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Association between Carcinogenic Potency and Tumor Pathology in Rodent Carcinogenesis Bioassays[1]

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*Biology and Medicine Division, Lawrence Berkeley Laboratory, Berkeley, California 94720; †Tumor Pathology and Pathogenesis Section, Laboratory of Comparative Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, NIH, Frederick, Maryland, 21701; and ‡Department of Preventive Medicine, University of Southern California School of Medicine, Los Angeles, California 90033

Association between Carcinogenic Potency and Tumor Pathology in Rodent Carcinogenesis Bioassays. GOLD, L. S., WARD, J. M., BERNSTEIN, L., AND STERN, B. (1986). *Fundam. Appl. Toxicol.* 6, 677–690. Carcinogenic potency (TD50) estimated from the results of 88 NCI/NTP carcinogenesis bioassays was examined by common target sites in rats and mice. Other indicators of a chemical's hazard were investigated, including whether tumors were induced at more than one site in a single sex–species group of test animals, whether tumors may have caused the death of the animal or were found at sacrifice, and whether metastases of induced tumors occurred. These hazard indicators are sometimes interrelated; however, the potency (TD50) values of chemicals which are hazardous by each of these measures spanned a wide range. Carcinogens which caused some type of fatal tumor were more likely than other carcinogens to cause tumors in multiple organ sites and multiple sex–species groups. Since these other hazard indicators were not related to carcinogenic potency, they should be included along with potency estimates such as the TD50 in summarizing the potential dangers of human exposures to a carcinogen and in comparisons of hazard among carcinogens.

Humans are exposed to a large variety of natural and synthetic chemical compounds from various environmental sources such as air, water, food (Ames, 1983), and occupation. The number of established associations between chemicals or industrial processes and cancer in man is limited (IARC, 1982). Although several hundred chemicals have been found to induce tumors in laboratory animals, the doses to which humans are exposed vary enormously. On the one hand, high dose exposures may occur in occupational environments, but to limited numbers of individuals; on the other hand, many people may be exposed to low levels of mycotoxins, pesticide residues, or plant toxins in foods. In addition, the degree of bioaccumulation and persistence in the environment varies for different chemicals. Thus, establishing the link between human chemical exposure and cancer risk is a difficult task. As a result, government regulatory agencies, public and private organizations, and individuals are confronted with complex decision-making processes in their attempts to reduce the suspected dangers of various chemicals to humans.

Since information about the carcinogenic potential of chemicals to humans is rarely available, the results of chronic-exposure animal carcinogenesis bioassays have been used to evaluate hazards to humans. To further utilize such results, we have proposed a measure of carcinogenic potency, the TD50 (tumorigenic dose 50), and have calculated its value for approximately 3000 chronic laboratory animal experiments on 770 chemicals (Peto *et al.*, 1984; Gold *et al.*, 1984). This measure provides a single numerical description of the

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carcinogenic potency of a chemical which allows one to make quantitative comparisons among experiments, as well as across various laboratory measures.

In the absence of tumors for a given target site in control animals, the TD50 is defined as the chronic dose rate (in mg/kg body wt/day) which would induce tumors in half the test animals at the end of a standard life span for the species (Sawyer *et al.*, 1984; Peto *et al.*, 1984). Since tumors at the site of interest often do occur in control animals, TD50 is more precisely defined as that dose rate which will halve the probability of remaining tumor-free throughout the standard life span of the species.

In earlier papers we have described our conventions for estimating TD50 from experimental data (Peto *et al.*, 1984; Gold *et al.*, 1984), presented a plot of the carcinogenic potency database (Gold *et al.*, 1984), analyzed the relationship of TD50 values in rats and mice (Bernstein *et al.*, 1985), and compared estimates of TD50 based on lifetable vs summary incidence data (Gold *et al.*, 1986).

The tumorigenic dose rate (TD50) is only one measure of potency, however, and therefore cannot fully summarize the results of animal cancer tests on a particular chemical. In this paper we identify chemicals which are hazardous by four other measures, and examine the relationship between carcinogenic potency and these other hazard indicators. We investigate (1) the common target site(s); (2) whether a chemical induces tumors of more than one type in a single sex–species group of test animal; (3) whether the tumors caused the death of the animal or whether they were discovered incidentally at necropsy after terminal sacrifice; and (4) whether metastases of the induced tumor occurred. The latter two characteristics are indicative of the malignancy and biology of the tumor.

Data for this analysis are from the National Cancer Institute/National Toxicology Program (NCI/NTP) Carcinogenesis Technical Reports published prior to July 1980. Of the 189 chemicals tested, we report results for the 88 which were evaluated as providing evidence for carcinogenicity in at least one sex–species group. (See Haseman *et al.* (1984) for results of more recent NCI/NTP Technical Reports.)

METHODS

The TD50 values for the NCI/NTP carcinogenesis bioassays published prior to July 1980 have been reported in detail by Gold *et al.* (1984). Briefly, this group of studies includes long-term bioassays of 185 chemicals conducted in both sexes of B6C3F1 mice and either F344, Osborne–Mendel, or Sprague–Dawley rats, as well as bioassays of 4 chemicals conducted only in F344 rats. For the purposes of this paper an experiment is defined as the test of one chemical in one sex–species group. In most experiments, two dose levels were used, and tumor incidence in dosed animals at a particular target site was compared to that of matched control animals. Among the resulting 776 experiments, (note, some chemicals were tested more than once), 85% of those in mice and 80% of those in rats had 50 or more animals in each dose group; however, 60% of the matched control groups had no more than 20 animals. Most exposures were long term, with more than 88% of both species exposed for at least 18 months; however, exposure for a full 2 years occurred in only 16% of these experiments. Overall, the length of experiment to terminal sacrifice was shorter in mice than in rats; only 32% of the mouse tests lasted 2 years, compared to 85% of the rat tests.

A complete necropsy examination was performed at each NCI/NTP contracted laboratory by contractor pathologists using standard nonmenclature for reporting results of animal tests (Goodman *et al.*, 1979; Ward *et al.*, 1979). Because of the large number of organs examined and the reasonably consistent reporting and evaluation, these experiments provide the most complete and reliable data available for comparing target sites across many tests (Linhart *et al.*, 1974). In this analysis, only target sites evaluated in the NIC/NTP Technical Reports as providing evidence for carcinogenicity are used, and those for which the evidence was considered suggestive or equivocal are excluded in order to restrict the comparison to clearly positive results.

Statistical comparisons of results on hazard measures were made using the χ^2 test; geometric means were compared between groups using the Student's t test.

RESULTS

Carcinogenic Potency by Common Target Sites

There is a ten million-fold range of potency among carcinogens tested in female B6C3F1

mice by NCI/NTP (Ames *et al.*, 1982). There is a similar range of potency for each of the other sex–species groups tested (Gold *et al.*, 1984).

We have previously shown (Bernstein *et al.*, 1985) that the estimated tumorigenic dose rate, TD50, for a carcinogen is restricted to an approximately 30-fold range surrounding the maximum dose tested, in the absence of 100% tumor incidence in all of the treated groups. Since the doses of a particular chemical administered to males and females of each species as well as to rats and mice are highly correlated and vary over many orders of magnitude, it necessarily follows statistically that the potencies of carcinogens will be highly correlated when comparisons are made between males and females or rats and mice.

Below, we compare the TD50 values for chemicals carcinogenic at various target sites in order to determine whether there is some relationship between the potency of a chemical and the site at which tumors are induced. In Table 1 we present the carcinogenic potency values for each of 12 "frequent" target sites in which at least five chemicals were evaluated as carcinogens in either rats or mice. When a chemical was judged carcinogenic in both males and females at a given site, the lower TD50 value is presented.

As illustrated in Table 1, there is no obvious relationship between the tumorigenic dose of carcinogen and the particular organ in which tumors are induced. For each target site the TD50 values span a wide range. For example, the potencies for the ear and Zymbal's gland in rats range from 0.1 mg/kg body wt/day for thio-TEPA to 665.0 mg/kg body wt/day for 2,4-diaminoanisole sulfate. We have also found a wide range of TD50 values for each target site among carcinogens reported in the general literature that were not tested by NCI/NTP and which are included in the carcinogenic potency database (see Gold *et al.*, 1984).

Since nearly all NCI bioassays were conducted in both rats and mice, Table 1 can be used to compare the most frequent target sites in the two species. Five organs (liver, thyroid gland, stomach, hematopoietic system, and vascular system) were target sites for at least five chemicals in both species. Six sites were frequent target organs in the rat but not the mouse (ear or Zymbal's gland, kidney, skin, urinary bladder, mammary gland, and uterus); however, only the lung was a frequent target organ in the mouse but not the rat. Thus, there was a greater variety of frequent target organs in the rat (11) than in the mouse (6). For both species the liver was the most common target site; 50 chemicals induced liver tumors in the mouse compared to eighteen in the rat.

Tumors at Multiple Target Sites in a Single Sex-Species Group

The induction of tumors at more than one target site in a single experiment by a carcinogen is another indicator of chemical hazard. This phenomenon is common in the NCI bioassays where a large number of organs are routinely examined histologically. Since individual animal pathology data are available for each NCI/NTP experiment, we estimated a composite TD50 based on the presence of a tumor at any site at which a compound was judged carcinogenic in the NCI/NTP Technical Report, as well as the TD50 for each site individually. The composite TD50 was based on the number of animals with tumor(s) rather than the number of tumors. For example, in the male rat, cupferron caused liver, stomach, and vascular tumors; and we have estimated the TD50 for each of these separately as well as for the combination of all three. In all cases but one, the composite TD50 value is within a factor of 2 of the lowest TD50 for an individual target site.

In Table 2 we report the multiple-site carcinogens, and the lowest composite TD50 value estimated for any sex–species group with multiple target sites. Other sex–species groups with multiple target sites are noted.

Among the 88 carcinogens tested by NCI/NTP, 40 (45%) were evaluated in the Technical Reports as carcinogenic at more than one site in at least one sex–species group. Twenty-eight of these had such an effect in two or more groups. The phenomenon of tumor induction

TABLE 1

CARCINOGENIC POTENCY (TD50) (IN mg/kg/body wt/day) BY COMMON TARGET SITES

Organ	Species	Sex	TD50	Chemical[a]
Ear, Zymbal's gland	Mouse (1)	F	3930.0	Cupferron
	Rat (9)	M	0.1	Thio-TEPA
		F	2.1	beta-Thioguanine deoxyriboside
		M	7.9	5-Nitroacenaphthene
		M	20.3	4,4′-Thiodianiline
		M	38.5	Hydrazobenzene
		F	58.0	3-Amino-9-ethylcarbazole mixture
		M	105.0	5-Nitro-*o*-anisidine
		F	171.0	Cupferron
		F	665.0	2,4-Diaminoanisole sulfate
Hematopoietic system	Mouse (8)	F	0.2	Thio-TEPA
		F	0.7	Phenesterin
		F	0.7	Procarbazine · HCl
		F	2.2	Estradiol mustard
		F	5.1	Isophosphamide
		F	23.7	ICRF-159
		F	3160.0	2-Aminoanthraquinone
		M	10900.0	C.I. vat yellow 4
	Rat (5)	M	0.2	Thio-TEPA
		F	0.32	Lasiocarpine
		M	2.1	Procarbazine · HCl
		M	405.0	2,4,6-Trichlorophenol
		M	1270.0	3,3′-Dimethoxybenzidine-4,4′-diisocyanate
Kidney	Mouse (2)	M	256.0	Tris(2,3-dibromopropyl)phosphate
		M	1470.0	Nitrilotriacetic acid
	Rat (7)	M	1.6	Tris(2,3-dibromopropyl)phosphate
		M	119.0	Chloroform
		M	131.0	1-Amino-2-methylanthraquinone
		M	511.0	Nitrilotriacetic acid, trisodium salt monohydrate
		M	1040.0	*o*-Anisidine · HCl
		F	1450.0	Nitrilotriacetic acid
		M	2080.0	Chlorothalonil
Liver	Mouse (50)	M	87 ng	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
		M	876 ng	HCDD mixture
		M	0.70	Kepone
		M	0.74	Aldrin
		M	1.09	Heptachlor
		M	2.15	Chlordane
		M	4.08	Toxaphene
		F	6.13	2,4,5-Trimethylaniline
		F	9.45	*p,p′*-DDE
		F	26.00	Hydrazobenzene
		F	26.70	2,4-Diaminotoluene
		F	30.50	3-Amino-9-ethylcarbazole mixture
		M	32.90	Dicofol
		M	33.20	4,4′-Thiodianiline
		F	35.40	1,1,2,2-Tetrachloroethane
		F	45.80	5-Nitroacenaphthene

TABLE 1—*Continued*

Organ	Species	Sex	TD50	Chemical[a]
		F	47.60	1,1,2-Trichloroethane
		F	48.00	Chloroform
		F	53.00	Michler's ketone
		M	62.20	Piperonyl sulfoxide
		F	64.20	Nitrofen
		F	69.30	Selenium sulfide
		F	71.10	Phenazopyridine · HCl
		F	75.60	Tetrachloroethylene
		F	95.00	Tris(2,3-dibromopropyl)phosphate
		F	115.00	1,5-Naphthalenediamine
		F	174.00	1-Amino-2-methylanthraquinone
		F	180.00	5-Chloro-*o*-toluidine
		F	207.00	4,4'-Methylenebis(*N*,*N*-dimethyl)benzenamine
		M	228.00	Tetrachorvinphos
		M	235.00	Chlorobenzilate
		M	266.00	5-Nitro-*o*-toluidine
		F	301.00	*p*-Cresidine
		F	319.00	Hexachloroethane
		F	330.00	Trifluralin
		M	340.00	*p*-Nitrosodiphenylamine
		F	354.00	6-Nitrobenzimidazole
		F	413.00	Cupferron
		M	421.00	Trichloroethylene
		F	594.00	1,4-Dioxane
		F	614.00	2 Nitro-*p*-phenylenediamine
		F	754.00	*o*-Toluidine · HCl
		M	755.00	2-Aminoanthraquinone
		M	758.00	Nithiazide
		M	856.00	2,4,6-Trichlorophenol
		M	957.00	4-Chloro-*o*-phenylenediamine
		F	1230.00	4-Chloro-*m*-phenylenediamine
		M	2270.00	3-Nitro-*p*-acetophenetide
		F	3720.00	5-Nitro-*o*-anisidine
		F	5230.00	Chloramben
Liver	Rat (18)	F	127 ng	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
		F	596 ng	HCDD mixture
		F	2.96	Kepone
		M	3.70	Hydrazobenzene
		F	4.87	Michler's ketone
		F	5.38	1,2-Dibromoethane (gavage)
		F	6.14	Selenium sulfide
		M	7.18	4,4'-Thiodianiline
		M	8.11	2,4-Diaminotoluene
		M	9.28	Cupferron
		M	17.10	3-Amino-9-ethylcarbazole mixture
		F	27.20	2,4,5-Trimethylaniline
		M	39.90	1-Amino-2-methylanthraquinone
		M	48.80	2-Methyl-1-nitroanthraquinone
		M	101.00	2-Aminoanthraquinone
		F	160.00	1,4-Dioxane
		M	201.00	*p*-Nitrosodiphenylamine
		M	406.00	*p*-Cresidine

TABLE 1—*Continued*

Organ	Species	Sex	TD50	Chemical[a]
Lung	Mouse (11)	F	0.42	Phenesterin
		M	0.62	Procarbazine · HCl
		F	1.14	Estradiol mustard
		M	1.82	1,2-Dibromo-3-chloropropane (inhalation)
		M	13.40	1,2-Dibromoethane (gavage, inhalation)
		M	89.70	1,2-Dichloroethane
		M	92.50	Sulfallate
		F	137.00	Selenium sulfide
		M	211.00	Tris(2,3-dibromopropyl)phosphate
		F	331.00	1,5-Naphthalenediamine
		F	1360.00	Trifluralin
	Rat (3)	F	9.63	5-Nitroacenaphthene
		F	83.50	1,2-Dibromoethane (inhalation)
		F	88.10	2,4,5-Trimethylaniline
Mammary gland	Mouse (4)	F	3.58	Reserpine
		F	26.20	1,2-Dibromoethane (inhalation)
		F	27.30	Sulfallate
		F	133.00	1,2-Dichloroethane
	Rat (12)	F	0.39	Acronycine
		F	0.40	Procarbazine · HCl
		F	0.52	Phenesterin
		F	1.46	2,4-Diaminotoluene
		F	2.33	1,2-Dibromo-3-chloropropane
		F	3.60	1,2-Dibromoethane (inhalation)
		F	5.49	1,2-Dichloroethane
		F	11.40	Hydrazobenzene
		F	17.20	Sulfallate
		F	77.90	5-Nitroacenaphthene
		F	115.00	*o*-Toluidine · HCl
		F	131.00	Nithiazide
Skin	Mouse (1)	M	0.28	Thio-TEPA
	Rat (5)	M	0.15	Thio-TEPA
		M	30.50	5-Nitro-*o*-anisidine
		M	45.00	3-Amino-9-ethylcarbazole mixture
		M	358.00	2,4-Diaminoanisole sulfate
		M	1330.00	3,3′-Dimethoxybenzidine-4,4′-diisocyanate
Stomach	Mouse (6)	M	2.36	1,2-Dibromoethane (gavage)
		F	4.29	1,2-Dibromo-3-chloropropane (gavage)
		F	5.17	Estradiol mustard
		F	127.00	Tris(2,3-dibromopropyl)phosphate
		M	161.00	3-(Chloromethyl)pyridine · HCl
		F	3090.00	Trifluralin
	Rat (8)	F	0.91	1,2-Dibromo-3-chloropropane (gavage)
		F	1.26	1,2-Dibromoethane (gavage)
		M	6.28	Cupferron
		M	46.30	1,2-Dichloroethane

TABLE 1—*Continued*

Organ	Species	Sex	TD50	Chemical[a]
		M	53.90	Sulfallate
		M	154.00	Pivalolactone
		M	433.00	3-(Chloromethyl)pyridine · HCl
		M	2500.00	4-Chloro-*o*-phenylenediamine
Thyroid gland	Mouse (5)	F	1.59 μg	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
		M	51.80	4,4′-Thiodianiline
		M	276.00	1,5-Naphthalenediamine
		M	791.00	2,4-Diaminoanisole sulfate
		M	2070.00	3-Amino-4-ethoxyacetanilide
	Rat (7)	M	101 ng	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
		M	5.59	4,4′-Thiodianiline
		F	16.40	4,4′-Methylenebis(*N*,*N*-dimethyl)benzenamine
		F	23.80	*N*,*N*′-Diethylthiourea
		F	25.80	Trimethylthiourea
		M	192.00	2,4-diaminoanisole sulfate
		M	235.00	*o*-Anisidine · HCl
Urinary bladder	Mouse (2)	M	44.70	*p*-Cresidine
		M	935.00	*o*-Anisidine · HCl
	Rat (10)	F	27.80	*o*-Anisidine · HCl
		M	88.40	*p*-Cresidine
		F	106.00	*p*-Quinone dioxime
		F	116.00	*N*-Nitrosodiphenylamine
		F	175.00	*o*-Toluidine · HCl
		F	212.00	4-Chloro-*o*-phenylenediamine
		M	309.00	4-Amino-2-nitrophenol
		M	470.00	*m*-Cresidine
		F	1570.00	Nitrilotriacetic acid
		F	1990.00	Nitrilotriacetic acid, trisodium salt monohydrate
Uterus	Mouse (3)	F	0.25	Procarbazine · HCl
		F	230.00	1,2-Dichloroethane
		F	335.00	Trimethylphosphate
	Rat (7)	F	0.74	Isophosphamide
		F	8.79	4,4′-Thiodianiline
		F	10.70	ICRF-159
		F	69.60	1,5-Naphthalenediamine
		F	106.00	3-Amino-9-ethylcarbazole · HCl
		F	2500.00	Daminozide
		F	2740.00	3,3′-dimethoxybenzidine-4,4′-diisocyanate
Vascular tumors	Mouse (9)	M	1.34	2-Methyl-nitroanthraquinone
		F	24.10	1,2-Dibromoethane (inhalation)
		F	40.00	4-Chloro-*o*-toluidine · HCl
		M	203.00	Michler's ketone
		M	213.00	5-Chloro-*o*-toluidine
		F	419.00	Cupferron
		M	926.00	*o*-Toluidine · HCl
		M	1490.00	Nitrofen
		F	1860.00	5-Nitro-*o*-toluidine

TABLE 1—*Continued*

Organ	Species	Sex	TD50	Chemical[a]
	Rat (6)	F	0.35	Lasiocarpine
		M	5.50	Cupferron
		M	9.60	1,2-Dibromoethane (gavage, inhalation)
		M	15.00	1,2-Dichloroethane
		M	160.00	Aniline · HCl
		F	450.00	*o*-Toluidine · HCl

[a] TD50 values are calculated to three significant figures. For chemicals which cause tumors at the site in both sexes of rat or mouse, the lower TD50 value is reported.

at multiple sites was more common in the rat than in the mouse. Twenty-one of 71 carcinogens in the mouse (30%) induced multiple tumors, compared with 29 of 62 carcinogens in the rat (47%) (χ^2 test two-sided p, $0.05 < p < 0.1$). Since the average mouse test for multiple-site carcinogens was shorter than the average mouse test for single-site carcinogens, this difference between species is not likely to be due to the fact that rat experiments generally last longer than mouse experiments.

We observe in Table 2 that multiple-site carcinogens span a broad range of potency as measured by TD50. The frequency distribution of TD50 values for multiple-site chemicals is similar to the distribution for other carcinogens. For both rats and mice, there were no statistically significant differences between the geometric means of multiple-site and single-site carcinogens (Student's t test). However, multiple-site carcinogens are significantly more likely than other chemicals to induce tumors in both rats and mice (71% vs 39%) rather than in only one of the species (χ^2 test two-sided $p < 0.01$).

A variety of chemical classes are represented among the multiple-site compounds. It is especially noteworthy that chlorinated compounds are underrepresented since most of them induced only liver tumors. Of 17 chemicals containing only carbon, hydrogen, chlorine, and optionally oxygen, only 3 (18%) induced multiple tumors, compared to 37 of the 71 (52%) other carcinogens.

Fatal (Nonincidental) Tumors

The malignancy and biology of induced tumors are additional measures of a chemical's hazard. Since NCI evaluations for more than 95% of the identified carcinogens included at least one target site in one sex–species group which is composed solely of malignant tumors, we have not investigated with this dataset whether a carcinogen induces malignant vs only benign tumors. However, we have investigated whether some induced tumor types may have been fatal and whether metastases occurred.

Few reports of animal experiments attempt to distinguish between those tumors which are the cause of the animal's death (fatal) and those tumors which are not (incidental). Although no data are available on cause of death for the NCI/NTP bioassays, we can, with life-table data, identify those cases where induced tumors were found in animals that died during the experiment rather than at sacrifice. Thus we are able to differentiate between tumors which may have been fatal because the animal died "naturally," and tumors found at sacrifice. We are not able to determine whether tumors discovered at death in nonsacrificed animals actually caused that death, nor can we judge whether tumors found in sacrificed animals might later have killed the animal if it had not been sacrificed.

For a given target site we computed the ratio of TD50 based on all tumors (including those

TABLE 2

CARCINOGENIC POTENCY (TD50) (IN mg/kg body wt/day) OF 40 CHEMICALS WHICH INDUCED TUMORS AT MORE THAN ONE TARGET SITE IN A SEX–SPECIES GROUP

Chemical	TD50[a] Multiple-site groups[b]
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	526 ng FM
1,2-Dibromo-3-chloropropane (inhalation)	0.11 MR (FM, MM, FR)
Lasiocarpine	0.14 FR (MR)
Thio-TEPA	0.15 MR (FR, MM)
Procarbazine · HCl	0.19 FM (MM, FR, MR)
Phenesterin	0.21 FM (MM)
Acronycine	0.50 MR (FR)
Estradiol mustard	0.68 FM (MM)
1,2-Dibromo-3-chloropropane (gavage)	0.86 FR
1,2-Dibromomethane (inhalation)	1.10 MR (FM, MM, FR)
1,2-Dibromomethane (gavage)	1.26 FR (FM, MM, MR)
2,4-Diaminotoluene	1.43 FR
Hydrazobenzene	3.55 MR (FR)
Cupferron	5.33 MR (FM, FR)
4,4′-Thiodianiline	5.52 MR (FM, MM, FR)
5-Nitroacenaphthene	5.98 FR (FM, MR)
1,2-Dichloroethane	11.50 MR (FM)
3-Amino-9-ethylcarbazole mixture	11.80 MR (FR)
Azobenzene	19.20 MR (FR)
2,4,5-Trimethylaniline	20.40 FR
Dapsone[c]	22.40 MR
o-Toluidine · HCl	23.30 MR (FR)
5-Nitro-*o*-anisidine	28.10 MR (FR)
o-Anisidine · HCl	31.90 MR
1-Amino-2-methylanthraquinone	34.10 MR
Selenium sulfide	46.80 FM
1,1,2-Trichloroethane	47.60 FM (MM)
1,5-Naphthalenediamine	50.80 FR (FM)
p-Cresidine	69.00 FM (FR, MR)
2,4-Diaminoanisole sulfate	72.60 MR (FR)
Tris(2,3-dibromopropyl)phosphate	80.10 FM (MM)
Nitrofen[c]	85.30 MM
Aniline · HCl	88.00 MR
1,4-Dioxane	126.00 FR
5-Chloro-*o*-toluidine	134.00 MM (FM)
4-Chloro-*o*-phenylenediamine	197.00 MR (FR)
5-Nitro-*o*-toluidine	242.00 FM (MM)
Trifluralin[c]	330.00 FM
Nitrilotriacetic acid, trisodium salt monohydrate	511.00 MR (FR)
3,3′-Dimethoxybenzidine-4,4′-diisocyanate	742.00 MR (FR)
Nitrilotriacetic acid	1450.00 FR (MR)
2-Aminoanthraquinone	1490.00 FM

[a] The TD50 value listed is for the combination of all tumors evaluated in the NCI/NTP Technical Reports as treatment related. For chemicals causing tumors at multiple sites in more than one sex–species group, the lowest composite TD50 value is listed followed by the appropriate sex–species group. The other sex-species groups are given in parentheses. TD50 values are calculated to three significant figures.

[b] FM = female mouse; MM = male mouse; FR = female rat; MR = male rat.

[c] For these experiments, carcinogenicity at multiple sites was evaluated using pooled controls and no composite TD50 was calculated. The reported TD50 values are those calculated on the basis of the most potent individual target site.

TABLE 3

CARCINOGENIC POTENCY (TD50) (IN mg/kg/body wt/day) OF 19 CHEMICALS FOR WHICH TUMORS AT SOME SITE OCCUR PRIMARILY IN ANIMALS DEAD BEFORE TERMINAL SACRIFICE

Chemical	TD50[a] "Fatal tumor" groups[b]
Thio-TEPA*	0.22 FM (MM, MR)
Lasiocarpine	0.35 MR
Acronycine	0.70 MR (FR)
Procarbazine · HCl*	0.76 FM (MM, FR)
1,2-Dibromo-3-chloropropane (inhalation)*	1.15 MR (FM, MM, FR)
Estradiol mustard	2.34 FM
Phenoxybenzamine · HCl	2.36 FR (FM, MM)
1,2-Dibromoethane (gavage)*	13.40 MM
1,2-Dichloroethane*	15.00 MR (FR)
1,2-Dibromoethane (inhalation)*	16.50 MR (FM, FR)
4,4'-Thiodianiline*	20.30 MR (FR)
1,2-Dibromo-3-chloropropane (gavage)*	30.10 FR
2,4-Diaminotoluene	58.00 FR
o-Toluidine · HCl*	93.20 MR
Sulfallate	98.30 FM
3-Amino-9-ethylcarbazole mixture*	104.00 MR (FR)
5-Nitro-*o*-anisidine	105.00 MR (FR)
1,4-Dioxane*	476.00 FR
2,4-Diaminoanisole sulfate*	665.00 FR (MR)
Nitrilotriacetic acid, trisodium salt, monohydrate	826.00 MR
p-Cresidine*	1190.00 FR

Note. *Chemicals tested in all four sex-species groups which are carcinogenic in all four, and also are potent carcinogens according to the criteria of (a) inducing tumors at multiple sites in at least one sex-species group and (b) inducing tumors in a target site found in animals dead before terminal sacrifice.

[a] TD50 values are calculated to three significant figures. The value given is the lowest TD50 per chemical calculated for a site at which nearly all tumors occur prior to terminal sacrifice in a particular sex–species group. Other sex–species groups which induce tumors that occur primarily before sacrifice are given in parentheses.

[b] FM = female mouse; MM = male mouse; FR = female rat; MR = male rat.

found at sarifice) to TD50 based only on tumors discovered in nonsacrificed animals. If all tumors occurred in animals that died before sacrifice, the TD50 would be the same in both cases and the ratio would equal one. If tumors were found in dosed animals sacrificed at the termination of the experiment, the TD50 for all animals would be lower (more potent) than that based only on animals that died before sacrifice and the ratio would be less than one.

We identified all experiments with a ratio of one or nearly one to determine carcinogens which induced tumors that may have been lethal. We omitted those tumor sites where less than 10% of the animals had the tumor of interest, even though the evaluation in the NCI/NTP Technical Report indicated that the tumors were induced by compound administration. The purpose of this selection procedure was to exclude cases where a few tumors would have a great effect on the value of the ratio.

Experiments for 19 of the 88 carcinogens in the NCI/NTP dataset (22%) were classified as inducing fatal tumors (i.e., ratio ~ 1). The fact that the induced tumors were primarily carcinomas and sarcomas supports the validity of this measure of lethality. Table 3 lists these compounds, their TD50 values, and the sex–species groups in which lethal tumors were induced; the lowest TD50 value is reported when fatal tumors were induced in more than one sex–species group, and that group is listed first.

The TD50 values for these carcinogens range from 0.22 mg/kg body wt/day for thio-TEPA in the female mouse to 1.19 g/kg body wt/day for *p*-cresidine in the female rat.

Chemicals which produce fatal tumors are more likely than other carcinogens to induce tumors in both rats and mice and males and females. Of the 17 carcinogens in Table 3 which were tested in both species, 15 were carcinogenic in rats and mice (88%), compared to only 30/67 (45%) of the carcinogens which did not induce lethal tumors by our criterion. Moreover, 73% of these chemicals were carcinogenic in all four sex–species groups compared to less than 20% of the other carcinogens. Similarly, these substances are more likely than other carcinogens to induce multiple tumors (all but 2 of the chemicals in Table 3 also appear in Table 2).

We have indicated with an asterisk in Table 3 the 11 compounds which are hazardous according to several measures—induction of lethal tumors, induction of multiple-site tumors, and carcinogenicity in all four sex–species groups. The potency values of these 11 substances vary, as do their chemical classes and their uses in the environment. For example, the list includes aromatic amines and halogenated alkylating agents; some of these substances are chemical intermediates in the manufacture of dyes; some are cancer chemotherapy agents; others are pesticides and fumigants.

Metastases of Hepatocellular Carcinomas and Mammary Adenocarcinomas to the Lung

The extent to which induced malignant tumors metastasize to other tissues is another indicator of chemical hazard. We have examined metastases to the lung for the rat and mouse liver carcinogens and the rat mammary carcinogens tested by NCI/NTP. The lung is generally the most common site of tumor metastases in rodents. We define the metastatic rate as the percentage of malignant neoplasms which metastasized to the lung in a given study. Several authors have reported such metastatic rates for selected tissues and chemicals, and they range from 0 to 100% (Ward, 1984; Vessilinovitch *et al.*, 1978).

Generally, the metastatic rates of malignant liver and mammary tumors among NCI/NTP bioassays are quite low; a few chemicals have high rates. Of 50 mouse liver carcinogens, 28 had at least one metastasis in a dosed animal, but only the 8 listed in Table 4 had metastatic rates greater than the rate for historical controls. Among 5000 control B6C3F1 mice, Ward *et al.* (1979) reported a 12% rate of hepatocellular carcinomas metastatic to the lung (in males, 43 metastases among 349 carcinomas, and in females, 7 metastases among 58 carcinomas). Similarly, we found that for the 28 NCI mouse liver carcinogens which had at least one pulmonary metastasis in a dosed animal, the metastatic rate among controls was 12.6% (12/95). In Table 4 for mice, only the metastatic rate for HCDD mixture (60%) was statistically significantly different from the historical rate.

In rats there were too few spontaneous tumors to estimate a historical metastasis rate (Goodman *et al.*, 1979), and we therefore report all cases of induced metastatic liver and mammary gland carcinomas (Table 4). Of the 18 chemicals which induced liver tumors in rats, there were metastases to the lung for 8; of the 12 chemicals which induced mammary adenocarcinomas there were metastases to the lung for 4. Although the numbers are small, there is no apparent association between TD50 and the metastatic rate to the lung for hepatocellular carcinomas in rats or mice, or for mammary carcinomas in the rat.

DISCUSSION

We earlier proposed the TD50 as a standard numerical index of the carcinogenic potency of a compound in a particular strain and sex of animal at a particular target site (Sawyer *et al.*, 1984; Peto *et al.*, 1984), and we reported our estimates of the value of TD50 for approximately 3000 experiments on 770 com-

TABLE 4

METASTATIC RATE TO THE LUNG OF SELECTED INDUCED HEPATOCELLULAR CARCINOMAS AND MAMMARY GLAND ADENOCARCINOMAS

TD50[a] (mg/kg/day)	Chemical	Sex	Dose group	Number of metastases to lung	Number of primary liver or mammary carcinomas	Metastatic rate (%)
Mouse liver[b]						
876 ng	HCDD mixture	M	lo	3	5	60
			hi	3	9	33
33.30	4,4′-Thiodianiline	F	hi	4	30	13
45.80	5-Nitroacenaphthene	F	lo	3	23	13
62.20	Piperonyl sulfoxide	M	lo	4	31	13
71.10	Phenazopyridine · HCl	F	hi	2	14	14
392.00	6-Nitrobenzimidazole	M	hi	3	21	14
856.00	2,4,6-Trichlorophenol	M	hi	1	7	14
3720.00	5-Nitro-*o*-anisidine	F	hi	1	8	13
Rat liver						
127 ng	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	F	hi	1	3	33
3.70	Hydrazobenzene	M	lo	1	5	20
			hi	5	31	16
4.87	Michler's ketone	F	lo	8	41	20
			hi	25	44	57
		M	lo	1	9	11
			hi	9	40	22
5.38	1,2-Dibromoethane (gavage)	F	hi	1	5	20
7.18	4,4′-Thiodianiline	M	lo	7	21	33
			hi	1	10	10
48.80	2-Methyl-1-nitroanthraquinone	M	hi	1	9	22
58.00	2,4-Diaminotoluene	F	hi	1	3	33
406.00	*p*-Cresidine	M	hi	1	1	100
Rat mammary gland						
0.52	Phenesterin	F	hi	1	12	8
2.33	1,2-Dibromo-3-chloropropane	F	lo	4	24	17
			hi	3	30	10
5.49	1,2-Dichloroethane	F	hi	2	18	11
17.20	Sulfallate	F	hi	2	11	18

[a] Value reported is the most potent TD50 (in mg/kg body wt/day) calculated for tumors in the designated organ. TD50 values are calculated to three significant figures.

[b] Includes only experiments in which the metastatic rate is greater than the rate in historical controls (>12%).

pounds (Gold *et al.*, 1984). Such a standard index of the chronic tumorigenic dose rate may improve efforts to use long-term animal bioassay data in the quantitative evaluation of the danger various chemical exposures pose to humans.

One measure, however, cannot fully describe the results of animal cancer tests, and in this paper we have investigated several indicators of chemical hazard from the results of animal tests. These include target site, the induction of tumors at multiple sites in the

same sex–species of test animal, tumors which may have been lethal, and tumors which metastasized to the lung. We have identified particular substances which are hazardous by each of these indicators and have discussed the number of species in which they induce tumors.[3] Our findings show that these hazard indicators are sometimes interrelated, but that the TD50 values for each hazard vary considerably. For example, the distribution of potencies for chemicals which produce tumors at multiple sites is similar to that of carcinogens which produce tumors at only one site.

Theoretically, if the levels of human exposure to two carcinogens are equal, then the compound with the lower TD50, i.e., the more potent carcinogen, is likely to pose a more significant health hazard to humans than the carcinogen with a higher TD50. Since the other measures of hazard investigated here are not related to carcinogenic potency (TD50), they can provide important additional information about potential dangers to humans. For example, assuming a constant exposure level, the potentially most hazardous carcinogen would be one in which the dose rate to induce tumors in animals was low (i.e., low value of TD50), the tumors were malignant and had metastasized, tumors were found in multiple organs and were responsible for early mortality, and the compound induced tumors in several sex–species groups. Another carcinogen with a similarly low tumorigenic dose rate (i.e., low value of TD50) which induced tumors in only one tissue of one sex–species and did not cause early mortality might be considered less hazardous, other parameters being equal.

Currently, the indicators we have discussed are generally used to characterize the qualitative strength of evidence for a compound's carcinogenicity. We have shown here that these measures of hazard are not related to quantitative estimates of potency; that is, that compounds which are hazardous according to one or more of these criteria span a wide range of potency. Therefore, information about such hazards should be included in summaries of the potential dangers to humans of chemical exposures, and in comparisons of the relative hazard of various carcinogens. (See recently proposed guidelines, Environmental Protection Agency, 1984.) The methods for incorporating this qualitative information into estimates of potential dangers to humans in a consistent manner still remains to be determined.

[3] While our investigation has shown that carcinogens which induce multiple or fatal tumors are more likely than other carcinogens to induce tumors in several sex–species groups, we have not addressed the question of whether the number of sex–species groups in which tumors are induced is related to the potency of carcinogens. An analysis of this issue is currently in progress.

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