIES

Duration of toxicology studies with rodents may range from 3 to 18 months depending on the toxicity of the chemical (Frederick, 1986). In general a 3- to 6-month toxicity study may be long enough for many chemicals with considerable acute toxic effects and limited cumulative toxicity. However, with a high proportion of chemicals, due to the cumulative nature of their toxicity, 3 to 6 months duration may not be long enough and studies of 9 to 12 months duration may be necessary. Chemicals that can cause delayed toxicity, such as neurotoxicity, may have to be evaluated in studies of 12 to 18 months duration. However, when conducting studies longer than 12 months, one should be aware of and consider the influence of age-associated changes and complications in interpretation of the toxic responses.

Duration of chemical carcinogenesis studies in rodents usually covers a major portion of the life span. Length of generally accepted chemical carcinogenesis studies in rats and mice include: (a) 21 to 24 months, (b) 30 months or 20% survival, whichever occurs first, and (c) until most or all animals in the chemically exposed groups die or are euthanatized due to moribund condition. For potent or "early" carcinogens, studies longer than 18 months may not be necessary (e.g., 1,3-butadiene, Huff *et al.*, 1985). For weaker carcinogens with delayed expression of carcinogenic potential, studies of longer than 21 months may be necessary. The median life span (50% survival) of rodent species and strains used for chemical carcinogenesis studies is 20 to 30 months and, therefore, studies lasting 21 to 27 months will cover 60–80% of the life span, assuming 90% mortality is close to the life span. In these long-term studies, especially in studies longer than 18 to 21 months, there are several age-associated

TABLE 6

PREVALENCE (%) OF AMYLOIDOSIS IN ICR–SWISS (CD-1) MICE[a]

Site	23 months of age[b]		25 months of age[c]	
	Male	Female	Male	Female
Liver	40	34	5–14	7–21
Kidney	55	57	13–33	17–52
Spleen	19	12	2–16	7–19
Heart	33	28	5–19	3–20
Thyroid	24	36	2–33	8–40
Adrenal	45	42	10–26	15–40
GI tract/stomach	48	52	0–19	0–25
Ovary	—	41	—	12–21

[a] From Rao *et al.*, 1988.
[b] Mean of five studies with 500 animals from Merck Sharp & Dohme Research Laboratories.
[c] Range of nine studies with 540 animals from Procter and Gamble Co. Studies conducted at different toxicology testing facilities.

changes that may compromise the health of the animals and complicate the expression and interpretation of carcinogenic response. Tables 6 and 7 and Figs. 1 to 3 illustrate some of the age-associated changes that may complicate long-term carcinogenesis studies.

Male F344 rats attain a maximum body weight at 80.2 ± 9.6 (mean ± SD) weeks of age (Haseman *et al.*, 1985) or some time during the 15th to 20th month of a chronic study and may lose as much as 20% of their body weight by the 24th month. The decline in body weight is accompanied by an increase in water consumption or vice versa (Fig. 1). The increase in water consumption (polydipsia) is due to nephrosis leading to polyuria with substantial changes in physiologic processes such as distribution, metabolism, and excretion of chemicals (Grice, 1984). These changes could complicate the interpretation of a toxicology study. Even in a carcinogenesis study where the animals are steadily losing body weight, it may not be scientifically prudent to continue the study after a substantial decline in body weight.

Female B6C3F1 mice reach maximum body weight at 98.1 ± 10.2 weeks of age (Haseman *et al.*, 1985) or during the 19th to 23rd month of a chronic study. A steep increase in mortality and persistent loss of body weight (Fig. 2) starts after 21 to 24 months of study. Even though the life span (90% mortality) of female B6C3F1 mice is about 35 months, median survival is reached at about the 28th month of a chronic study and coincides with a 15 to 20% loss of body weight. Furthermore, the prevalence of lymphoma in control mice shows a marked increase between the 21st and 24th months (Fig. 2), indicating that it may not be appropriate to con-

TABLE 7

PREVALENCE OF AMYLOIDOSIS (%) IN OUTBRED SYRIAN HAMSTERS[a]

Site	Males[b]		Females[c]	
	Mean	Range[d]	Mean	Range[d]
Liver	17	6–33	72	49–87
Kidney	26	17–42	81	77–86
Spleen	15	6–24	70	60–80
Adrenal	20	9–33	67	57–87
Thyroid	18	3–38	57	46–64

Note. From Newcomer *et al.*, 1987.
[a] 20–25 months of age.
[b] *N* of 217 to 236.
[c] *N* of 183 to 188.
[d] Range of Six Studies with 30–48 animals per study.

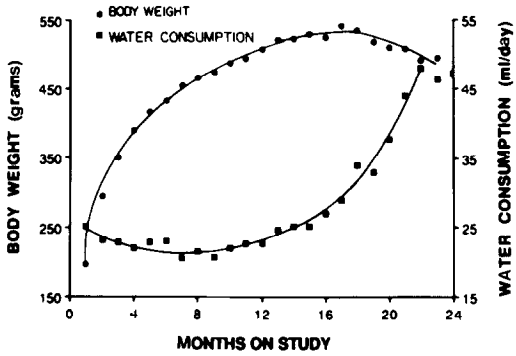

FIG. 1. Body weight and water consumption patterns of male F344 rats. Average of two control groups with 50 rats per group. (NTP unpublished data.)

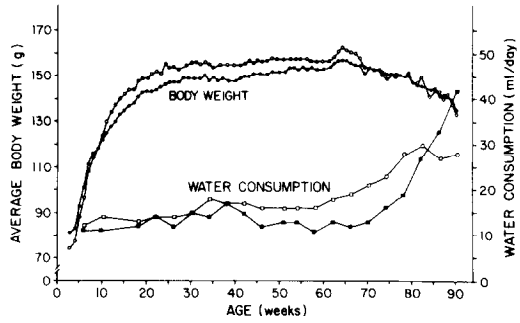

FIG. 3. Body weight and water consumption patterns of F1D Alexander hybrid hamsters. Average of a group of 87 hamsters of each sex. (■, ●) Males, (□, ○) females (adapted from Homburger *et al.*, 1983).

tinue carcinogenesis studies in B6C3F1 mice beyond 24 months, especially when the lymphoreticular organs are the target tissues.

Table 6 shows the prevalence of amyloidosis in ICR–Swiss (or CD-1) outbred mice (Rao *et al.*, 1988b). High prevalence of amyloidosis in major organs such as kidney, liver, and heart at 23–25 months of age (or 22–24 months of a chronic study) may impair the ability of these organs to metabolize and dispose the chemicals and may complicate the interpretation of neoplastic lesions.

Body weight and water consumption patterns of a strain of Syrian hamsters (Homburger *et al.*, 1983) are shown in Fig. 3. A marked increase in water consumption begins at 65–75 weeks of age together with a start of persistent decrease in body weight, indicating changes in physiologic processes. Furthermore, there is a high prevalence of amyloidosis (Newcomer *et al.*, 1987) in major organs such as liver, kidney, and spleen in 20- to 25-month-old Syrian hamsters as shown in Table 7. The body weight and water consumption patterns (Fig. 3) and the prevalence of amyloidosis (Table 7) indicate that the physiology and health of these animals may have been compromised by about 20 months of age and so carcinogenesis study of longer than 18 months may not be appropriate with Syrian hamsters.

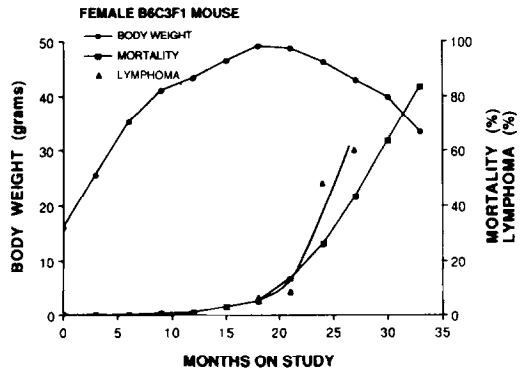

FIG. 2. Average body weight and mortality patterns of a group of 300 mice and incidences of lymphoma in groups of 50 mice necropsied at 18, 21, 24, and 27 months of a study. (NTP unpublished data.)

Tables 6 and 7 and Figs. 1 to 3 identified a few of many age-associated changes observable in the whole animal. These changes are due to several pertubations at the subcellular, cellular, and organ level. Some of these changes relevant to chemical toxicology and carcinogenesis may include chemical absorption, distribution, metabolism, and excretion; protein, lipid, and carbohydrate metabolism; hepatic drug metabolism, renal excretion, and morphologic changes in liver, kidney, and other organs (Grice, 1984).

A variety of diseases, tumors, and age-associated changes present in laboratory rodents most often increase in severity and incidence with increasing age, resulting in marked alter-

ations in physiologic processes. These diseases and tumors are associated with alterations in function and morphology of many organs, making it difficult to differentiate between age-associated changes and chemically induced toxicity and lesions. Changes in organ function are associated with changes in metabolism and toxic responses to chemicals. With few exceptions, the aging animal metabolizes chemicals less effectively and is more susceptible to the toxic effects of chemicals (Grice, 1984). Studies beyond 18 months in hamsters and 24 months in mice and rats may result in greater variability and nonneoplastic disease-associated complications in interpretation of results. Increasing the duration beyond these general limits may not be a refinement of long-term toxicity and carcinogenesis studies. However, for weak carcinogens with delayed expression of carcinogenic potential, studies of longer than 24 months in strains with long life spans and low prevalence of spontaneous lesions and tumors may be necessary.

OTHER REFINEMENTS

Most of the National Cancer Institute–National Toxicology Program long-term chemical carcinogenesis studies included male and female F344 inbred rats and male and female B6C3F1 hybrid mice. Haseman and Huff (1987) reported species correlation in 266 long-term toxicology and carcinogenesis studies. Retrospective analyses of sex–species combinations for identifying potential chemical carcinogens are shown in Table 8. Combinations of male rats and male mice or male rats and female mice detected 124 (92%) of the 135 identified carcinogens. The male rats and female mice combination "missed" 11 (8%) chemicals shown to be positive in one of the other sex–species, of which 7 were positive in male mice only, 3 in female rats only, and 1 in female rats and male mice. The male rats and male mice combination also "missed" 11 (8%) carcinogens, of which 6 were positive in female mice only, 3 in female rats only, and 2 in female rats and female mice. Prospective predictions based on these retrospective evaluations may not be appropriate for unknown chemicals. When a class of chemicals is evaluated (e.g., benzidine dyes, nitrotoluenes, glycol ethers), both sexes of rats and mice may have to be included to assess full carcinogenic potential and mechanism of carcinogenesis of a few representative chemicals of the class. However, for evaluation of other chemicals in that class, the most sensitive sex of one or more species may be adequate. This refinement may decrease the number of animals for selected chemicals and reduce costs of some long-term studies.

Chemical carcinogenesis is a multistep process and may involve the activation of one or more protooncogenes. Genetically engineered animal models such as transgenic mice with *ras, myc, neu* or other oncogenes may be useful in refinement of chemical carcinogenesis studies. The advantages of transgenic animal models could be (a) carcinogenic potential of chemicals may be detected with shorter studies, e.g., 6 to 12 months instead of 18 to 24 months; (b) sensitivity and possibly specificity may be increased with proper animal models for different types or classes of potentially carcinogenic chemicals; and (c) the number of animals per chemical may be reduced with a reduction in cost. The NTP has started studies with transgenic strains TG.SH with *ras,* TG.M with *myc,* and TG.NK with *neu* oncogenes using known carcinogens and noncarcinogens. The purpose of these studies is to assess the usefulness and advantages of transgenic mouse models for detecting chemical carcinogens. In addition, the NTP (NIEHS) is developing an extensive program for development and evaluation of transgenic models to refine and optimize not only carcinogenesis studies but also genetic toxicology, possibly immunotoxicity, and reproductive and developmental toxicology studies.

In summary, alternative *in vitro* assays or other methods will not replace live animals

TABLE 8

RETROSPECTIVE ANALYSES OF SEX-SPECIES COMBINATIONS FOR IDENTIFYING POTENTIAL CHEMICAL CARCINOGENS[a]

Sex-species combinations	Positive (%)	All (%)[b]
Male and female rats	99/135 (73)	230/266 (86)
Male and female mice	103/135 (76)	234/266 (88)
Male rats and male mice	124/135 (92)	255/266 (96)
Male rats and female mice	124/135 (92)	255/266 (96)
Female rats and male mice	112/135 (83)	243/266 (91)
Female rats and female mice	114/135 (84)	245/266 (92)
Male and female rats and mice	135/135 (100)	266/266 (100)

[a] Haseman and Huff (1987).

[b] 131 studies were negative in male and female rats and mice.

for toxicology research. If a study must be done with animals, then we are obligated to do it right the first time. This is the best refinement one can do to decrease the use of animals. Essential components of refining toxicity and carcinogenesis studies include: (a) selection of animal models that can metabolize a chemical in a similar manner to humans, that are small enough to house in large numbers in a laboratory setting, that have a low prevalence of spontaneous lesions, and that have a long life span; (b) control of variables such as environmental conditions, diseases, and genetic characters of laboratory animals; (c) euthanasia of animals during the course of a study to alleviate pain and suffering, thus preventing development of secondary lesions and facilitating detection of chemical effects; and (d) limiting the duration of studies in rodents; which may decrease variability and complications due to nonneoplastic lesions and spontaneous tumors. All of these procedures will help to decrease the variability and increase the sensitivity—thus refining toxicity and carcinogenesis studies. Further refinements include the use of genetically engineered animal models such as transgenic mouse strains with known oncogenes to decrease the duration and increase the sensitivity of carcinogenesis studies with a decrease in the use of animals.

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