Reproducibility of Results in "Near-Replicate" Carcinogenesis Bioassays [1,2]

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ABSTRACT—Reproducibility of results was examined in 70 "near-replicate" comparisons consisting of 2 or more long-term carcinogenesis bioassays of the same chemical administered by the same route and using the same sex and strain of rodent. Overall, there was good reproducibility of positivity, target site, and carcinogenic potency in hamsters, mice, and rats. The published authors' opinions about whether the test was positive disagreed in only 9 of the 70 comparisons. Among the 35 comparisons in which all tests of the chemical were positive, 33 of the near-replicates had at least 1 identical target site. The carcinogenic potency values estimated from near-replicate tests in these 35 comparisons were within a factor of 2 of each other in 40% of the comparisons, within a factor of 5 in 80%, and within a factor of 10 in 90%. For the few cases in which the carcinogenic response was not reproduced, analyses suggest two explanations: In mice the discrepant cases tended to have shorter experiment times than average; in both rats and mice the discrepant results tended to be tests of weakly active compounds.—JNCI 1987; 78:1149-1158.

Rodent bioassays are widely used to detect potential human carcinogens and to assess possible hazards to humans from various chemical exposures. The utility of the animal data depends on two major assumptions: 1) that the biological processes involved in the development of cancer are similar in rodents and humans and 2) that the results of animal bioassays are reproducible. Two or more experiments testing the same chemical in the same test animal under comparable conditions are reproducible if they obtain similar results. An experiment is defined here as the dose and control groups for a particular species, strain, and sex in a single research report, and near-replicate experiments are those conducted in the same species, strain, and sex of animal with the use of a reasonably similar protocol. Knowledge of the variation in results between near-replicate experiments provides a reference point for assessing differences in results between species, strains, sexes, or routes of chemical administration. If there are significant differences when the test animals and route are identical, this may reflect the biological, experimental, and/or statistical variation that occurs even when species, strain, and route are constant.

Factors that are important in determining the degree of similarity of the outcomes of two experiments include *a*) whether or not the test agent is carcinogenic in both experiments, *b*) whether the particular target sites are the same, and *c*) whether the estimates of carcinogenic potency are similar. In this paper we compare these three experimental outcomes in chronic, long-term carcinogenesis bioassays, which we define as "near-replicates" because they test the same chemical agent in the same strain and sex of rat, mouse, or hamster by the same route of administration.

The source of data for this analysis is the CPDB, a large compendium of standardized results from chronic long-term animal tests (*1*). The CPDB includes results of approximately 3,000 experiments on 770 chemicals; these results either were published in the general literature prior to July 1981 or were from bioassays carried out under the auspices of the National Cancer Institute-National Toxicology Program published prior to July 1980. All experiments in the data base met a set of inclusion criteria that were designed to allow the estimation of carcinogenic potency; therefore, reasonable consistency in experimental protocols is assured. For example, experiments are included if the test agent was administered alone rather than in combination with other substances, if the protocol included a control group, if the route of administration was diet, water, gavage, inhalation, iv injection, or ip injection, and if the length of the experiment in rodents was at least 1 year with dosing for at least 6 months. [*See* Gold et al. (*1*) for further details.]

METHODS

Selection of near-replicate comparisons.—The CPDB was searched for "near-replicate" comparisons by identifying all cases where 2 or more experiments were conducted of the same chemical, with the use of the same sex and strain of rodent (hamster, mouse, or rat) and the same route of administration. The experiments may have been conducted in the same laboratory at different times or in different laboratories. Seventy near-replicate comparisons were identified, including 161 individual experiments on 38 different chemicals. There are 4 comparisons in hamsters, 25 in mice, and 41 in rats. About a quarter of the comparisons involve 3 or 4 near-replicate experiments, and the rest compare 2 experiments.

ABBREVIATIONS USED: CPDB = carcinogenic potency data base; MTD = maximally tolerated dose.

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Although only 38 chemicals met the criteria for near-replicate experiments, the retesting of compounds generally is not a rare event. One-quarter of the chemicals in the CPDB (190/770) have at least 2 tests conducted at different times, ignoring the requirement that the species, strain, and sex be the same. The 38 compounds in this analysis cover a wide range of applications and chemical classes. Included, for example, are pesticides (DDT, dieldrin), drugs (isoniazid, diethylstilbestrol), compounds found in foods (saccharin, safrole), nitroso compounds (dimethylnitrosamine), metallic compounds [nickel(II) acetate], and recognized human carcinogens (vinyl chloride). A variety of strains or stocks were used in these near-replicate experiments, including inbred and outbred strains and F_1 hybrids.

In two-thirds of the comparisons the individual experiments were conducted in the same laboratory at different times and were reported in separate papers; the purpose of the second test was rarely to reevaluate the carcinogenicity of the compound. The remaining comparisons contain tests conducted by different researchers in different laboratories. We did not consider tests of the same chemical conducted concurrently in the same laboratory as near-replicate experiments, because generally such experiments were designed to assess the effects of dosing schedules, dose levels, or type of diet. If the chemical was tested at another time in the same laboratory or else in another laboratory and the concurrent experiments qualified as near-replicates, we included only 1 of the concurrent experiments in this analysis, that with a protocol most similar to that of the comparison test.

Although these 70 comparisons are defined as near-replicates, the experimental protocols may vary in terms of length of the experiment, length of exposure to the test agent, proportion of experiment time that the animals were dosed, average daily dose rate, and total dose. To summarize the differences within a near-replicate comparison, we have computed the ratio of the value of a protocol characteristic in one test to the value in each of its near-replicate comparison tests using the higher value in the numerator so that all ratios are equal to or greater than 1. These results are reported in table 1 and indicate that for length of experiment, duration of dosing, and proportion of experiment that dosing continued, all ratios are within 3.0; this restricted range is due to the criteria for inclusion into the CPDB. To compare dose levels, we use both the average daily dose rate and the maximum total dose (i.e., the average daily dose rate × length of experiment). All of the dose ratios in mice and three-quarters of the dose ratios in rats are low; however, the range in rats extends to 44.1 for vinyl chloride in both sexes of Sprague-Dawley rats and to 9,366 for vinyl chloride in male Wistar rats. Overall, these results indicate that the term "near-replicate" describes these comparisons well; while the protocols are usually quite similar, they are not identical. [Full details for these characteristics in each experiment are published in (*1*).]

Measures of reproducibility.—For each of the 70 near-replicate comparisons identified in the carcinogenic potency data base, we compare the results of the replicate experiments by examining 1) carcinogenicity, 2) target site, and 3) carcinogenic potency. To determine whether there was evidence for carcinogenicity in each experiment, we use the author's opinion in the published paper to classify the results as either positive (+) or negative (−). In a few papers the authors did not clearly evaluate the carcinogenicity of the compound; in this analysis we consider these "no opinion" (n) cases as lacking evidence of carcinogenicity and categorize them as "negative."

Generally, the designation of positivity by these authors' opinions corresponds well with the statistical significance of the results based on the *P*-value of the carcinogenic potency estimate. Of the mouse tests that were evaluated as positive by the author, 95% (37/39) were also statistically significant at 1 or more target sites (two-tail $P<.01$). The corresponding proportion for rat tests was 78% (38/49); however, 98% of the rat tests were significant at the $P<.10$ level (two-tail), suggesting that

TABLE 1.—*Differences in experimental design factors of near-replicate experiments in mice and rats*[a]

Species	No. of comparisons	Factor	Median ratio	Range of ratios
Mice	25	Experiment time	1.2	1.0-1.7
		Exposure time	1.1	1.0-2.4
		Proportion of experiment time animals were dosed	1.0	1.0-3.0
		Highest daily dose rate	1.3	1.0-4.1
		Maximum total dose	1.2	1.0-3.5
Rats	41	Experiment time	1.2	1.0-2.6
		Exposure time	1.3	1.0-2.6
		Proportion of experiment time animals were dosed	1.3	1.0-3.0
		Highest daily dose rate	2.3	1.0-44.1[b]
		Maximum total dose	2.0	1.0-44.1[b]

[a] A summary ratio compares larger to smaller value of a factor in each near-replicate comparison (i.e., a comparison consisting of the results for ≥2 expts of the same chemical administered by the same route to the same strain and sex of rodent), with the median ratio used for comparisons consisting of >2 expts.

[b] Vinyl chloride in male Wistar rats is an outlier with a median ratio for daily dose rate of 9,366 and for maximum total dose of 8,122.

borderline statistical results are considered evidence of carcinogenicity more often in rats than in mice. Six of 7 cases without an author's opinion were not statistically significant; the seventh was of borderline significance ($P<.03$). For each near-replicate comparison, we consider the results to be concordant if all (2, 3, or 4) of the experiments are positive or all are negative; otherwise we consider the results to be discordant. We emphasize that our analysis is not an evaluation of the carcinogenicity of particular substances, but rather an assessment of replicability of similar experiments. Whenever all experiments in a comparison are positive, we have determined whether the neoplasms were induced in the same target organ, again using the author's opinion for each target site. We consider the results reproducible if any 1 target site was the same in all of the near-replicate tests.

When the individual experiments in a comparison are all positive, we also examine the estimates of carcinogenic potency based on the results of the different experiments. Previously, we proposed the TD_{50} (tumorigenic dose-rate 50) as a numerical description of carcinogenic potency and calculated its value for the 3,000 experiments in the CPDB. Briefly, TD_{50} is defined as follows: In the absence of tumors for a given target site in control animals, TD_{50} is the chronic dose rate in milligrams per kilogram body weight per day that would induce tumors in half the test animals at the end of a standard life-span for the species (*2, 3*). Since tumors at the site of interest often do occur in control animals, TD_{50} is more precisely defined as that dose rate that will halve the probability of remaining tumor-free throughout the standard life-span of the species. The range of TD_{50} values for the chemicals in this near-replicate analysis is from 932 ng/kg/day for aflatoxin B_1 to 31 g/kg/day for saccharin; this is similar to the range in the entire CPDB (*1, 4*).

In our comparison of TD_{50} values computed from the results of 2 or more near-replicate experiments of the same test agent, we use the lowest TD_{50} (i.e., the most potent value) calculated for the target sites identified by the author in each experiment. To assess the reproducibility of the potency estimate, we simply compute a ratio of the TD_{50} values obtained from the individual tests within a comparison and use the median ratio when the comparison consists of more than 2 experiments.

RESULTS

Overall, the carcinogenic response is highly reproducible in hamsters, mice, and rats. Among the 70 comparisons, only 13% (9/70) have discordant author's opinions about whether neoplasms were induced in the individual experiments (table 2). Of the 4 comparisons in hamsters, all are concordant in positivity. In rats, among 41 comparisons, only 3 are discordant (7%). Results in mice are discordant more often, with 6 of 25 comparisons (24%) disagreeing on the compound's carcinogenicity. Approximately half of the comparisons in each species are concordantly positive. Most comparisons are reproducible whether the experiments were conducted in the same laboratory or in different laboratories, although there are not enough data for conducting a detailed analysis.

When we base the positivity analysis on statistical significance of the TD_{50} ($P<.01$) rather than on author's opinion, the number of discordant results is nearly the same (14/70); however, there are slightly more discrepancies in rats (10/41) than in mice (4/25) due to the author's positive evaluations of rat tests that were significant at the $P<.10$ (two-tail) level.

In tables 3–6 we report the results for each of the 70 near-replicate comparisons including chemical name, sex, strain, route of administration, TD_{50} value and its statistical significance, target organs, and reference number. (*See* "Appendix: References Cited in Tables.") Within each comparison the experiments are listed in the order in which the articles were published. The tables are organized according to whether the results on positivity are concordant positive (tables 3, 4), concordant negative (table 5), or discordant (table 6). In tables 5 and 6 we have indicated with an "n" those tests for which the author did not provide an opinion as to carcinogenicity and which we consider as negative. Minuses represent tests designated as "negative" by the authors. Taken together, the tables show that a variety of strains, routes, chemical classes, and target sites are included among the concordant as well as the discordant comparisons.

Reproducibility with respect to the particular organ in which neoplasms are induced is excellent among the positive near-replicate bioassays in all 3 species (tables 3, 4). In all but 2 of the 35 positive comparisons, at least 1 target site is identical in all of the near-replicate tests

TABLE 2.—*Summary of reproducibility of positivity in near-replicate comparisons[a] of chronic exposure carcinogenesis bioassays in hamsters, mice, and rats*

Species	No. discordant in positivity (%)	Concordant in positivity		Total No. (%)
		No. all positive (%)	No. all negative (%)	
Hamsters	0 (0)	2 (50)	2 (50)	4 (100)
Mice	6 (24)	13 (52)	6 (24)	25 (100)
Rats	3 (7)	20 (49)	18 (44)	41 (100)
All species	9 (13)	35 (50)	26 (37)	70 (100)

[a] A comparison consists of the results for ≥2 expts of the same chemical administered by the same route to the same strain and sex of rodent.

TABLE 3.—*Near-replicate comparisons[a] of chronic bioassays in hamsters and mice: Concordant-positive results*

Species	Chemical name	Sex	Strain[b]	Route	TD_{50}[c]	*P*-value	Target organ	Appendix reference[d]
Hamsters	Urethan	♀	syg	Water	102	<.004	Forestomach	*52*
					44.5	<.0005	Forestomach, skin, cecum, thyroid gland, adrenal gland	*67*
		♂	syg	Water	74.2	<.0005	Forestomach, skin	*52*
					64.2	<.0005	Forestomach, skin, cecum	*67*
Mice	DDT	♀	cf1	Diet	52.2	<.01	Liver	*65*
					9.11	<.0005	Liver	*73*
					5.82	<.0005	Liver	*64*
					43.0	<.0005	Liver	*66*
		♂	cf1	Diet	4.55	<.0005	Liver	*65*
					15.0	<.0005	Liver	*73*
					8.04	<.0005	Liver	*64*
					34.7	<.0005	Liver	*66*
	Dieldrin	♀	cf1	Diet	0.606	<.002	Liver	*73*
					0.567	<.0005	Liver	*64*
		♂	cf1	Diet	0.547	<.0005	Liver	*73*
					100%[e]	<.0005	Liver	*64*
	Diethylstilbestrol	♀	c3h	Diet	0.029	<.0005	Mammary gland	*21*
					0.026	<.0005	Mammary gland	*22*
	Formic acid, 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide	♀	swi	Diet	8.85	<.002	Forestomach, hematopoietic system	*11*
					13.8	<.0005	Forestomach	*8*
	Isoniazid	♀	swa	Water	153	<.0005	Lung	*72*
					386	<.0005	Lung	*71*
		♂	swa	Water	124	<.0005	Lung	*72*
					104	<.0005	Lung	*71*
	N-Methyl-*N*-formyl-hydrazine	♀	swa	Water	6.44	<.0005	Liver, lung, gallbladder	*68*
					2.16	<.0005	Liver, lung, gallbladder, vascular system	*70*
					0.921	<.0005	Lung, vascular system	*69*
		♂	swa	Water	16.8	<.0005	Liver, lung, gallbladder, bile duct	*68*
					1.03	<.0005	Liver, lung, gallbladder, bile duct	*70*
					1.72	<.0005	Liver, lung, gallbladder, vascular system	*69*
	N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide	♀	swi	Diet	18.2	<.0005	Urinary bladder, hematopoietic system, lung	*15*
					7.72	<.0005	Urinary bladder, hematopoietic system, forestomach	*11*
					19.8	<.0005	Urinary bladder	*8*
	Nitrofen	♀	b6c	Diet	406	<.0005	Liver	*48*
					64.2	<.0005	Liver	*49*
		♂	b6c	Diet	204	<.0005	Liver	*48*
					133	<.007	Liver	*49*

[a] A comparison consists of the results for ≥2 tests of a single chemical agent administered by the same route to the same strain and sex of hamster or mouse.
[b] Strain abbreviations: syg = Syrian golden; cf1 = CF-1; c3h = C3H; swi = Swiss; swa = Swiss albino; b6c = $(C57BL/6 \times C3H)F_1$.
[c] In mg/kg body wt/day, to 3 significant figures.
[d] Complete reference is given in "Appendix: References Cited in Tables." Within each comparison the expts are listed in the order in which the articles were published.
[e] 100% = all animals had the tumor of interest, and therefore no TD_{50} could be calculated.

TABLE 4.—*Near-replicate comparisons[a] of chronic bioassays in rats: Concordant-positive results*

Chemical name	Sex	Strain[b]	Route	TD_{50}[c]	*P*-value	Target organ	Appendix reference[d]
Aflatoxin B_1	♂	f34	Diet	0.00093	<.0005	Liver	*76*
				0.00113	<.003	Liver	*51*
				0.00134	<.005	Liver	*50*
				0.0558	<.06	Liver, colon	*74*
2,4-Diaminoanisole sulfate	♀	f34	Diet	301	<.0005	Thyroid gland, Zymbal's gland	*41*
				113	<.0005	Thyroid gland, mammary gland, clitoris	*19*
Formic acid, 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide	♀	sda	Diet	5.85	<.0005	Mammary gland, kidney, liver	*17*
				3.54	<.0005	Mammary gland, kidney-pelvis, lymphoid tissue	*10*
2-Hydrazino-4-(5-nitro-2-furyl)thiazole	♀	sda	Diet	2.83	<.0005	Mammary gland, kidney	*17*
				3.66	<.0005	Mammary gland	*9*
2-Hydrazino-4-(*p*-Nitrophenyl)thiazole	♀	sda	Diet	8.66	<.0005	Mammary gland	*9*
				1.97	<.0005	Mammary gland	*10*
1′-Hydroxysafrole	♂	cdr	Diet	47.8	<.0005	Liver	*75*
				12.1	<.0005	Liver, forestomach	*4*
Lasiocarpine	♂	f34	Diet	0.250	<.0005	Liver	*44*
				0.688	<.004	Liver	*55*
2-Methoxy-3-amino-dibenzofuran	♂	wis	Diet	100%[e]	<.004	Urinary bladder	*25*
				35.2	<.02	Urinary bladder	*53*
4,4′-Methylenebis(2-chloroaniline)	♂	cdr	Diet	20.8	<.0005	Lung, liver, mammary gland, Zymbal's gland, vascular system	*31*
				42.3	<.0005	Lung, liver	*60*
N-Nitrosodimethylamine	♂	wis	Diet	0.782	<.0005	Liver	*2*
				0.136	<.0005	Testis	*63*
N-Methyl-*N*′-nitro-*N*-nitrosoguanidine	♂	wis	Water	2.09	<.2	Glandular stomach	*62*
				0.693	<.0005	Glandular stomach	*61*
				0.523	<.002	Glandular stomach	*35*
3-Methylcholanthrene	♀	wis	Gavage	0.669	<.0005	Mammary gland	*24*
				0.506	<.0005	Mammary gland	*59*
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide	♀	sda	Diet	5.07	<.0005	Urinary bladder	*18*
				100%[e]	<.0005	Urinary bladder	*16*
Phenacetin	♀	sda	Diet	1,380	<.08	Mammary gland, ear duct	*30*
				2,560	<.0005	Kidney-pelvis, urinary bladder, nasal cavity	*28*
	♂	sda	Diet	1,300	<.02	Kidney cortex and pelvis, urinary bladder, forestomach	*29*
				741	<.0005	Kidney-pelvis, urinary bladder, nasal cavity	*28*
N-Propyl-*N*′-Nitro-*N*-nitrosoguanidine	♂	wis	Water	0.919	<.002	Glandular stomach	*56*
				2.27	<.06	Glandular stomach	*36*
Safrole	♂	cdr	Diet	627	<.05	Liver	*75*
				425	<.3	Liver	*4*
Vinyl chloride	Both sexes combined	sda	Inhalation	361	<.003	Vascular system, liver, Zymbal's gland, kidney, brain	*34*
				3.69	<.008	Vascular system, liver, Zymbal's gland, kidney, mammary gland	*34*
				91.5	<.0005	Vascular system, liver, Zymbal's gland, kidney, mammary gland	*34*
				555	<.0005	Vascular system	*34*
	Both sexes combined	sda	Gavage	14.2	<.02	Vascular system, liver, Zymbal's gland	*34*
				54.1	<.0005	Vascular system, kidney	*34*
	♂	wis	Inhalation	330	<.03	Vascular system, liver, nasal, Zymbal's gland	*20*
				1,550	<.0005	Vascular system	*34*
				0.761	<.01	Vascular system	*34*

[a] A comparison consists of the results for ≥2 tests of a single chemical administered by the same route to the same strain and sex of rat.
[b] Strain abbreviations: f34 = Fischer 344; sda = Sprague-Dawley; cdr = Charles River CD; wis = Wistar.
[c] In mg/kg body wt/day, to 3 significant figures.
[d] Complete reference is given in "Appendix: References Cited in Tables." Within each comparison the expts are listed in the order in which the articles were published.
[e] 100% = all animals had the tumor of interest, and therefore no TD_{50} could be calculated.

TABLE 5.—*Near-replicate comparisons[a] of chronic bioassays in hamsters, mice, and rats: Concordant-negative results*

Species	Chemical name	Route	Strain[b]	Author's opinion for:[c]		Appendix reference[d]
				Females	Males	
Hamsters	DDT	Diet	syg	– –	– –	*1, 5*
Mice	Lead acetate	Water	cd1	– –	– –	*57, 58*
	Nickel(II) acetate	Water	cd1	– –	– –	*57, 58*
	Titanium oxalate, potassium	Water	cd1	– –	– –	*57, 58*
Rats	Aldrin	Diet	osm	– –	– –	*13, 39*
				– –	–	*12, 14*
	Aramite	Diet	osm	– –	– –	*54, 12*
	DDT	Diet	osm	– –	– –	*54, 12*
				–	–	*40*
	Dieldrin	Diet	osm	– –	– –	*13, 42*
	Endrin	Diet	osm	– –	– –	*13, 43*
	Methoxychlor	Diet	osm	– n	– n	*54, 12*
				–	–	*45*
	Nitrite, sodium	Water	mrc	– –	– –	*23, 33*
	Saccharin, sodium	Diet	cdr	– n		*38, 3*
	Thiourea	Diet	osm	– –	– –	*54, 12*
	Vinylidene chloride	Inhalation	cdr	– n		*32, 27*

[a] A comparison consists of the results for ≥2 tests of a single chemical administered by the same route to the same strain and sex of hamster, mouse, or rat. Each *minus sign* or "n" represents a separate expt.

[b] Strain abbreviations: syg = Syrian golden; cd1 = Charles River CD1; osm = Osborne-Mendel; mrc = MRC; cdr = Charles River CD.

[c] – = indicates the author's opinion that the compound did not induce tumors in the expt; "n" indicates that the authors did not clearly state an opinion regarding carcinogenicity of the compound.

[d] Complete reference is given in "Appendix: References Cited in Tables." Within each comparison the expts are listed in the order in which the articles were published.

(whether there be 2, 3, or 4 tests per comparison). Reproducibility of target site includes a variety of organs: liver, lung, thyroid gland, Zymbal's gland, mammary gland, kidney, urinary bladder, stomach, vascular system, and hematopoietic system. The liver is a common target organ, with reproducible results in 7 of 13 positive mouse comparisons (table 3) and 6 of 20 positive rat comparisons (table 4). Most often the overlapping histopathology occurs in only 1 target organ; however, sometimes it includes as many as 5 sites. For example, in table 3 we report results from 3 tests of *N*-methyl-*N*-formylhydrazine in male Swiss albino mice, and in all 3 tests neoplasms were induced in the liver, lung, and gallbladder. (The tumor histologies were also similar.)

The estimates of carcinogenic potency (TD_{50}) for each positive test are reported in tables 3, 4, and 6. We determine the reproducibility of the estimates by computing a ratio of the high to the low TD_{50} values estimated for each pair of tests within a concordant-positive comparison. For comparisons with multiple tests we have chosen the median ratio from among the ratios calculated for each pair of tests in the near-replicate comparison. For 3 experiments no TD_{50} could be calculated because 100% of the animals had the tumor of interest; we have used the upper 99% confidence limit of TD_{50} in the ratios for these cases.

In hamsters there are only 2 concordant-positive comparisons (both for urethan), and the TD_{50}s from the individual tests are within a factor of 2 of each other. In mice 46% of the TD_{50} values for the 13 positive comparison tests are within a factor of 2 of one another, 77% are within a factor of 5, and all are within a factor of 10. In rats there are a few more extreme cases among the 20 positive comparisons, but still 40% are within a factor of 2, 80% are within a factor of 5, and 90% are within a factor of 20.

The inhalation bioassays of vinyl chloride in male Wistar rats show the most extreme differences in the magnitude of TD_{50} (table 4); the median ratio is 433. Vascular system tumors were induced in experiments with large differences in the daily dose rate (table 1, footnote *b*). Thus the carcinogenic action of vinyl chloride occurs at very small doses as well as at large doses. Vinyl chloride is one of only a few chemicals to have been tested chronically over such a broad range of dose levels. There is also a wide range of TD_{50} estimates for the near-replicate comparisons of aflatoxin B_1 administered in the diet to Fischer 344 rats; the median ratio is 21.5.

Although there are some exceptions, potency estimates from near-replicate tests are generally similar; in about 80% of the comparisons the TD_{50} estimates are within fivefold, and in more than 90% they are within tenfold. This similarity in results is to be expected, given the usual similarity in the average daily dose rate administered in the comparison tests (table 1). Whereas within a species the potency estimates for a single compound usually vary less than tenfold, the estimates for all carcinogens in the CPDB vary more than 10 millionfold. [For a discussion of the relationship between administered dose and estimated values of TD_{50}, *see* Bernstein et al. (*5, 6*).]

DISCUSSION

This comparison of chronic, near-replicate experiments demonstrates reproducibility of results in terms of positivity, target site, and carcinogenic potency in each of 3 rodent species. These results are based on 70 comparison groups in which more than 1 experiment was conducted with the use of the same chemical, administered by the same route to the same sex, strain, and species of test animal; the tests used for comparison were not designed to study replication and generally differ with respect to several aspects of experimental protocol.

While noting that the number of discrepant cases is small, we have investigated possible reasons for the discrepant findings on carcinogenicity in 6 of 25 mouse comparisons and 3 of 41 rat comparisons (table 6). We first examined whether the differences between the protocols of the individual experiments within a comparison were greater among the discrepant cases in table 6 than among the concordant-positive cases in tables 3 and 4. We took the ratio of each protocol characteristic within a comparison and found the median value for the combination of concordant-positive and discordant cases within each species. We then analyzed whether the ratios of the discrepant cases more commonly were above the median, i.e., whether there was more variation in the ratios of the discordant comparisons than the concordant-positive comparisons. The ranges of ratios among the discrepant comparisons are comparable to those of concordant-positive comparisons in terms of experiment length, exposure duration, proportion of the experiment that exposure lasted, highest average daily dose rate, and maximum total dose. Therefore, overall differences in these protocol characteristics do not account for differences in reproducibility of carcinogenicity.

We also have examined whether the individual experiments in the discrepant cases may have shared characteristics of length or dose that might tend to produce inconsistent results. We hypothesized that if experiments were of short duration, then a carcinogenic response might be detected in some cases but not in others, since tumors generally appear late in life. In animals that might have developed tumors if they were permitted to live longer, there would be no observed tumors in a short test. Our analysis shows that in mice the discrepant cases were more often below the median experiment length than the concordant-positive cases (Fisher's exact probability test, two-sided, $P<.01$); however, in rats the opposite was true—the discrepant tests tended to be longer. In mice, among the 12 individual experiments from the discrepant comparisons, only 17% were longer than 78 weeks (2/12); in contrast, 73% of the experiments in the concordant-positive comparisons (24/33) lasted that long. Thus we conclude that in mice but not in rats the generally short experiments may have contributed to the discrepancy in carcinogenic response.

With respect to dose we have found that the highest

TABLE 6.—*Near-replicate comparisons[a] of chronic bioassays in mice and rats: Nonreproducible results*

Species	Chemical name	Sex	Strain[b]	Route	Author's opinion[c]	TD_{50}[d]	*P*-value	Target organ	Appendix reference[e]
Mice	AF2	♀	icr	Diet	−		<.9		37
					+	95	<.0005	Forestomach	77
		♂	icr	Diet	−		=1.0		37
					+	90.3	<.0005	Forestomach	77
	Butylated hydroxy-toluene	♂	bal	Diet	+	368	<.03	Lung	6
	1'-Hydroxysafrole	♂	cd1	Diet	n		<.8		7
					+	429	<.0005	Vascular system	4
	Safrole	♂	cd1	Diet	n		=1.0		75
					n		<.03		4
	Vinylidene chloride	♀	cd1	Inhalation	+	249	<.0005	Liver	75
					−		=1.0		32
					+	170	<.3	Vascular system	27
Rats	Nitrilotriacetic acid, trisodium salt, monohydrate	♀	f34	Diet	+	783	<.0005	Kidney, urinary bladder, ureter	46
					−		=1.0		47
		♂	f34	Diet	+	511	<.0005	Kidney, ureter	46
					−		<.7		47
	Saccharin, sodium	♂	cdr	Diet	−		=1.0		38
					−		=1.0		26
					+	31,100	<.05	Urinary bladder	3

[a] A comparison consists of the results for ≥2 tests of a single chemical agent administered by the same route to the same strain and sex of mouse or rat.

[b] Strain abbreviations: icr = ICR/Jcl; bal = BALB/c; cd1 = Charles River CD1; f34 = Fischer 344; cdr = Charles River CD.

[c] + and − represent single bioassays with results that authors identified as positive and negative, respectively; "n" indicates authors did not clearly state an opinion regarding carcinogenicity of the compound.

[d] In mg/kg body wt/day to 3 significant figures.

[e] Complete reference is given in "Appendix: References Cited in Tables." Within each comparison the expts are listed in the order in which the articles were published.

average daily dose levels of the chemicals in the discrepant cases are generally larger than the dose levels in the concordant-positive cases for both mice and rats (Fisher's exact probability test, two-sided, $P<.01$). In mice, only 17% (2/12) of the discrepant experiments had average daily dose rates below 70 mg/kg/day compared to 70% (23/33) of the concordant-positive experiments. In rats, the dose levels in all of the discrepant experiments were above 400 mg/kg/day compared to only 9% (4/46) of the concordant experiments. Thus there appears to be a relationship between dose level and reproducibility.

In other ongoing analyses of the CPDB we find similar results for concordance in positivity between rats and mice: Among compounds that were tested in both rats and mice and found to be carcinogenic in at least 1 species, the administered dose level is inversely related to the probability that the compound is carcinogenic in both species. For example, compounds administered at less than 1 mg/kg/day to either rats or mice are always positive in both species if they are positive at all, whereas compounds administered above 100 mg/kg/day are positive in both species only about half the time if they are positive at all. Put somewhat differently, chemicals that induce tumors in only 1 species tend to have been administered at higher dose levels than chemicals that induce tumors in 2 species.

If we assume that the maximum dose tested in carcinogenesis bioassays approximates the chronic MTD (*7, 8*), then our results indicate that the nonreproducible experiments tend to be for chemicals that are toxic only at higher doses, whereas chemicals in the positive reproducible experiments are more often toxic at lower doses. Similarly, for the entire CPDB we find that positivity in more than 1 species is less likely among substances that are toxic only at high doses.

Thus both concordance in positivity between rats and mice and reproducibility within a single species are related to the toxicity of the chemical. In other words, chemicals for which the MTD is relatively large are more likely to be discordant in species comparisons and reproducibility than chemicals with small MTDs. The explanation for this finding is not clear, though it could conceivably be related to saturating metabolic systems when chemicals are given in large doses or that MTDs of weakly active substances are less often achieved. It may also be that fewer of the weaker substances are genotoxic, although there are both mutagens and nonmutagens among the discrepant compounds in this analysis.

As a second general approach to understanding the discrepant cases, we have searched on a case-by-case basis for idiosyncratic factors or combinations of factors that may explain the discordance. In mice, for the tests of AF2, there was a 6-year time lapse between the 2 experiments. Because the mouse strain ICR/Jcl is outbred, genetic drift may have occurred that produced important changes in the test animals. In the case of butylated hydroxytoluene, the authors suggest that the positive result is aberrant, since they have obtained only negative results in several subsequent tests using the same strain, BALB/c mice (Clapp NK: Personal communication). For vinylidene chloride, the evaluation of carcinogenicity was based on a statistically nonsignificant result (two-sided $P<.3$); in addition, a larger dose was administered in the test evaluated as positive, and the duration of dosing was longer. For safrole, although the authors did not give an opinion in one case, the results are of borderline statistical significance ($P<.03$). For 1′-hydroxysafrole, there were survival problems in the mice in the negative test. We note that results for both safrole and 1′-hydroxysafrole were reproducible in male Charles River CD rats (table 4).

In male and female rats administered nitrilotriacetic acid trisodium salt monohydrate, the highest daily dose rates in the positive tests were higher than in the negative tests; tumors were induced only in the highest dose group in the positive experiments. Also, whereas dosing was continued for the length of the experiment in the positive tests, it was stopped 6 months before the terminal sacrifice in the negative tests. For sodium saccharin, both negative tests were shorter, and the animals were older when dosing began than in the positive test.

In "Results" we describe the variation in estimates of carcinogenic potency (TD_{50}) from different positive experiments of the same test agent administered by the same route to the same species, strain, and sex of test animal. We find that about 40% of the time the estimates are within a factor of 2 of each other, 80% of the time they are within a factor of 5, and 85% or more of the time they are within a factor of 10. A few outliers occur in rat comparisons with wider ranges in TD_{50} estimates. Although the number of cases considered is small, the TD_{50} estimates of a single compound do vary by more than fivefold within a species in 20% of the comparisons.

We have also evaluated the 99% confidence limits for TD_{50} to determine whether the intervals for all of the near-replicate tests within a concordant-positive comparison overlap. In hamsters the intervals overlap in both cases. In mice the intervals do not overlap in 5 of 13 comparisons, and in rats they do not overlap in 6 of 20 comparisons. The nonoverlapping cases include tests of 3 different chemicals in mice and 3 in rats. These numbers are derived from similar experiments that use the same strain and sex within a species. Therefore, when making comparisons in potency between rats and mice, we cannot assume that observed differences reflect true species differences. The observed differences may be a function of the variability that we have shown to occur within each species.

In conclusion, we find that near-replicate experiments conducted in mice, hamsters, and rats generally provide reproducible results in terms of carcinogenic response, target site, and carcinogenic potency. For the few discordant cases, analyses suggest two explanations for the lack of reproducibility of carcinogenicity. In mice the discrepant cases tended to have shorter experiment times than average; in both rats and mice the discrepant results tend to be tests of weakly active compounds.

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