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INTERPRETATION OF BIOASSAY RESULTS — SPECIES, SEX, AND TUMOR-SITE SPECIFIC CARCINOGENIC EFFECTS IN LONG-TERM STUDIES IN RATS AND MICE —

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Abstract: Correlations among species, sex, and tumor-site of neoplastic responses in 25 chemicals tested for carcinogenicity at the An-Pyo Center in 1981-1993 were evaluated. Of the 25 chemicals tested in rats and mice, 44 percent (11 of 25) were positive in at least one species and 56 percent (14 of 25) were negative in both rats and mice. Rats and mice exhibited a similar sensitivity to carcinogens as evidenced by 8 positive studies in rats and 9 positive studies in mice for a total of 17 positive studies. Three of the 25 chemicals evaluated were genotoxic and all tested positive in the carcinogenic bioassays in all groups and in both species and sexes. In addition, target tissues were similar between males and females. However, species specific or sex-related responses were evident at different dose levels with the non-genotoxic compounds tested.

Compilation of historical tumor development data has revealed biological mechanisms whereby three classes of compounds produce characteristic positive results in rodent bioassays. Criteria for defining genotoxic, non-genotoxic and promotor/pseudo-carcinogens are presented and discussed.

Results of current bioassays together with mechanistic and refined, short-term studies may be evaluated collectively to aid in interpretation of results and in the mechanistic identification of these three classes of compounds. (J Toxicol Pathol 9 : 1~12, 1996)

Key words: Rodent carcinogenicity, Interspecies correlation, Chemical carcinogens, Site-specific neoplasms

Introduction

The results of the total bioassay testing experience of the An-Pyo Center, a not-for-profit contract laboratory, is available for studies conducted on the carcinogenic potential of 25 chemicals in rats and mice from 1981 to 1993. Criteria were established to evaluate current rodent bioassays as a useful way of identifying chemicals that may pose a carcinogenic threat to humans.

There are many inherent problems in the analysis and interpretation of long-term carcinogenicity studies in laboratory rodents, such as interlaboratory variations in animal husbandry and environmental factors. Different standard operating procedures together with the lack of uniform criteria for evaluations may be causative factors in the interpretation of bioassay results[1-4]. Perhaps most significant is the wide spread variability that may arise in the appearance of spontaneously occurring tumors in rats and mice as the results of interlaboratory heterogeneity[2,5-7]. The compilation of findings and establishment of a large historical control data base on 50 carcinogenicity studies conducted at the An-Pyo Center, coupled with a rational statistical analysis of tumor incidence data, could contribute to minimize interlaboratory variability and lead to more meaningful evaluations and interpretations of rodent bioassays[8-10].

In this paper, the species distribution of individ-

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ual findings in the 50 carcinogenicity studies conducted on 25 chemicals in rats and mice, and the species and sex correlations in site-specific neoplastic responses were examined. These data between genotoxic and non-genotoxic chemicals were also compared to evaluate species, sex, and site correlations in the distribution of carcinogenic manifestation.

Materials and Methods

Table 1 shows the standard design of a bioassay for carcinogenicity at the An-Pyo Center. Long-term animal bioassays were conducted in animal rooms with barrier systems in compliance with GLP regulations. This design includes statistical analyses of animal survival, body weight data, and incidences of neoplastic and non-neoplastic lesions in accordance with the Toxicity Test Guidelines, collection of notifications related to the pharmaceutical affairs law (IV), issued from the Government of Japan (MHW), 1984[11], and the Guidelines (59 Nohsan No. 4200, 1985). Details of animal husbandry of the An-Pyo Center have been described elsewhere[12,13].

Twenty-five compounds were tested for various industries and included pharmaceutical products, pesticides, and chemicals. Of the 17 pharmaceuticals tested, 2 were genotoxic and 15 were non-genotoxic. The classes of medicinal drugs tested included : central nervous stimulants, psychomimetics, gastrointestinal and anti-ulcer drugs, analgesics, anti-inflamatories, urinary tract anti-bacterials, anti-asthmatics, anti-neoplastics, and others. Seven non-genotoxic and one genotoxic pesticide were tested and included : herbicides, insecticides, germicides, and plant growth regulators. One other industrial chemical was genotoxic. The majority of all compounds tested were administered by dietary admixture and fed to the animals *ad libitum* for up to 104 weeks (Table 1). One anti-neoplastic agent and one industrial chemical were administered in the drinking water.

The present study consists of the analyses that were performed on the 50 studies that were conducted in rats and mice on the aforementioned 25 compounds. Pathology data were compiled by nine An-Pyo Center pathologists, using the same diagnostic criteria over the past 12 years. Their procedures have been described previously[9,10]. Pathological evaluations consisted of a two-phase process conducted by both study pathologists and chief pathologists, including two full members of the Society of Toxicologic Pathologists, North America, and two certified members of Japanese College of Veterinary Pathologists. To avoid any discrepancies in the terminology used, practical and generally accepted definitions of commonly used terms following the definition of terminology of JE Huff *et al.*[4] and RR Maronpot[14,15] were used. Equivocal evidence of carcinogenic activity was defined as a study that showed a false positive effects as explained in detail in the Results and Discussion sections of the report.

Results

Analysis of the An-Pyo Center's bioassay results indicated that 17 bioassays (34%), 11 out of 25 chemicals tested (44%), were positive for carcinogenicity

Table 1. Representative Design Outline for Long Term Toxicity and Carcinogenicity Studies in the An-Pyo Center

Chemicals :	Medical drugs, pesticides and other chemicals
Animals :	Fischer 344/DuCrj rats and B6C3F$_1$ mice
Sex :	Males and females
Group size :	70-80 animals/sex/group/dose for pesticides, 50 animals/sex/group/dose for medical drugs and other chemicals
No. of groups :	Control and three to four exposure groups/species/sex
Doses :	Chosen as proportions of a predicted estimated maximally tolerated dose (EMTD). Selection based on body weight and food consumption, clinical signs, some on biochemical and blood examination and histopathologic observations, from prechronic studies (14 days and/or 28-90 day exposure)
Duration :	104 weeks (6 studies using mice, terminated at week 78)
Interim kill :	At 26, 52 and 78 weeks. 10 animals/sex/group/dose
Necropsy :	All animals
Diagnoses :	Two-phase histopathology process (see text for explanation)

Table 2. Bioassay Results Including Species Involved and Hepatic Tumors of Mice in Positive Carcinogen Bioassays in the An-Pyo Center

Category	Number (%) 25 chemicals	Category	Number (%) 50 studies for 25 chemicals
Positive bioassays	11/25 (44%)	Positive bioassays	17/50 (34%)
No evidence in one species	5/25 (20%)	Rat involved	8/17 (47%)
No carcinogenic effects	14/25 (56%)	Positive in rat only	2/17 (12%)
Equivocal (Pseudocarcinogen)	3/25 (12%)	Mouse involved	9/17 (53%)
		Positive in mouse only	3/17 (18%)
		Only liver in mouse positve	6/ 9 (67%)
		Mouse liver, only site in total positives	3/17 (18%)

Table 3. Carcinogenicity Results for 25 Chemical Studies in Rodents

Proportion of positive studies	Rats		Mice		Number of chemicals	%
	Male	Female	Male	Female		
4/4	+	+	+	+	3*	
					Subtotals: 3*	12.0
3/4	+	+	+	−	1	
	+	+	−	+	0	
	+	−	+	+	0	
	−	+	+	+	1	
					Subtotals: 2	8.0
2/4	+	+	−	−	0	
	+	−	+	−	0	
	+	−	−	+	1	
	−	+	+	−	0	
	−	+	−	+	0	
	−	−	+	+	2	
					Subtotals: 3	12.0
1/4	+	−	−	−	(2)	
	−	+	−	−	0	
	−	−	+	−	0	
	−	−	−	+	(1)	
					Subtotals: (3)	12.0
0/4	−	−	−	−	14	
					Subtotals: 14	56.0
Number of positive chemicals	7 (2)	5	7	8 (1)		
Totals:					25	100

( ): Number of chemical showing equivocal carcinogenicity.

*: All genotoxic compounds.

(**Table 2**). Fourteen chemicals (56%) were conclusively negative in both rats and mice. Five of the compounds tested were positive in only one species. Rats and mice displayed similar sensitivities to carcinogenesis; rat (8 out of 17 positive studies, 49%) and mouse (9 out of 17 positive studies, 53%). Three studies (18%) were positive in the mouse only and 2 studies (12%) were positive in the rat only. One-half of the rat and one-third of the mouse studies were judged to be positive due to the formation of both benign and malignant tumors. However, one-half of the rat and two-thirds of the mouse studies; four rat and three mouse studies were associated with the development of benign tumors only. Two of these rat and one of these mouse studies were judged to show equivocal evidence of carcinogenicity. This was probably the result of a pseudo-carcinogenic effect because of the development of benign tumors only in one sex and/or the enhanced development of naturally occurring tumors, such as, pituitary adenoma, or C-cell adenoma of the thyroid and liver cell adenoma in mice. In addition, although statistically higher incidences ($p<0.05$ vs. concurrent control groups) of tumors were observed in the high-dose groups, a dose response was not evident.

Table 3 summarizes the distribution of individual findings of the 25 chemicals in all four sex-species groups, as done by Huff, Haseman, and Rall (1991)[4]. All three genotoxic chemicals were positive in all four groups. Non-genotoxic compounds exhibited species or sex-related responses to tumor development. The occurrence of tumor sites by species, incuced by 11 of the 25 chemicals testes, is shown in **Table 4**. The underlined numbers indicate the numbers of non-genotoxic compound. The extensive bioassay program conducted by the NTP, U.S.A. revealed that the liver, especially the mouse liver, was the most frequent and common organ site of chemically-induced carcinogenicity[4]. The mouse liver appeared to be more sensitive to carcinogenic effects in the An-Pyo Center studies[8]. Six (67%) out of 9 positive studies in mice showed tumor development in the liver only with non-genotoxic chemicals. The mouse liver was the only site where cancer was induced in 3 (18%) of the 17 positive studies in both rats and mice. Of the 6 chemicals that induced hepatocellular tumors only in mice, one compound showed an equivocal evidence of carcinogenicity. The other 5 chemicals, including two MFO-inducers, induced liver enlargement in both mice and rats. Only one compound incuced hepatocellular adenoma in femals rats at the same high dose-level that caused hepatocellular adenoma and carcinoma in mice. Histologically, the liver cells showed swelling associating with

Table 4. Interspecies Correlation in Carcinogenic Response for Selected Target Sites (An-Pyo Center; 25 Chemicals)

Number of chemicals Site of carcinogenicity	Carcinogenicity outcome (rat/mouse)			
	+/+ 6 (3)	+/− 2*	−/+ 3 (1*)	−/− 14
Liver	2 (1)		5 (1*)	
Hematopoietic system	1			
Lung			1	
Kidney		2	1	
Pituitary		1*	2	
Thyroid		2 (1*)		
Harderian gland			1	
Tongue	1			
Esophagus	1			
Forestomach			1	
Zymbal's gland		1		
Female reproduct. organs	1	2 (1)		

Underlined numbers: Numbers of studies with non-genotoxic chemicals.

*: Number of chemical showing equivocal carcinogenicity.

Table 5. Sites Showing Significant Correlation between Males and Females for Chemically Related Carcinogenicity (An-Pyo Center; 50 Studies for 25 Chemicals)

Number of studies (rats and mice) Site of carcinogenicity	Carcinogenicity outcome (male/female)			
	+/+	+/−	−/+	−/−
	10 (4)	4 (2*)	3 (1*)	33
Rats				
Number of studies	4 (1)	3 (2*)	1	17
Liver		1	1	
Hematopoietic system	1		1	
Kidney		2		
Pituitary		1*	1	
Thyroid		2 (1*)		
Tongue	1			
Esophagus	1			
Zymbal's gland	1			
Female reproduct. organs			3 (1)	
Mice				
Number of studies	6 (3)	1	2 (1*)	16
Liver	4 (3)	1	2 (1*)	
Hematopoietic system			1	
Lung	1			
Kidney		1		
Pituitary		1	1	
Harderian gland	1			
Tongue	1			
Esophagus	1			
Forestomach	1			
Female reproduct. organs			1	

Underlined numbers: Numbers of studies with non-genotoxic chemicals.
*: Number of studies showing equivocal carcinogenicity.

fatty changes or other cytoplasmic alterations and necrosis at very high dose-levels.

In mice, all chemicals that were carcinogenic to the organs other than the liver were genotoxic. Two out of three compounds showing positive interspecies correlation were genotoxic. Their correlated target organs were the liver, tongue, and esophagus. No clear correlation between species was evident in other organs in the other positive carcinogenicity studies. All tumors of the kidney, thyroid, pituitary, and endometrial stromal polyp of the uterus in rats were induced by non-genotoxic chemicals. Mice showed no corresponding target sites with the same chemicals.

Table 5 summarizes the correlation between males and females with respect to site-specific carcinogenic responses for 50 carcinogenicity studies on 25 chemicals. A relatively low association between males and females was seen in either the rat or mouse. All tumors of the kidney and thyroid were induced by non-genotoxic compounds in male rats. There was good correlation between male and female rats with genotoxic compound that induced tumors in hematopoietic organs, the tongue, esophagus, and Zymbal's gland in rat and in the lungs, Harderian glands, tongue, esophagus, and forestomach in mice.

Discussion

Interpretation of bioassay results from 50 carcinogenicity studies in rats and mice on 25 chemicals

that were conducted at the An-Pyo Center from 1981 to 1993, are presented to aid in the scientific evaluation of long-term carcinogenicity studies as predictors of potential human hazards. Current guide-lines for conducting rodent bioassays on a variety of substances including drugs, pesticides, and other industrial chemicals require well-designed, properly conducted and rationally evaluated studies[4,16,17]. As described in the Materials and Methods section, all studies were conducted in strict compliance with the appropriate Good Laboratory Regulations and all protocols (**Table 1**), were designed in accordance with the Toxicity Test Guidelines issued from the Government of Japan after 1984. The An-Pyo Center's historical control data over the past 10 years on F344/CuCrj rats and B6C3F$_1$ mice which includes mortality, body weight, food, and water consumption, clinical signs, organ weight data, gross post-mortem findings, as well as neoplastic, non-neoplastic and spontaneously occurring lesions have been reported previously[9,10,12,13,18].

Bioassay results

Future development of more sensitive assays to detect neoplastic responses to insults will require the utilization of the most currently published data focused on the evaluation and interpretation of results of the U.S. National Toxicology Program[3,4,19-23] coupled with analysis of species, sex, and tumor-site specific carcinogenic effects in long-term studies using both rats and mice[20-22]. Decision on the use or attenuated study designs, the utilization of only one sex of each species and/or the exclusion of the mouse in future bioassays, whthout producing false negative results[20,22], may be possible following careful evaluation of and deliberation on previous bioassays conducted on numerous chemical substances with a variety of biological activities.

Current results of the An-Pyo Center's data base reveal that in 17 bioassays of 25 chemicals, 11 (44%) were positive for carcinogenicity (**Table 2**). Eight out of the 17 positive studies (49%) were positive in rat and 9 (53%) were positive in mouse. Correlation of carcinogenicity between species, as well as between the sexes, was seen in all studies on three genotoxic chemicals, in three of eight non-genotoxic compounds between species (**Table 4**), in one of five rat studies and in three of six mouse studies on non-genotoxic compounds between sexes (**Table 5**). The major target sites that showed significant, chemically related carcinogenic responses between species were seen in the studies on genotoxic compounds, and included the liver, tongue and esophagus. Six(67%) out of 9 positive studies with non-genotoxic chemicals in mice showed tumor development in the liver only. These results indicate that non-genotoxic chemicals were not carcinogenic to the organs other than the liver. However, this may be due to the limited number of positive carcinogens tested in these studies. Tumor induction caused by non-genotoxic agents were also evident in the forestomach[24,25], skin[26], kidney[27] and urinary bladder[28] of mice. All tumors of the kidney, thyroid, pituitary and endometrial stromal polyp of the uterus in rats were induced by non-genotoxic compounds. None of liver carcinogens produced thyroid tumors in either species in these studies.

Another significant characteristic of neoplastic responses of rodents to the exposure of chemicals is the development of malignancy in the induced tumors. One-half of the rat and one-third of the positive mouse studies were malignant. However, half of the rat and two-thirds of the mouse studies with non-genotoxic materials were associated with the development of benign tumors only. As described in the Results section, three chemicals showing benign tumor development showed statistically greater incidences as compared to those of concurrent control groups. This finding was considered to demonstrate equivocal evidence of carcinogenicity and may be more indicative of pseudo-carcinogenicity.

Selection of the mouse in carcinogenicity studies

Recent recommendations for the use of the rat only for carcinogenicity bioassays include the following reasons ; 1) a high correlation in carcinogenic and non-carcinogenic responses between the rat and mouse[20,21], 2) carcinogens, which would be missed by use of rat only, would be predicted, in the light of today's knowledge, not to pose a human risk, 3) the possibility of false positive results in the liver due to the high sensitivity of the mouse liver, 4) difficulty with the statistical analysis of induced neoplasms in organs which have a high incidence of naturally, spontaneously occurring neoplasms as evident by liver

tumors in aged mice. The historical control incidence data for liver tumors (hepatocellular adenoma and carcinoma) at the An-Pyo Center is 1.1% for male F344 rats, 0.4% for female F344 rats[9], 62.1% for male B6C3F$_1$ mice, and 23.8% for female B6C3F$_1$ mice[10]. However, significant associations in chemically-related carcinogenic responses between species is not always observed in the studies with non-genotoxic substances as seen in our present bioassay results. Therefore, the use of both rats and mice are necessary at present to better predict and prevent false negative responses in one species[14,29] (**Table 2**).

Recent progress in the study of molecular mechanisms of carcinogenesis has shed much light on genetic similarities and differences between mice and humans[30–34]. The mouse has been extensively used as the experimental animal of choice in human gene sequencing experiments. Although multiple blood sampling from mice is difficult for mechanistic studies, there appears to be little other justification for excluding the mouse as a test species simply because more relevant models are not available.

The maximum tolerated dose (MTD) and induction time

An important aspect in the analysis and evaluation of bioassay results is the impact of dose-levels on carcinogenicity and the estimation of induction time to tumor. Although the use of high doses to assess human risks has frequently been debated[29,35–37], a MTD less than 30,000 ppm was established to give the greatest chance of detecting effects of chemicals in studies at the An-Pyo Center. The results of these studies suggest that chemical carcinogens are likely to have both an upper and lower limit in induction dose. Selecting one or two dose levels close to but below the lowest level necessary to induce carcinogenicity would be necessary to demonstrate the existence of a threshold[8].

A variety of factors such as species, strain, sex, age, and target tissue may influence the effects of the dose[38]. However, recent well-designed and properly conducted studies employing at least four dose-levels with the detailed histopathological evaluations of both neoplastic and non-neoplastic lesions revealed no evidence of treatment-related tumor induction at a less than a ten to 100-fold lower limit for the vast majority of carcinogenic chemicals[8,39]. Only exceptionally rare and/or strong genotoxic carcinogens have possible carcinogenic effects at a 1,000-fold lower limits such as, aflatoxin B$_1$[40–42] and/or a few nitrosoamine compounds[43–45].

Concerning the induction time of tumors, most of the carcinogen-induced tumors showing progression appeared between 3 to 18 months. One approach to estimate the induction time of a tumor is to refer to its growth rate based on the so-called doubling time of a tumor[46]. Doubling time is calculated by measuring the change in tumor volume as an index which is known to range from one to 10 days; 2 to 3 days on the average in rat and mouse, and from 7 to 2,300 days; 30 to 120 days on the average in human tumors[47,48]. It may be emphasized in the induced tumor profile that the induction time of tumors by chemical carcinogens ranges within a determined time frame for each animal species and for each tissue.

Genotoxic carcinogens

Analyses and evaluation of the data obtained by long-term rodent bioassay have revealed three groups of chemicals, each having unique characteristics in the mode of tumor development and in their mechanism of action as follows.

Genotoxic carcinogens are classical, direct-acting carcinogens which are also genotoxic. As shown in **Table 6**, they induced carcinogenicity following relatively brief period of exposure from 9 to 12 months. Tumors develop in multiple tissues and induction of rare neoplasms may be observed. The incidence of malignant tumor generally exceeds 25 percent. Another characteristic is that they are generally carcinogenic in more than one species, generally in both the rat and mouse, and at low doses.

The genotoxic potential and induction of DNA-adducts have been demonstrated for the hepatocarcinogenic, estrogenic or anti-estrogenic hormones including diethylstibestrol and tamoxifen by 32P-postlabeling techniques[49–53]. Some genotoxic chemicals induced tumors only at dose levels, which produce tissue damage[54]. With such chemicals, tumor induction is considered to be caused by non-genotoxic or dual, genotoxic and non-genotoxic mechanisms.

A short term bioassay for genotoxic chemicals was described by Duskin in 1949[55]. The "radiomimetic cytotoxicity" which elucidated this

Table 6. Genotoxic Carcinogens

Rodent carcinogenicity bioassay
• Carcinogenic by relatively brief exposure
— only one exposure may suffice —
• Carcinogenic in multiple tissues
• Carcinogenic in more than one species
• Induction of "rare" neoplasms
• Malignant neoplasms, more than 25% in incidence
• Carcinogenic at low doses
Short term bioassay
• "Radiomimetic (Duskin, 1949)" cytotoxicity: Hematopoietic organs, Gastrointestinal tract, Gonads, Liver (Upton *et al.*, 1963)
Genotoxicity tests
• Genotoxic *in vivo* and *in vitro* in several different test systems

biological effect was performed by Upton, Asano and ohers[56]. Our studies on derivatives of genotoxic carcinogens, including 4-nitroquinoline-N-oxide, 2-acetylaminofluorene and nitrosoamines, also revealed similar cytotoxicity in hematopoietic organs, the gastrointestinal tract, liver, and gonads, where cell proliferation is continuous[57]. The hepatotoxic compound, 2-acetylaminofluorene causes liver cell necrosis while aflatoxin B_1 causes nuclear damage of liver cells including condensation of the chromatin and karyorrhexis following short term exposure[58].

Results of life time rodent bioassays conducted in Japan, from 1972 to 1980 revealed that some chemicals that tested positive in various *in vitro*, mutagenicity assays were negative with no carcinogenic potential in life time studies[59]. These discrepancies between the positive, *in vitro*, mutagenicity tests and the negative bioassay results are probably due to the presence of various, inherent factors present in the *in vivo* models, which suppress or diminish carcinogenic potential[54]. Sodium benzoate[60] and dichlorvos (DDVP)[61] did not show any radiomimetic cytotoxicity in acute studies.

Non-genotoxic carcinogens **(Table 7)**

High-dose, long-term administration of some chemicals to rodents revealed the existence of non-genotoxic mechanisms of carcinogenicity, that is, induced by maintaining persistent tissue damage, increased metabolic demands, or hormonal imbalance through a receptor-mediated system[62,63]. The target organs of non-genotoxic carcinogens are generally

Table 7. Non-genotoxic Carcinogens

Rodents carcinogenicity bioassay
• Carcinogenic by maintaining persistent tissue damage or increased metabolic demands, or hormonal imbalance
— Reparative/adaptive or hormonal in mechanistic —
• Carcinogenic in limited number of tissues
• Carcinogenic in limited number of species
• Carcinogenic only at high level of exposure
• Demonstrable thresholds or no-effect level
Short term bioassay
• Tissue damage and the consequent induction of cellular proliferation are demonstrable in the target tissue by repeated exposure for 13 weeks
Genotoxicity tests
• Negative in genotoxicity or positive in only a few of several tests for genotoxicity

Table 8. Promoter or Pseudo-carcinogen

Rodent carcinogenicity bioassay
• Chemicals showing an increase in the incidence of naturally-occurring tumors only in treated animals at termination of exposure for 2 years
• Highly conditional in occurrence of tumors such as;
Single species affected
Single sex affected
Single tissue affected
• No or low-level increase in incidence of malignant neoplasms. Spontaneous incidence in appropriate control group of neoplasms induced in treated groups; more than 5%
• Absence of evident dose responsiveness
Short term bioassay
• No "Radiomimetic" cytotoxicity
• No evident tissue damages or no induction of cellular proliferation
Genotoxicity tests
• Negative

limited to specific organs such as the liver, kidney, urinary bladder, forestomach, thyroid, and gonads. However, their carcinogenic potential is evident only at high levels of exposure. Consequently, thresholds or no-effect levels are evident in these studies. Since the carcinogenic activity of non-genotoxic carcinogens is mainifest in only a limited number of species, due to quantative or qualitative difference in metabolism, extrapolation of animal carcinogenicity to man is difficult[64,65].

Tissue damage and the consequent induction of cellular proliferation are demonstrable in target tissues by repeated exposure to these chemicals for 13 weeks. Short-term bioassays are therefore essential to reveal reparative/adaptive or hormonal responses in and mechanistic characteristics of non-genotoxic carcinogens[15,54,66,67]. Non-genotoxic carcinogens are generally negative in genotoxicity tests, but may be positive in only a few.

Promoters or pseudo-carcinogens (**Table 8**)

Rodent bioassays, which show an increase in the incidence of naturally-occurring tumors in the treated animals at termination of long-term exposure may be the result of promoting effects of chemicals coupled with an increased sensitivity of aged animals to carcinogenesis. Analyses of the neoplastic lesions showing dose-related increases or decreases in their incidence rates in long-term bioassays using both F344 rats and B6C3F$_1$ mice in our Center for the past 12

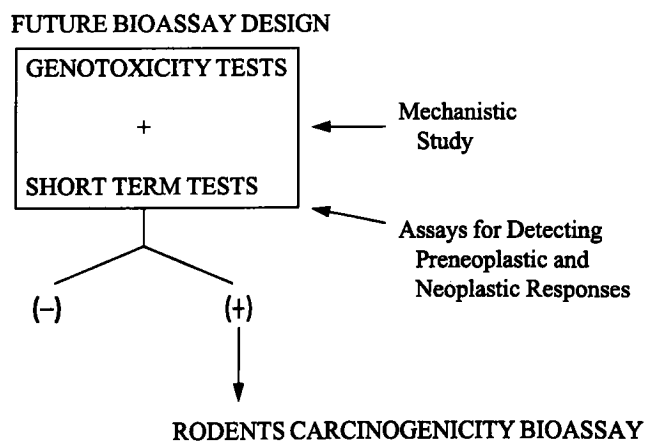

Fig. 1. Future bioassay design

years revealed that they were exclusively naturally-occurring tumors commonly seen in these strains of animals. These lesions included pituitary tumors, C-cell tumors of the thyroid and mononuclear cell leukemia (LGL) in F344 rats and bronchiolar/alveolar adenoma of the lung, hepatocellular adenoma and carcinoma, malignant lymphoma and forestomach papilloma in $B6C3F_1$ mice[8].

Accumulating information on factors influencing the occurrence of naturally-occurring tumors have contributed to the evaluation of long-term bioassay data, which show an increase or decrease in the incidence rates of these tumors in rats or mice. Hormonal and nutritional factors are known to be related to tumor promotion[8,68-71]. Consequently, this group of chemicals which show an increase in tumor incidence rates that occur later than 78 weeks in the bioassay are probably the results of promotion. Consequently, these materials are not true carcinogens but rather preudo-carcinogens.

Bioassay designs for the future (Fig. 1)

Results of current bioassays together with mechanistic and refined, short-term studies should be evaluated and interpreted collectively to aid in differentiating genotoxic from non-genotoxic effects and for detecting chemicals, which act as promoters and enhance tumor production for future bioassays. Refined, short-term assays for detecting preneoplastic and neoplastic responses will be developed by the utilization of and incorporation of the most current scientific knowledge. Future studies will concentrate only on positive chemicals predicted by short-term studies to elucidate target organs, dose responses, sex and species differences in determining their carcinogenic potential and estimating what dose levels will induce tumor formation.

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