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Tumor site concordance and genetic toxicology test correlations in NTP 2-year gavage, drinking water, dermal, and intraperitoneal injection studies

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Abstract

The National Toxicology Program has conducted 594, 2-year studies exposing various strains of rats and mice via different routes of exposure. In the current study, we analyze the results from 108 chemicals tested in 106, 2-year studies conducted by exposing F334/N rats and B6C3F$_1$ mice via gavage. An additional 18, 2-year gavage studies have been conducted in Osborne–Mendel rats and B6C3F$_1$ mice on 19 different chemicals. We analyze the results from 23 chemicals tested in 21, 2-year studies conducted by exposing F334/N rats and B6C3F$_1$ mice via drinking water; 18 chemicals tested in 18, 2-year studies conducted by exposing F334/N rats and B6C3F$_1$ mice via dermal application; and 11 chemicals tested in 11, 2-year studies conducted by exposing F334/N rats and B6C3F$_1$ mice via intraperitoneal injection. The results from these 174 studies are analyzed and discussed separately. The neoplasticity of each chemical was analyzed for tumor incidence by species–sex category, tumor site concordance across species, and tumor site concordance across sex within species. When available the Ames *Salmonella* mutagenicity assay results, and any results from a test for genotoxicity other than the Ames test, were correlated with the neoplasticity results. Tumor site concordance across sex within species is generally higher than tumor site concordance across species. In addition, the high degree of variability of Ames test results suggests that historical Ames test data are less reliable than recent results conducted under good laboratory practices and employing Organization for Economic Cooperation and Development protocols relevant to the physicochemical characteristics of the test chemical.

Keywords

Dermal, drinking water, gavage, intraperitoneal injection, NTP, tumor site concordance

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Introduction

The National Toxicology Program (NTP) is a branch of the US Department of Health and Human Services. A major current emphasis of NTP is "The Toxicology in the 21st Century: The Role of the National Toxicology Program."[1] NTP describes this program as follows:

> The Role of the National Toxicology Program is to support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations.

NTP's intent is to expand the scientific basis for making public health decisions on the potential toxicity of environmental agents. Over the history of the NTP testing program, 594 different 2-year animal bioassays have been conducted

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via different routes of exposure including inhalation, feed, drinking water, intraperitoneal injection, and dermal. In an earlier publication, we analyzed the results from 60, 2-year inhalation studies conducted by NTP and showed a high level of discordance in tumor formation between rats and mice.[2] In addition, we analyzed the results from 212, 2-year feed studies conducted in F334/N rats and $B6C3F_1$ mice and 31, 2-year feed studies conducted in Osborne–Mendel rats and $B6C3F_1$ mice. The feed studies showed a higher degree of concordance within either male or female rats, or male and female mice, than between male rats and male mice or female rats and female mice.[3]

In the current study, we analyze the results from 108 chemicals tested in 106, 2-year studies conducted by exposing F334/N rats and $B6C3F_1$ mice via gavage (Online Appendix A, supplemental materials). An additional 18, 2-year gavage studies have been conducted in Osborne–Mendel rats and $B6C3F_1$ mice on 19 different chemicals (Online Appendix A-1, supplemental materials). We also analyze the results from 23 chemicals tested in 21, 2-year studies conducted by exposing F334/N rats and $B6C3F_1$ mice via drinking water (Online Appendix B, supplemental materials); 18 chemicals tested in 18, 2-year studies conducted by exposing F334/N rats and $B6C3F_1$ mice via dermal application (Online Appendix C, supplemental materials); and 11 chemicals tested in 11, 2-year studies conducted by exposing F334/N rats and $B6C3F_1$ mice via intraperitoneal injection (Online Appendix D, supplemental materials). The results from these 174 studies are analyzed and discussed separately. The neoplasticity of each chemical was analyzed for tumor incidence by species–sex category, tumor site concordance across species, and tumor site concordance across sex within species. When available the Ames *Salmonella* mutagenicity assay results, and any results from a test for genotoxicity other than the Ames test, were correlated with the neoplasticity results.

NTP considers results from the Ames assay test to be very important in its deliberations as illustrated by the following statement from a recent report on carcinogens.[4]

> DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[5] A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).[6,7] Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone . . . .

To eliminate the introduction of selection bias into this analysis, all positive Ames assay *Salmonella* bacterial mutagenicity test results reported in the literature were accepted at face value. Similarly, any positive result in a test of genetic toxicity other than the Ames test was also accepted at face value. NTP's categorization of neoplastic evidence as either "positive" or "clear" was used to determine the tumorigenicity of the tested chemicals.

Statistical methods

The following tests were applied to assess the statistical significance of the differences in proportions.[8]

Pooled test

The null hypothesis is

$$H0 : p_1 - p_2 = 0$$

The formula for the pooled test statistic comparing two proportions is

$$z = \frac{(\hat{p}_1 - \hat{p}_2) - 0}{\sqrt{\hat{p}(1-\hat{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where

$\hat{p}_1$ is the proportion in the first sample with the characteristic of interest.

$\hat{p}_2$ is the proportion in the second sample with the characteristic of interest.

$\hat{p}$ is the proportion in the combined sample (all the individuals in the first and second samples together) with the characteristic of interest, and z is a value on the Z distribution.

$$\hat{p} = \frac{x_1 + x_2}{n_1 + n_2}$$

The standard error is

$$\sqrt{\hat{p}(1-\hat{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

Unpooled test

The null hypothesis is

$$H0 : p_1 - p_2 = 0$$

$$z = \frac{\hat{p}_1 + \hat{p}_2}{\sqrt{\frac{\hat{p}_1(1-\hat{p}_1)}{n_1} + \frac{\hat{p}_2(1-\hat{p}_2)}{n_2}}}$$

This test works well for medium to large populations. For small populations, the exact binomial probability test is preferred.

Exact binomial probability test

Exact binomial probabilities are calculated through repeated applications of the standard binomial formula

$$P\,(k \text{ successes in } n \text{ trial}) = \binom{n}{k} p^k q^{n-k}$$

Binomial probabilities can be calculated and/or estimated for situations of the general "k out of n" type, where k is the number of times a binomial outcome is observed or stipulated to occur, p is the probability that the outcome will occur on any particular occasion, q is the complementary probability $(1 - p)$ that the outcome will not occur on any particular occasion, and n is the number of occasions.

The method of exact binomial probabilities is preferable in all cases, as it involves direct calculation of exact binomial probabilities. Its limitation is that it is not computationally feasible with very large samples.[9]

Chi-square statistic

The chi-square (χ^2) statistic is defined as the sum of the squares of the Z square values. If there are d degrees of freedom, then let this process of calculating χ^2 continue until d different Z values are selected from the distribution.

If $Z_1, \ldots, Z_k$ are independent, standard normal random variables, then the sum of their squares

$$Q = \sum_{i=1}^{k} Z_i^2$$

is distributed according to the χ^2 distribution with k degrees of freedom. This is usually denoted as

$$Q \sim \chi^2\,(k) \text{ or } Q \sim \chi_k^2$$

The χ^2 distribution has one parameter: k—a positive integer that specifies the number of degrees of freedom (i.e. the number of Z_is).[9] Statistical results are found in Table 1.

Results

Results for 106 NTP 2-year gavage studies conducted in F344/N rats and B6C3F$_1$ mice (108 chemicals tested)

Clear evidence of neoplasms in male rats, female rats, male mice, and female mice. Of the 108 chemicals tested, 14 produced neoplasms in male rats, female rats, male mice, and female mice including a polybrominated biphenyl mixture (Firemaster FF-1) CASRN 36355-01-8; diglycidyl resorcinol ether (DGRE) (technical grade) (CASRN 101-90-6); ethyl acrylate (CASRN 140-88-5); benzene (CASRN 71-43-2); 3-chloro-2-methylpropene (technical grade containing 5% dimethylvinyl chloride) (CASRN 563-47-3); chlorinated paraffins (C12, 60% chlorine) (CASRN 108171-26-2); dimethylvinyl chloride (1-chloro-2-methylpropene) (CASRN 513-37-1); bromodichloromethane (CASRN 75-27-4); glycidol (CASRN 556-52-5); furan (CASRN 110-00-9); methyleugenol (CASRN 93-15-2); riddelliine (CASRN 23246-96-0); 2,4-hexadienal (89% trans, trans isomer, CASRN 142-83-6) 11% cis, trans isomer; and *N*,*N*-dimethyl-*p*-toluidine (CASRN 99-97-8). Of the 14 ubiquitously neoplastic chemicals, only 5 were correctly predicted by a positive Ames test (5/14 observed vs. 14/14, $p_{\text{pooled}} = 0.0001$; $p_{\text{unpooled}} = 0.00043$), for the equivalent of a false negative rate of 64.3% (9/14 observed vs. 0/14 expected, $p_{\text{pooled}} = 0.0001$; $p_{\text{unpooled}} = 0.00043$). In contrast, for the 13 chemicals having definitive genetic toxicology results in a test other than Ames, 9 correctly predicted the positive neoplastic results (9/13 observed vs. 13/13 expected, $p_{\text{pooled}} = 0.0148$; $p_{\text{unpooled}} = 0.08190$), with a false negative rate of 30.8% (4/13 observed vs. 0/13 predicted, $p_{\text{pooled}} = 0.0148$; $p_{\text{unpooled}} = 0.08190$; Table 1, Online Appendix A).

Clear evidence of neoplasms in three of four sex/species categories. Of the 108 chemicals tested, only five displayed neoplasms in three of the four sex/species categories including 3-(chloromethyl) pyridine hydrochloride (CASRN 6959-48-4); selenium sulfide (CASRN 7488-56-4); commercial grade 2,4 (80%)- and 2,6 (20%)-toluene diisocyanate (TDI) (CASRN 26471-62-5); 1,4-dichlorobenzene (CASRN 106-46-7); and pulegone (CASRN 89-82-7). Only two of these five chemicals were positive in the Ames test (2/5 observed vs. 5/5 expected, pooled = 0.0192; $p_{\text{unpooled}} = 0.10035$) for a false negative rate of 60% (3/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0192$; $p_{\text{unpooled}} = 0.07812$). The results for genetic toxicology tests other than the Ames were also not very accurate for these chemicals with three of the five positive, and two of the five negative for a false negative rate of 40% (2/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0569$; $p_{\text{unpooled}} = 0.15443$; Table 1).

Clear evidence of neoplasms in male rats and female rats, no clear evidence in male and female mice. Of the 108 chemicals tested, five produced tumors in both male and female rats but did not produce tumors in either male mice or female mice. These five chemicals were *m*-cresidine (CASRN 102-50-1); pivalolactone (CASRN 1955-45-9); methyl carbamate (CASRN 598-55-0); malonaldehyde, sodium salt (3-hydroxy-2-propenal, sodium salt) (CAS no. 24382-04-5); and tris(2-chloroethyl) phosphate (TRCP) (CASRN 115-96-8). Of the five chemicals, only pivalolactone was positive in the Ames test (1/5 observed vs. 5/5 expected, $p_{\text{pooled}} = 0.0049$; $p_{\text{unpooled}} = 0.02627$) for a false negative rate of 80% (4/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0049$; $p_{\text{unpooled}} = 0.03225$). For these five chemicals, there were only three definitive results in a genetic toxicity test other than the Ames test, with 2/3 being positive (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$; Table 1).

Table 1. False positive and false negative rates for the Ames test and genetic toxicology assays OAT by binomial tail test[a] (Clear, not clear, negative refer to NTP categorization of degree of neoplastic evidence.).

Test results	Observed	Expected	Pooled *p* value	Unpooled *p* value binomial tails
Gavage (F344/N rats and B6C3F$_1$ mice)				
Clear, clear, clear, clear	5/14	14/14	0.0001	0.00043
Ames positive				
Clear, clear, clear, clear	9/14	0/14	0.0001	0.00043
Ames negative				
Clear, clear, clear, clear	2/3	0/3	0.0072	NA
Ames negative				
Clear, clear, clear, clear	9/13	13/13	0.0148	0.08190
OAT positive				
Clear, clear, clear, clear	4/13	0/13	0.0148	0.08190
OAT negative				
Negative, negative, negative, negative	18/18	18/18	NA	0.00000
Ames negative				
Negative, negative, negative, negative	11/18	0/18	0.0000	0.00011
OAT positive				
Negative, negative, negative, negative	8/18	18/18	0.0001	0.00037
OAT negative				
Clear, clear, clear, not clear[b]	2/5	5/5	0.0192	0.10035
Ames positive				
Clear, clear, clear, not clear	3/5	0/5	0.0192	0.07812
Ames negative				
Clear, clear, clear, not clear[b]	2/5	0/5	0.0569	0.15443
OAT negative				
Rats—clear, clear	1/5	5/5	0.0049	0.02627
Mice—not clear, not clear				
Ames positive				
Rats—clear, clear	4/5	0/5	0.0049	0.03225
Mice—not clear, not clear				
Ames negative				
Rats—clear, clear	2/3	3/3	0.1367	0.46416
Mice—not clear, not clear				
OAT positive				
Mice—clear, clear	1/8	8/8	0.0002	0.00050
Rats—not clear, not clear				
Ames positive				
Mice—clear, clear	11/12	0/12	0.0000	0.00000
Rats—not clear, not clear				
Ames positive				
Mice—clear, clear	7/8	0/8	0.0002	0.00064
Rats—not clear, not clear				
Ames negative				
Mice—clear, clear	1/6	0/6	0.1481	0.26401
Rats—not clear, not clear				
OAT negative				
Male rats—clear	0/3	3/3	0.0072	0.01562
Female rats—not clear				
Mice—not clear				
Ames negative				
Male rats—clear	2/3	3/3	0.1367	0.46416
Female Rats—not clear				
Mice—not clear				
OAT positive				
Male rats—clear	3/3	0/3	0.0072	0.01562
Female rats—not clear				
Mice—not clear				
OAT negative				

(continued)

Table 1. (continued)

Test results	Observed	Expected	Pooled *p* value	Unpooled *p* value binomial tails
Female rats—clear	2/2	2/2	NA	0.00000
Male rats—not clear				
Mice—not clear				
Ames positive				
Female rats—clear	3/3	3/3	NA	0.00000
Male rats—not clear				
Mice—not clear				
OAT positive				
Male mice—clear	5/5	0/5	0.0008	0.00098
Female mice—not clear				
Rats—not clear				
Ames negative				
Male mice—clear	3/4	0/4	0.0142	0.07666
Female mice—not clear				
Rats—not clear				
OAT negative				
Female Mice—clear	2/2	2/2	NA	0.00000
Male Mice—not clear				
Rats—not clear				
Ames positive				
Female mice—clear	2/2	2/2	NA	0.00000
Male mice—not clear				
Rats—not clear				
OAT positive				
Male and female rats, male and female mice—clear and concordant site	4/6	6/6	0.0607	0.16107
Ames positive				
Male and female rats, male and female mice—clear and concordant site	4/6	6/6	0.0607	0.16107
OAT positive				
Male and female rats, male and female mice—clear and concordant site	5/5	0/5	0.0008	0.00098
Ames negative				
Male and female rats, male and female mice—clear and concordant site	2/5	0/5	0.0569	0.15443
OAT negative				
Male rats—clear but not concordant site	2/3	3/3	0.1367	0.46416
Female mice—clear but not concordant site				
Female rats—not clear				
Ames positive				
Male rats—clear but not concordant site	3/3	3/3	NA	0.00000
Female mice—clear but not concordant site				
Female rats—not clear				
OAT positive				
Overall Ames false negative	15/48	48/48	0.0000	0.00000
Overall positive predictive accuracy versus negative predictive accuracy	15/48	18/18	0.0000	0.00000
Drinking water				
Clear, clear, clear, clear	5/6	6/6	0.1481	0.26401
Ames positive				
Clear, clear, clear, clear	4/6	6/6	0.0607	0.16107
OAT negative				
Negative, negative, negative, negative	3/4	4/4	0.1425	0.26370
Ames negative				
Negative, negative, negative, negative	1/4	4/4	0.0142	0.05654
OAT negative				

(continued)

Table 1. (continued)

Test results	Observed	Expected	Pooled p value	Unpooled p value binomial tails
Dermal				
Negative, negative, negative, negative Ames negative	5/8	8/8	0.0273	0.10606
Negative, negative, negative, negative OAT negative	5/8	8/8	0.0273	0.10606
Overall summary of genotoxicity versus neoplasticity Ames positive	2/6	6/6	0.0072	0.04212
Overall summary of genotoxicity versus neoplasticity Ames negative	8/10	10/10	0.0680	0.24052
Intraperitoneal				
Clear, clear, clear, clear Ames positive	2/3	3/3	0.1367	0.46416
Clear, clear, clear, clear OAT positive	2/3	3/3	0.1367	0.46416

NTP: National Toxicology Program; OAT: other than Ames test, i.e. genetic toxicology test other than the Ames test.
[a]Binomial tail test for the Null Hypothesis that the true proportion is $\mu = (x_1 + x_2)/(n_1 + n_2)$. p-Values were calculated from summing the products of binomial probabilities $B(n_1, i, \mu) \times B(n_2, j, \mu)$ over all (i, j); where: $B(n_1, i, \mu) \times B(n_2, j, \mu) < B(n_1, x_1, \mu) \times B(n_2, x_2, \mu)$.
[b]Not clear can appear in any of the four possible positions representing species/sex.

Clear evidence of neoplasms in male mice and female mice, no clear evidence in male rats or female rats. Of the 108 chemicals tested, 8 induced tumors in both male and female mice but did not induce tumors in either male or female rats, including pentachloroethane (CASRN 76-01-7); 1,1,1,2-tetrachloroethane (CASRN 630-20-6); trichloroethylene (TCA) (without epichlorohydrin) (CASRN 79-01-6); *N*-methylolacrylamide (CASRN 924-42-5); benzofuran (CASRN 271-89-6); salicylazosulfapyridine (CASRN 599-79-1); androstenedione (CASRN 63-05-8); and *Ginkgo biloba* extract (CASRN 90045-36-6). Of these eight chemicals, only one, that is, *Ginkgo biloba* extract, was positive in the Ames test (1/8 observed vs. 8/8 expected, $p_{\text{pooled}} = 0.0002$; $p_{\text{unpooled}} = 0.00050$), with seven negative Ames test results for a false negative rate of 87.5% (7/8 observed vs. 0/8 expected, $p_{\text{pooled}} = 0.0002$; $p_{\text{unpooled}} = 0.00064$). In contrast, there were six definitive results in genetic toxicity tests other than the Ames test, and among these six results there were five positives for a false negative rate of 16.7% (1/6 observed vs. 0/6 expected, $p_{\text{pooled}} = 0.1481$; $p_{\text{unpooled}} = 0.26401$; Table 1).

Clear evidence of neoplasms in male rats only. Of the 108 chemicals tested, three showed clear evidence of neoplasia in only the male rats including allyl isothiocyanate (CASRN 57-06-7), dimethyl hydrogen phosphite (CASRN 868-85-9), and D-limonene (CASRN 5989-27-5). All three of these chemicals were negative in the Ames test (0/3 observed vs. 3/3 expected $p_{\text{pooled}} = 0.0072$; $p_{\text{unpooled}} = 0.01562$) for a false negative rate of 100% (3/3 observed vs. 0/3 expected, $p_{\text{pooled}} = 0.0072$; $p_{\text{unpooled}} = 0.01562$). In two of the three cases, the result was positive in a test of genetic toxicity other than the Ames (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$; Table 1).

Clear evidence of neoplasms in male mice only. Five of 108 chemicals tested showed clear evidence in only the male mice. These five chemicals were chlorinated paraffins (C23, 43% chlorine) (CASRN 108171-27-3), furfural (CASRN 98-01-1), formamide (CASRN 75-12-7), isoeugenol (CASRN 97-54-1), and kava kava extract (CAS no. 9000-38-8). All five chemicals were negative in the Ames test (5/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0008$; $p_{\text{unpooled}} = 0.00098$), representing a false negative rate of 100%. Four of the five chemicals had a definitive genetic toxicity test other than the Ames test with three results being negative (3/4 observed vs. 0/4 expected, $p_{\text{pooled}} = 0.0142$; $p_{\text{unpooled}} = 0.07666$) for a false negative rate of 75%; Table 1).

Clear evidence of neoplasms in female mice only. Three of 108 chemicals tested showed clear evidence in only the female mice, that is, dichlorvos (CASRN 62-73-7), 4-vinylcyclohexene (CASRN 100-40-3), and coumarin (CASRN 91-64-5). Two (dichlorvos and coumarin) of the three chemicals were positive in the Ames test (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$; Table 1). In addition, these same two chemicals were positive in at least one other test of genetic toxicity other than Ames. 4-Vinylcyclohexene was negative in the Ames test and in at least one other test of genetic toxicity other than Ames.

Clear evidence of neoplasms in male rats and male mice only. Only 1 of 108 chemicals tested, that is, β-myrcene (CASRN 123-35-3), displayed tumors in both male rats and male mice but not in female rats or female mice.

Table 2. Predictive accuracys of positive and negative Ames tests and OATs.[a]

Study type	Predictive accuracy of positive Ames test[b] (%)	Predictive accuracy of negative Ames test[c] (%)	Predictive accuracy of positive OAT[d] (%)	Predictive accuracy of negative OAT[e] (%)	*p* Value A (row)	*p* Value B (row)
Gavage (F344/N) (108)	23% (25/108)	19% (20/108)	50% (54/108)	8% (9/108)	0.00000	0.00000
Gavage (Osborne–Mendel) (18)	28% (5/18)	11% (2/18)	50% (9/18)	11% (2/18)	0.05826	0.04434
Drinking water (23)	43% (9/23)	19% (4/23)	33% (7/23)	9% (2/23)	0.12457	0.12849
Dermal (18)	18% (3/18)	29% (5/18)	41% (7/18)	24% (4/18)	0.63299	0.63458
Intraperitoneal (11)	45% (5/11)	0% (0/11)	55% (6/11)	0% (0/11)	0.00309	0.00489
p Value A (Column)	0.32439	0.48966	0.31933	0.43971		
p Value B (Column)	0.32324	0.46895	0.32311	0.41626		

OAT: other than Ames test, that is, genetic toxicology test other than the Ames test.
[a]*p* Value A is calculated by summing the tail probabilities from products of binomial probabilities; *p* value B is calculated from the value of a χ^2 statistic.
[b]Percentage of Ames test results that were positive thereby correctly predicting the development of at least one neoplasm in at least one sex/species category.
[c]Percentage of Ames test results that were negative thereby correctly predicting the absence of development of a neoplasm.
[d]Percentage of "Other than Ames test" assay results for genetic toxicity that were positive thereby correctly predicting the development of at least one neoplasm in at least one sex/species category.
[e]Percentage of "Other than Ames test" assay results for genetic toxicity that were negative thereby correctly predicting the absence of development of a neoplasm.

β-Myrcene was negative in both the Ames test in other tests of genetic toxicity.

Female rats and female mice only. There were no chemicals of the 108 tested that induced tumors in only female rats and female mice.

Male rats and female mice only. Three of the 108 chemicals tested induced tumors in only male rats and female mice but not in female rats or male mice. These three chemicals were trimethylphosphate (CASRN 512-56-1), allyl isovalerate (CASRN 2835-39-4), and Telone® (technical grade 1,3-dichloropropane (CASRN 542-75-6) containing 1.0% epichlorohydrin as a stabilizer. Two of the three chemicals were positive in the Ames test (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$), and all three were positive in another test of genetic toxicity other than Ames (3/3 observed vs. 3/3 expected, $p_{\text{pooled}} = \text{NA}$; $p_{\text{unpooled}} = 0.00000$; Table 1).

No evidence of neoplasia in male rats, female rats, male mice, and female mice. Of the 108 chemicals tested, 18 chemicals did not induce a tumor in male rats, female rats, male mice, or female mice. These 18 chemicals included food grade geranyl acetate (71% geranyl acetate, 29% citronellyl acetate) (CASRN 105-87-3); 1,2-dichlorobenzene (*o*-dichlorobenzene) (CASRN 95-50-1); tetrakis(hydroxymethyl) phosphonium sulfate (CASRN 55566-30-8) and tetrakis(hydroxymethyl)phosphonium chloride (CASRN 124-64-1); *n*-butyl chloride (CASRN 109-69-3); chlorpheniramine maleate (CASRN 113-92-8); xylenes (mixed) (60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene, and 17% ethylbenzene) (CASRN 1330-20-7); penicillin VK (CASRN 132-98-9); benzyl alcohol (CASRN 100-51-6); succinic anhydride (CASRN 108-30-5); monochloroacetic acid (CASRN 79-11-8); resorcinol (CASRN 108-46-3); promethazine hydrochloride (CASRN 58-33-3); scopolamine hydrobromide trihydrate (CASRN 6533-68-2); theophylline (CASRN 58-55-9); methacrylonitrile (CASRN 126-98-7); 5-(hydroxymethyl)-2-furfural (CASRN 67-47-0); and ginseng (CAS no. 50647-08-0).

Of these 18 chemicals, all tested negative in the Ames test (18/18 observed vs. 18/18 expected, pooled = NA; $p_{\text{unpooled}} = 0.00000$). Genetic tests other than the Ames test were far less predictive of these 18 chemicals with 8 instances of being negative (8/18 observed vs. 18/18 expected, $p_{\text{pooled}} = 0.0001$; $p_{\text{unpooled}} = 0.00037$) and 11 instances of being positive (11/18 observed vs. 0/18 expected, $p_{\text{pooled}} = 0.0000$; $p_{\text{unpooled}} = 0.00011$; Table 1).

Overall summary of genotoxicity versus neoplasticity. Of the 108 chemicals tested by exposing F334/N rats and B6C3F$_1$ mice via gavage, 49 chemicals induced a neoplasm in at least one sex/species category. Of these 49 chemicals, 48 had a definitive Ames test result. Of the 48 Ames test results, only 15 were positive for an overall predictive accuracy rate of 31.2% (15/48 observed vs. 48/48 expected, $p_{\text{pooled}} = 0.0000$; $p_{\text{unpooled}} = 0.00000$). In contrast with the poor predictive ability of positive Ames test results, negative Ames results were better with all 18 of the ubiquitously non-neoplastic chemicals being negative in the Ames for a predictive accuracy rate of 100% (15/48 positive predictive accuracy vs. 18/18 negative predictive accuracy, $p_{\text{pooled}} = 0.0000$; $p_{\text{unpooled}} = 0.00000$; Tables 1 and 2).

Tumor concordance in F334/N rats and B6C3F$_1$ mice

Yes, across species; yes, across sex within species. Of the 108 chemicals tested by exposing F334/N rats and B6C3F$_1$ mice via gavage, 12 induced tumors at the same anatomical site in male rats, female rats, male mice, and female mice.

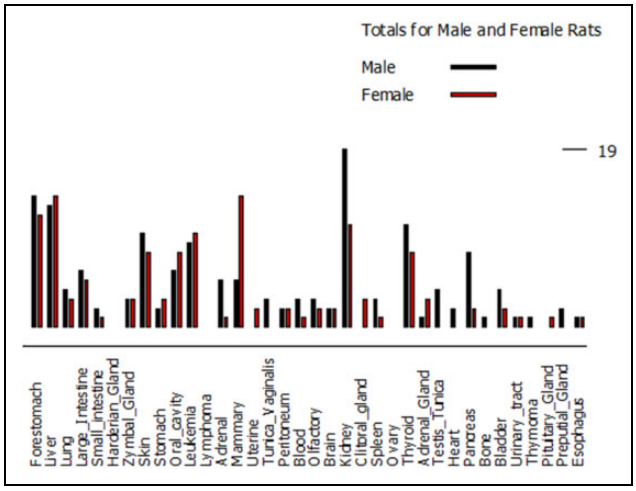

Figure 1. Distribution of tumor incidence in male and female rats treated by gavage.

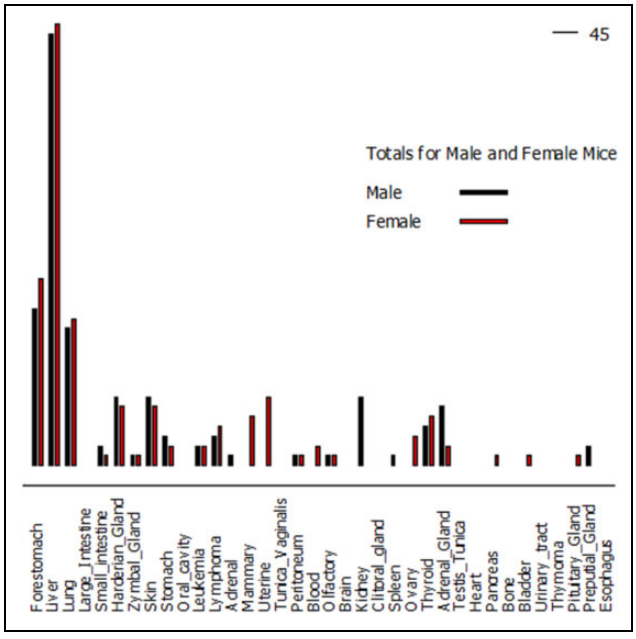

Figure 2. Distribution of tumor incidence in male and female mice treated by gavage.

The most common site of tumor induction was the placement site for gavage, that is, the forestomach. There were 6 chemicals of the 12 that induced forestomach tumors in male and female rats (Figure 1) and in male and female mice (Figure 2) including 3-(chloromethyl) pyridine hydrochloride (CASRN 6959-48-4); DGRE (technical grade) (CASRN 101-90-6); ethyl acrylate (CASRN 140-88-5); 3-chloro-2-methylpropene (technical grade containing 5% dimethylvinyl chloride) (CASRN 563-47-3); dimethylvinyl chloride (1-chloro-2-methylpropene) (CASRN 513-37-1); and 2,4-hexadienal (89% trans, trans isomer, CASRN 142-83-6; 11% cis-, trans-isomer. Of these six chemicals inducing forestomach tumors, four were positive in the Ames test (4/6 observed vs. 6/6 expected, $p_{\text{pooled}} = 0.0607$; $p_{\text{unpooled}} = 0.16107$) and four were positive in another genetic toxicity test other than Ames (4/6 observed vs. 6/6 expected, $p_{\text{pooled}} = 0.0607$; $p_{\text{unpooled}} = 0.16107$; Table 1). Although a greater number of chemicals caused forestomach tumors than liver tumors, an examination of Figures 1 and 2 shows that approximately the same number of forestomach tumors as liver tumors was formed in male and female rats, while significantly more liver tumors than forestomach tumors were induced in male and female mice (Table 3).

The second most common pattern of tumor induction in F334/N rats and B6C3F$_1$ mice via gavage was liver tumors in the male rats, female rats, male mice, and female mice. This pattern was seen for the following five chemicals: a polybrominated biphenyl mixture (Firemaster FF-1) CASRN 36355-01-8; methyleugenol (CASRN 93-15-2); *N*,*N*-dimethyl-*p*-toluidine (CASRN 99-97-8); chlorinated paraffins (C12, 60% chlorine) (CASRN 108171-26-2); and furan (CASRN 110-00-9). All five of these liver tumor-inducing chemicals were negative in the Ames test (5/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0008$; $p_{\text{unpooled}} = 0.00098$). Two of these five liver tumor-inducing chemicals were negative in a genetic toxicity test other than Ames (2/5 observed vs. 0/5 expected, $p_{\text{pooled}} = 0.0569$; $p_{\text{unpooled}} = 0.15443$; Table 1). Benzene displayed a unique tumor presentation of Zymbal gland tumors in male rats, female rats, male mice, and female mice. It was negative in the Ames test and positive in another test of genetic toxicity.

In addition to the three tumor types seen in male rats, female rats, male mice, and female mice, that is, forestomach, liver, and Zymbal gland, several other tumor types were seen in this series of chemicals that were concordant only within sex. These chemicals and their concordance combinations were a polybrominated biphenyl mixture (Firemaster FF-1) (CASRN 36355-01-8) and cholangiocarcinomas in rats; benzene (CASRN 71-43-2) and tumors of the oral cavity in rats and lung tumors in mice; dimethylvinyl chloride (1-chloro-2-methylpropene) (CASRN 513-37-1) and tumors of the nasal cavity, oral cavity, and esophagus in rats; *N*,*N*-dimethyl-*p*-toluidine (CASRN 99-97-8) and transitional epithelium adenoma of the nasal cavity in rats; and furan (CASRN 110-00-9) and mononuclear cell leukemia in rats and benign pheochromocytomas in mice.

No, across species; yes, across sex within species. Twenty-nine of the 108 chemicals tested in F334/N rats and B6C3F$_1$ mice via gavage induced tumors at the same anatomical location in just either rats or mice and induced tumors at the same anatomical site in either male rats and female rats or male mice and female mice. These 29 chemicals were as follows: *m*-cresidine (CASRN 102-50-1); pivalolactone (CASRN 1955-45-9); selenium sulfide (CASRN 7488-56-4); pentachloroethane (CASRN 76-01-7); 1,1,1,2-tetrachloroethane (CASRN 630-20-6); TCA (without epichlorohydrin) (CASRN 79-01-6); benzyl acetate

Table 3. Routes of administration and tumor site/number.

	Route of administration	Gavage	Drinking water	Dermal	Intraperitoneal
Tumor site/ number	Forestomach	Male rats (13); female rats (11); male mice (12); female mice (15)	Male mice (3); female mice (3)	Male rats (1); female rats (1); male mice (1); female mice (1)	
	Liver	Male rats (11); female rats (13); male mice (35); female mice (34)	Male rats (1); male mice (5); female mice (6)	Male rats (1); female rats (1); male mice (4); female mice (5)	
	Lung	Male rats (2); female rats (2); male mice (9); female mice (11)	Male rats (1); female rats (1); male mice (3); female mice (3)	Male rats (1); male mice (1)	Male mice (1); female mice (1)
	Large intestine	Male rats (3); female rats (2)	Male rats (2); female rats (2)	Male rats (1); female rats (1)	
	Small intestine	Male rats (1); male mice (1)	Male mice (1); female mice (1)	Male rats (1); female rats (1)	
	Harderian gland	Male mice (4); female mice (4)	Male mice (3); female mice (2)		
	Zymbal gland	Male rats (2); female rats (2); male mice (1); female mice (1)		Male rats (1); female rats (1)	
	Skin	Male rats (5); female rats (3); male mice (2); female mice (1)	Male rats (1); female rats (1); male mice (1); female mice (2)	Male rats (2); female rats (2); male mice (3); female mice (3)	Male rats (2); female rats (2); male mice (1)
	Stomach	Male rats (2); female rats (3); male mice (3); female mice (2)			
	Oral cavity	Male rats (2); female rats (3)	Male rats (3); female rats (4)	Male rats (1); female rats (1)	
	Leukemia	Male rats (9); female rats (7)	Female rats (3)		Male mice (2); female mice (2)
	Lymphoma	Male mice (3); female mice (4)			
	Adrenal	Male rats (5); female rats (1); male mice (1)			
	Mammary	Male rats (3); female rats (7); female mice (3)	Female rats (3); female mice (2)	Female rats (1)	Male rats (2); female rats (3)
	Uterine	Female mice (4)		Female mice (1)	Female rats (2); female mice (2)
	Tunica vaginalis	Male rats (1)		Male rats (1)	Male rats (1)
	Peritoneum				Male rats (2); female rats (2); male mice (1); female mice (1)
	Blood	Male rats (2); female rats (1)			Male rats (1); female mice (2)
	Olfactory	Male rats (2); female rats (1)			Male rats (1); female rats (1); male mice (1); female mice (1)
	Brain	Male rats (1); female rats (1)			Male rats (1); female rats (1)
	Kidney	Male rats (15); female rats (9); male mice (5)	Male rats (2)	Male rats (3); female rats (2); male mice (3)	
	Clitoral gland	Female rats (1)	Female rats (1)	Female rats (1)	
	Spleen	Male rats (2); female rats (1); male mice (1)		Male rats (1)	
	Ovary	Female mice (3)	Female Mice (1)	Female Mice (1)	
	Thyroid	Male rats (7); female rats (4); male mice (2); female mice (3)	Male rats (4); female rats (4); male mice (2); female mice (2)		

(continued)

Table 3. (continued)

	Route of administration	Gavage	Drinking water	Dermal	Intraperitoneal
	Adrenal gland	Male rats (1); female rats (3); male mice (5); female mice (2)	Male mice (1)		
	Testis tunica	Male rats (1)	Male rats (3)		
	Heart		Male rats (2)		
	Pancreas	Male rats (8); female rats (2)	Female mice (1)		
	Bone		Male rats (1)		
	Bladder	Male rats (4); female rats (2); female mice (1)			
	Urinary tract	Male rats (1); female rats (1)			
	Thymoma	Male rats (1)			
	Pituitary gland	Female rats (1); female mice (1)			
	Preputial gland	Male rats (2); male mice (2)			
	Esophagus	Male rats (1); female rats (1)			
Total tumors	Total for route	364	81	49	36

(CASRN 140-11-4); commercial grade 2,4 (80%)- and 2,6 (20%)-TDI (CASRN 26471-62-5); 1,2-dichloropropane (propylene dichloride) (CASRN 78-87-5); Telone (technical grade 1,3-dichloropropane (CASRN 542-75-6) containing 1.0% epichlorohydrin as a stabilizer); dimethyl morpholinophosphoramidate (DMMPA) (CASRN 597-25-1); 1,4-dichlorobenzene (CASRN 106-46-7); bromodichloromethane (CASRN 75-27-4); methyl carbamate (CASRN 598-55-0); malonaldehyde, sodium salt (3-hydroxy-2-propenal, sodium salt) (CAS no. 24382-04-5); dichlorvos (CASRN 62-73-7); tribromomethane (bromoform) (CASRN 75-25-2); *p*-chloroaniline hydrochloride (CASRN 20265-96-7); *N*-methylolacrylamide (CASRN 924-42-5); glycidol (CASRN 556-52-5); benzofuran (CASRN 271-89-6); TRCP (CASRN 115-96-8); pentachloroanisole (CAS no. 1825-21-4); salicylazosulfapyridine (CASRN 599-79-1); riddelliine (CASRN 23246-96-0); Elmiron® (CASRN 37319-17-8); androstenedione (CASRN 63-05-8); pulegone (CASRN 89-82-7); and *Ginkgo biloba* extract (CASRN 90045-36-6).

Of these 29 chemicals discordant by species but concordant by sex, the most common pattern by far was induction of liver tumors in mice by a compound that tested negative in the Ames test (Figure 2). This pattern of tumor induction was seen for 11 chemicals including pentachloroethane (CASRN 76-01-7); 1,1,1,2-tetrachloroethane (CASRN 630-20-6); TCA (without epichlorohydrin) (CASRN 79-01-6); 1,2-dichloropropane (propylene dichloride) (CASRN 78-87-5); 1,4-dichlorobenzene (CASRN 106-46-7); *N*-methylolacrylamide (CASRN 924-42-5); benzofuran (CASRN 271-89-6); salicylazosulfapyridine (CASRN 599-79-1); Elmiron® (CASRN 37319-17-8); androstenedione (CASRN 63-05-8); and pulegone (CASRN 89-82-7). Only a single chemical induced liver tumors in both male and female mice but not in rats and was positive in the Ames test, that is, *Ginkgo biloba* extract (CASRN 90045-36-6) (11/12 observed vs. 0/12 expected, $p_{\text{pooled}} = 0.0000$; $p_{\text{unpooled}} = 0.00000$; Table 1). In contrast with the 12 chemicals that induced liver tumors in both male and female mice, only three chemicals induced liver tumors in both male and female rats: selenium sulfide (CASRN 7488-56-4); methyl carbamate (CASRN 598-55-0); and riddelliine (CASRN 23246-96-0). Two of these three chemicals were negative in the Ames test, with only riddelliine giving a positive response (2/3 observed vs. 0/3 expected, $p_{\text{pooled}} = 0.0072$; $p_{\text{unpooled}} =$ NA; Table 1).

At the site of chemical administration during gavage, that is, the forestomach, three chemicals induced tumors in rats including pivalolactone (CASRN 1955-45-9), Telone (technical grade 1,3-dichloropropane (CASRN 542-75-6) containing 1.0% epichlorohydrin as a stabilizer), and glycidol (CASRN 556-52-5). All three of these chemicals were positive in the Ames test (3/3 observed vs. 3/3 expected, $p_{\text{pooled}} =$ NA; $p_{\text{unpooled}} = 00000$; Table 1). Three chemicals also induced tumors in the forestomach of mice including benzyl acetate (CASRN 140-11-4), dichlorvos (CASRN 62-73-7), and benzofuran (CASRN 271-89-6). Dichlorovos was positive in the Ames test and the other two were negative in Ames.

In rats, two chemicals induced mononuclear leukemia, that is, DMMPA (CASRN 597-25-1) and TRCP (CASRN

115-96-8). Both of these chemicals were negative in the Ames test. Only *m*-cresidine (CASRN 102-50-1) caused tumors in rat urinary bladder. Two chemicals induced adrenal tumors in rats, that is, *p*-chloroaniline hydrochloride (CASRN 20265-96-7) and pentachloroanisole (CAS no. 1825-21-4). Both were positive in the Ames test. Two chemicals induced kidney tumors in rats with both testing negative in the Ames assay, that is, bromodichloromethane (CASRN 75-27-4) and TRCP (CASRN 115-96-8). Other tumor sites in rats included subcutaneous fibromas and fibrosarcomas (commercial grade 2,4 (80%)- and 2,6 (20%)-TDI (CASRN 26471-62-5)); large intestine (bromodichloromethane (CASRN 75-27-4)); thyroid (malonaldehyde, sodium salt (3-hydroxy-2-propenal, sodium salt) (CAS no. 24382-04-5)); spleen (*p*-chloroaniline hydrochloride (CASRN 20265-96-7)); mammary gland (glycidol (CASRN 556-52-5)); and brain (glycidol (CASRN 556-52-5)).

In mice, two chemicals induced lung tumors, that is, *N*-methylolacrylamide (CASRN 924-42-5) and benzofuran (CASRN 271-89-6), both of which are negative in Ames. Also in mice, two chemicals induced Harderian gland tumors, that is, *N*-methylolacrylamide (CASRN 924-42-5) and glycidol (CASRN 556-52-5), the former Ames negative and the latter Ames positive. Glycidol (CASRN 556-52-5) also induced skin tumors in mice.

Yes, across species; no, across sex within species. Of the 108 chemicals tested in F334/N rats and $B6C3F_1$ mice via gavage, only two caused tumors in the same tissue in both male rats and female mice but not in female rats or male mice. The first, Telone (technical grade 1,3-dichloropropane (CASRN 542-75-6) containing 1.0% epichlorohydrin as a stabilizer), caused tumors of the forestomach in both male rats and female mice. This chemical was positive in both the Ames test and a test of genetic toxicity other than Ames. The second chemical, allyl isovalerate (CASRN 2835-39-4), caused blood cancers in both male rats (mononuclear leukemia) and female mice (lymphoma). This chemical was negative in the Ames test and positive in a test of genetic toxicity other than Ames. A third chemical, trimethylphosphate (CASRN 512-56-1), caused tumors in both male rats and female mice but induced different tumor types, that is, subcutaneous tissue tumors in male rats and uterus/endometrium tumors in female mice. This compound was positive in both the Ames test and another test of genetic toxicity.

Results from 18 NTP gavage studies in Osborne–Mendel rats and $B6C3F_1$ mice

Clear evidence of neoplasms in male rats, female rats, male mice, and female mice. Four chemicals demonstrated clear evidence of neoplasm induction in male Osborne–Mendel rats, female Osborne–Mendel rats, male mice, and female mice. Three of the four were positive in both the Ames test and another test of genetic toxicity, that is, dibromochloropropane (DBCP) (CASRN 96-12-8), 1,2-dichloroethane (CASRN 107-06-2), and 1,2-dibromoethane (CASRN 106-93-4). In contrast, the fourth ubiquitously neoplastic chemical, that is, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (CASRN 1746-01-6) was negative in both the Ames test and another test of genetic toxicity (Online Appendix A-1).

Three of four species/sex categories displayed clear evidence of neoplasms. Three of four species/sex categories displayed clear evidence of neoplasms in three of the four species/sex categories, that is, chloroform (CASRN 67-66-3) and a mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (CASRN 57653-85-7, CASRN 19408-74-3) (HxCDD). Chloroform was positive in both the Ames test and another test of genetic toxicity, while HxCDD was not tested in either Ames or another test of genetic toxicity.

Clear evidence of neoplasms in Osborne–Mendel male rats and Osborne–Mendel female rats, no clear evidence in male mice and female mice. None.

Clear evidence of neoplasms in male mice and female mice, no clear evidence in Osborne–Mendel male rats and Osborne–Mendel female rats. Four chemicals induced neoplasms in male mice and female mice but not in male rats or female rats. All four, that is, TCA (CASRN 79-01-6), hexachloroethane (CASRN 67-72-1), 1,1,2-trichloroethane (CASRN 79-00-5), and 1,1,2,2-tetrachloroethane (CASRN 79-34-5) were negative in the Ames test, with three of four positive in another test of genetic toxicity.

Clear evidence of neoplasms in male mice, female mice, inadequate studies in Osborne–Mendel male and female rats. Tetrachloroethylene (CASRN 127-18-4) showed clear evidence of neoplasms in male mice and female mice, but the studies in male rats and female rats were judged to be inadequate. This chemical was negative in both the Ames test and another test of genetic toxicity.

No evidence of neoplasms in Osborne–Mendel male and female rats, male mice, female mice. Two chemicals did not induce neoplasms in male rats, female rats, male mice, and female mice, that is, 3-sulfolene (CASRN 77-79-2) and iodoform (CASRN 75-47-8). 3-Sulfolene was negative in both the Ames test and another test of genetic toxicity, while iodoform was positive in both the Ames test and another test of genetic toxicity.

Tumor concordance in Osborne–Mendel rats and $B6C3F_1$ mice

Yes, across species; yes, across sex within species. DBCP (CASRN 96-12-8) induced tumors in the stomach of male and female rats and male and female mice. 1,2-dibromoethane (CASRN 106-93-4) induced tumors of the

forestomach in male and female rats and male and female mice. It also induced tumors in the lungs of male and female mice.

No, across species; yes, across sex within species. The most common pattern of tumor induction was the development of liver tumors in mice seen in five cases: chloroform (CASRN 67-66-3), TCA (CASRN 79-01-6), tetrachloroethylene (CASRN 127-18-4), hexachloroethane (CASRN 67-72-1), and mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (CASRN 57653-85-7, CASRN 19408-74-3) (HxCDD). 1,1,2-Trichloroethane (CASRN 79-00-5) induced tumors in both the liver and adrenals in mice. 1,2-Dichloroethane (CASRN 107-06-2) caused lung tumors in mice.

Yes, across species; no, across sex within species. TCDD (CASRN 1746-01-6) induced thyroid tumors in male rats and female mice but not in female rats and male mice. It also induced liver tumors in female rats and male mice but not in male rats and female mice.

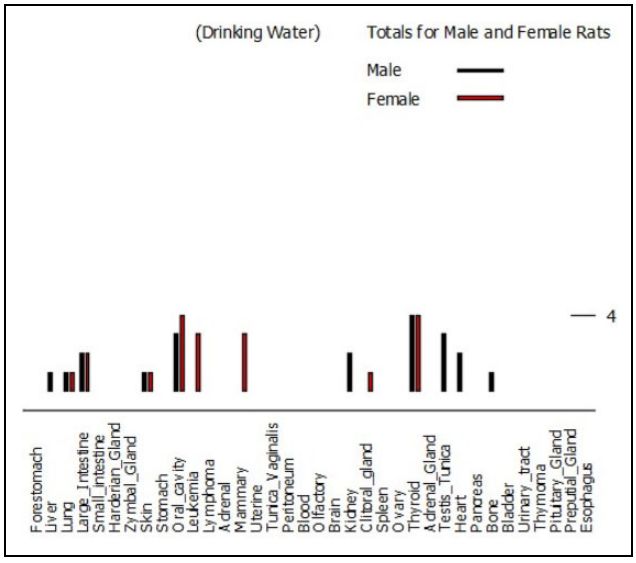

Figure 3. Distribution of tumor incidence in male and female rats treated in drinking water.

Results from 21 NTP drinking water studies in F344/N rats and B6C3F$_1$ mice (23 chemicals tested)

Clear evidence of neoplasms in male rats, female rats, male mice, and female mice. Of 23 chemicals tested, 6 induced tumors in male rats, female rats, male mice, and female mice including 4,4′-methylenedianiline (MDA) dihydrochloride (CASRN 13552-44-8), sodium dichromate dihydrate (CASRN 7789-12-0), bromochloroacetic acid (CASRN 5589-96-8), acrylamide (CASRN 79-06-1), bromodichloroacetic acid (CASRN 71133-14-7), and glycidamide (CASRN 5694-00-8). Of these six chemicals, five were positive in the Ames test (5/6 observed vs. 6/6 expected, $p_{\text{pooled}} = 0.1481$; $p_{\text{unpooled}} = 0.26401$) and four were positive in at least one other test of genetic toxicity (4/6 observed vs. 6/6 expected, $p_{\text{pooled}} = 0.0607$; $p_{\text{unpooled}} = 0.16107$; Table 1, Online Appendix B).

Clear evidence of neoplasms in three of four sex/species categories. Of the 23 chemicals tested via the drinking water route of exposure, only dibromoacetonitrile (CASRN 3252-43-5) displayed clear evidence of neoplasia in three of four sex/species categories, that is, it was neoplastic in male rats, male mice, and female mice. This chemical also tested positive in the Ames test and negative in another test of genetic toxicity.

Clear evidence of neoplasms in male rats and female rats, no clear evidence in male and female mice. Of the 23 chemicals tested in drinking water, the only one to show clear evidence of neoplasms in male rats and female rats but not in male mice and female mice was non-decolorized whole leaf extract of *Aloe barbadensis* Miller (*Aloe vera*) (CASRN 85507-69-3). This chemical was negative in the Ames test and positive in another test of genetic toxicity.

Clear evidence of neoplasms in male mice and female mice, no clear evidence in male rats or female rats. Pyridine (CASRN 110-86-1) and dibromoacetic acid (CASRN 631-64-1) displayed clear evidence of neoplasms in male mice and female mice but did not induce tumors in male rats or female rats following exposure to drinking water. Pyridine was negative in both the Ames test and another test of genetic toxicity, while dibromoacetic acid was positive in both the Ames test and another test of genetic toxicity.

Clear evidence of neoplasms in female mice only. Of the 23 chemicals tested in drinking water, the only one to show clear evidence of neoplasms in female mice only was β-picoline (CASRN 108-99-6). This chemical was negative in both the Ames test and another test of genetic toxicity.

No evidence of neoplasia in male rats, female rats, male mice, and female mice. Four of the 23 chemicals tested in drinking water did not induce tumors in male rats, female rats, male mice, or female mice. These chemicals were the following: phenol (CASRN 108-95-2), barium chloride dihydrate (CASRN 10326-27-9), 1-chloro-2-propanol (technical grade) (CASRN 127-00-4), and dipropylene glycol (CASRN 25265-71-8). Three of the four were negative in the Ames test (3/4 observed vs. 4/4 expected, $p_{\text{pooled}} = 0.1425$; $p_{\text{unpooled}} = 0.26370$) and only one of the four was negative in another test of genetic toxicity (1/4 observed vs. 4/4 expected, $p_{\text{pooled}} = 0.0142$; $p_{\text{unpooled}} = 0.05654$; Table 1).

Tumor concordance in drinking water studies. Figures 3 and 4 illustrate the distribution of tumors induced in rats and mice by chemical administration in the drinking water (Table 3).

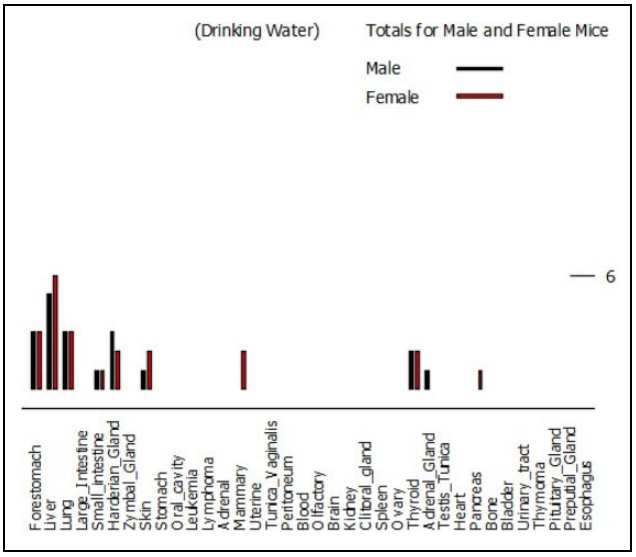

Figure 4. Distribution of tumor incidence in male and female mice treated in drinking water.

Yes, across species; yes, across sex within species. Of the 23 chemicals tested in drinking water, only the thyroid tumors induced by MDA dihydrochloride (CASRN 13552-44-8) were seen in male rats, female rats, male mice, and female mice. This Ames-positive and "other than Ames"-positive chemical also induced liver tumors in male and female mice.

No, across species; yes, across sex within species. Nine of the 23 chemicals tested in drinking water were discordant across species but were concordant across sex within species. Four of the nine within-sex concordant chemicals induced liver tumors in mice including pyridine (CASRN 110-86-1), dibromoacetic acid (CASRN 631-64-1), bromochloroacetic acid (CASRN 5589-96-8), and bromodichloroacetic acid (CASRN 71133-14-7). Forestomach tumors in mice were induced by dibromoacetonitrile (CASRN 3252-43-5) and glycidamide (CASRN 5694-00-8). Lung tumors in mice were caused by acrylamide (CASRN 79-06-1) and glycidamide (CASRN 5694-00-8). Tumors in the large intestine of rats were induced by bromochloroacetic acid (CASRN 5589-96-8) and non-decolorized whole leaf extract of *Aloe barbadensis* Miller (*Aloe vera*) (CASRN 85507-69-3). Harderian gland tumors were induced in mice by acrylamide (CASRN 79-06-1) and glycidamide (CASRN 5694-00-8). Other tumor sites seen in rats were the oral cavity (sodium dichromate dihydrate (CASRN 7789-12-0) and glycidamide (CASRN 5694-00-8)); thyroid ((acrylamide (CASRN 79-06-1) and glycidamide (CASRN 5694-00-8)); and skin (glycidamide (CASRN 5694-00-8)). Other tumor sites seen in mice were the small intestine (sodium dichromate dihydrate (CASRN 7789-12-0) and skin (glycidamide (CASRN 5694-00-8)).

Results for 18 NTP 2-year dermal studies conducted in F344/N rats and B6C3F$_1$ mice (18 chemicals tested)

Clear evidence of neoplasms in male rats, female rats, male mice, and female mice. Two of the 18 chemicals tested via the dermal route, that is, 4-vinyl-1-cyclohexene diepoxide (CASRN 106-87-6) and 2,3-dibromo-1-propanol (CASRN 96-13-9) induced tumors in male rats, female rats, male mice, and female mice. Both of these chemicals were positive in the Ames test and in at least one other test of genetic toxicity (Online Appendix C, supplemental materials).

Clear evidence of neoplasms in male mice and female mice, no clear evidence in male rats or female rats. Two of the 18 chemicals tested via the dermal route, that is, diethanolamine (CASRN 111-42-2) and coconut oil acid diethanolamine condensate (CASRN 68603-42-9) induced tumors in male mice and female mice but did not induce tumors in male rats or female rats. Both of these chemicals were negative in the Ames test, with diethanolamine (CASRN 111-42-2) also being negative in another test of genetic toxicity, and coconut oil acid diethanolamine condensate (CASRN 68603-42-9) testing negative in another test of genetic toxicity.

No clear evidence of neoplasia in male rats, female rats, male mice, and female mice. Of the 18 chemicals tested via the dermal route, 8 displayed no neoplasticity including 2-chloroethanol (ethylene chlorohydrin) (CASRN 107-07-3); benzethonium chloride (CASRN 121-54-0); sodium xylene sulfonate (CASRN 1300-72-7); oleic acid diethanolamine condensate (CASRN 93-83-4); diisopropylcarbodiimide (CASRN 693-13-0); bis(2-chloroethoxy)methane (CASRN 111-91-1); 1,2-dibromo-2,4-dicyanobutane (CASRN 35691-65-7); and methyl trans-styryl ketone (CASRN 1896-62-4). Of the eight not neoplastic chemicals, the Ames test correctly predicted a negative result in five cases (5/8 observed vs. 8/8 expected, $p_{\text{pooled}} = 0.0273$; $p_{\text{unpooled}} = 0.10606$). There were also five cases where a genetic toxicity test other than Ames predicted a negative result (5/8 observed vs. 8/8 expected, $p_{\text{pooled}} = 0.0273$; $p_{\text{unpooled}} = 0.10606$; Table 1).

Overall summary of genotoxicity versus neoplasticity for the dermal route. Six of the 18 chemicals tested positive in the Ames test. Only two of these six Ames-positive chemicals were associated with a positive neoplastic result (2/6 observed vs. 6/6 expected, $p_{\text{pooled}} = 0.0072$; $p_{\text{unpooled}} = 0.04212$) for an accuracy of 33.3%. Eleven of 17 chemicals tested negative in the Ames test. (Note, all Figures are in Appendix E). Eight of the 10 Ames-negative chemicals were associated with "not clear" neoplastic results (8/10 observed vs. 10/10 expected, $p_{\text{pooled}} = 0.0680$; $p_{\text{unpooled}} = 0.24052$) for an accuracy of 80% (Tables 1 and 2).

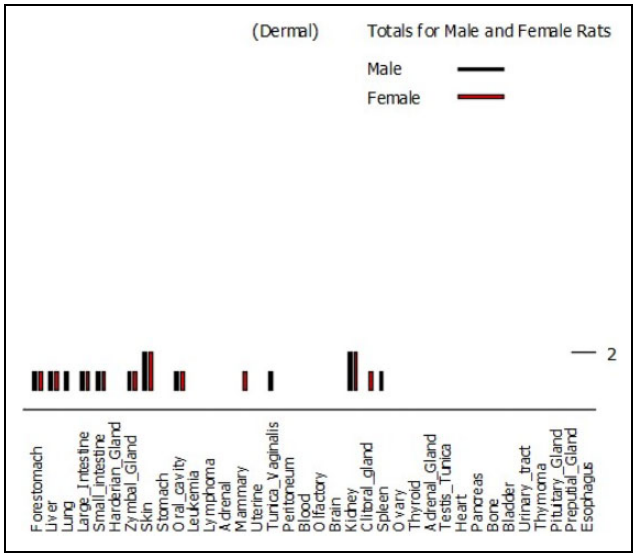

Figure 5. Distribution of tumor incidence for male and female rats following dermal exposure.

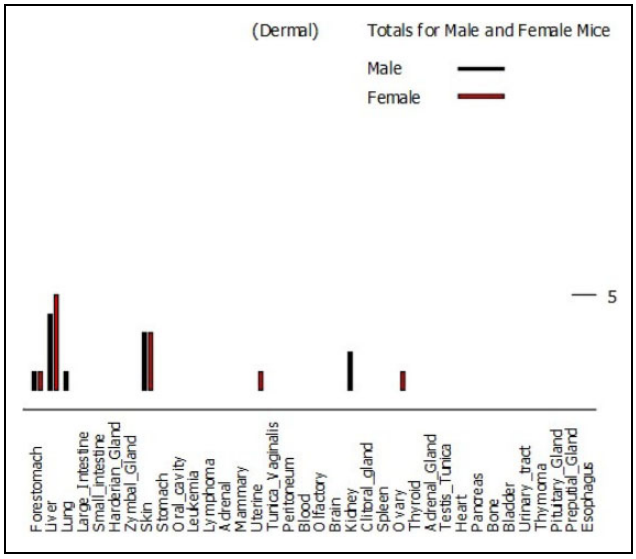

Figure 6. Distribution of tumor incidence in male and female mice following dermal exposure.

Tumor concordance for the dermal route. Figures 5 and 6 illustrate the distribution of tumors induced in rats and mice by chemical administration via the dermal route (Table 3).

Yes, across species; yes, across sex within species. 2,3-Dibromo-1-propanol (CASRN 96-13-9) induced skin tumors and tumors of the forestomach in male rats, female rats, male mice, and female mice. In male and female rats, it also induced neoplasms of the nose, oral mucosa, esophagus, small and large intestine, Zymbal's gland, liver, and kidney. 4-Vinyl-1-cyclohexene diepoxide (CASRN 106-87-6) induced skin tumors in male rats, female rats, male mice, and female mice.

No, across species; Yes, across sex within species. Diethanolamine (CASRN 111-42-2) induced liver tumors in male and female mice. Coconut oil acid diethanolamine condensate (CASRN 68603-42-9) also induced liver tumors in male and female mice.

Results for 11 NTP intraperitoneal injection studies conducted in F344/N rats (and Sprague-Dawley rats) and B6C3F$_1$ mice (11 chemicals tested)

Clear evidence of neoplasms in male rats, female rats, male mice, and female mice. Three of the 11 chemicals tested via intraperitoneal injection displayed tumors in male rats, female rats, male mice, and female mice including procarbazine (CASRN 366-70-1), thio-TEPA (CASRN 52-24-4) *N*,*N*′,*N*″-triethylenethiophosphoramide, and phenoxybenzamine hydrochloride (CASRN 63-92-3). Two of these three chemicals were positive in the Ames test (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$), and two of these three were also positive in a genetic test other than Ames (2/3 observed vs. 3/3 expected, $p_{\text{pooled}} = 0.1367$; $p_{\text{unpooled}} = 0.46416$; Table 1 and Online Appendix D, supplemental materials).

Clear evidence of neoplasms in male rats and female rats, no clear evidence in male and female mice. Cytembena (CASRN 21739-91-3) displayed clear evidence of neoplasms in male rats and female rats and no clear evidence in male and female mice. It was positive in both the Ames test and at least one other test of genetic toxicity.

Clear evidence of neoplasms in male rats and female rats, inadequate studies conducted on male mice and female mice. Acronycine (CASRN 7008-42-6) showed clear evidence of neoplasms in male rats and female rats. The studies on male mice and female mice were inadequate. The Ames test was not conducted on this chemical, and the result in a genetic test other than Ames was negative.

Clear evidence of neoplasms in female rats and female mice only. Isophosphamide (CASRN 3778-73-2) and ICRF -159 (CASRN 21416-87-5) 4-[1-(3,5-dioxopiperazin-1-yl)propan-2-yl]piperazine-2,6-dione (Razoxane) induced neoplasms in female rats and female mice only, with the former positive in the Ames test and the latter negative in the Ames test, respectively. Genetic tests other than Ames displayed a pattern opposite to the Ames test with isophosphamide negative and Razoxane positive.

Clear evidence of neoplasms in female mice only and inadequate studies in male rats, female rats, and male mice. 5-Azacytidine (CASRN 320-67-2) induced neoplasms in only the female mice, with inadequate studies in male rats, female rats, and male mice. It was positive in both the Ames test and a genetic toxicology test other than the Ames test.

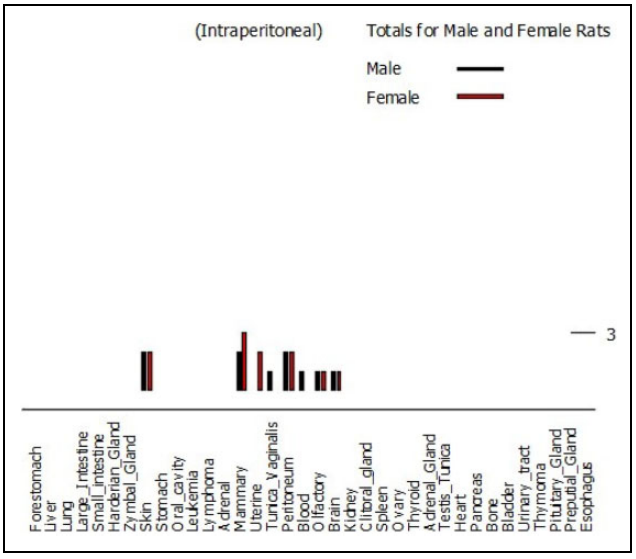

Figure 7. Distribution of tumor incidence in male and female rats following intraperitoneal injection.

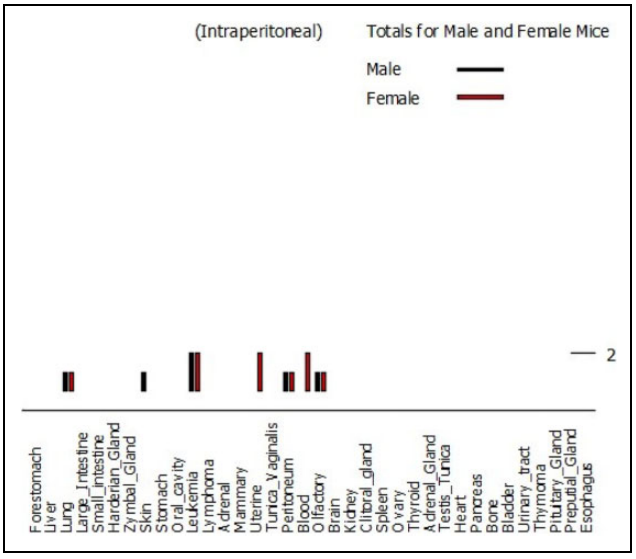

Figure 8. Distribution of tumor incidence in male and female mice following intraperitoneal injection.

Clear evidence of neoplasms in female rats, no clear evidence in male rats, inadequate studies in male mice and female mice. β-2′-Deoxy-6-thioguanosine monohydrate (b-TGdR) (CASRN 64039-27-6) displayed clear evidence of neoplasms in female rats, an absence of clear evidence in male rats, with inadequate studies in male mice and female mice. This compound was not tested in either Ames or other tests of genetic toxicity.

Tumor concordance via intraperitoneal injection. Figures 7 and 8 illustrate the distribution of tumors induced in rats and mice by chemical administration via intraperitoneal injection (Table 3).

Yes, across species; yes, across sex within species. Procarbazine (CASRN 366-70-1) induced lymphomas and olfactory tumors in male rats, female rats, male mice, and female mice. Phenoxybenzamine hydrochloride (CASRN 63-92-3) caused tumors in the peritoneum of male rats, female rats, male mice, and female mice. Thio-TEPA (CASRN 52-24-4) *N*,*N*′,*N*″-triethylenethiophosphoramide induced hematopoietic tumors in male rats, male mice, and female mice. It also induced skin tumors in male rats, female rats, and male mice. Thio-TEPA also induced squamous cell carcinoma of the ear canal in male and female rats.

No, across species; yes, across sex within species. Cytembena (CASRN 21739-91-3) caused mesotheliomas in the *tunica vaginalis* in male rats and female rats.

Different tumor sites. Isophosphamide (CASRN 3778-73-2) induced tumors at different anatomical sites across species. In female Sprague-Dawley rats, there was an increased incidence of leiomyosarcomas of the uterus and fibroadenoma of the mammary gland. Isophosphamide (CASRN 3778-73-2) was also carcinogenic in female $B6C3F_1$ mice, producing malignant lymphomas of the hematopoietic system. In female rats, ICRF-159 (CASRN 21416-87-5) 4-[1-(3,5-dioxopiperazin-1-yl)propan-2-yl]piperazine-2,6-dione (Razoxane) produced uterine adenocarcinomas and lymphomas in female $B6C3F_1$ mice.

Results from analysis of structural alerts

Of the 179 chemicals tested by way of gavage, drinking water, dermal administration, or intraperitoneal injection, 59 contained structural alerts (SAs). Twenty-nine of these compounds showed clear evidence of neoplasia in male and female rats and in male and female mice. Twenty eight of these 29 ubiquitously neoplastic chemicals possessed SAs suggestive of carcinogenic potential[10] (Table 4). The 28 ubiquitously neoplastic chemicals containing SAs were as follows: *N*,*N*-dimethyl-*p*-toluidine (CASRN 99-97-8), 2,4-hexadienal (89% trans, trans isomer, CASRN 142-83-6; 11% cis, trans isomer), riddelliine (CASRN 23246-96-0), furan (CASRN 110-00-9), glycidol (CASRN 556-52-5), bromodichloromethane (CASRN 75-27-4), dimethylvinyl chloride (1-chloro-2-methylpropene) (CASRN 513-37-1), chlorinated paraffins (C12, 60% chlorine) (CASRN 108171-26-2), 3-chloro-2-methylpropene (technical grade containing 5% dimethylvinyl chloride) (CASRN 563-47-3), benzene (CASRN 71-43-2), ethyl acrylate (CASRN 140-88-5), DGRE (technical grade) (CASRN 101-90-6), a polybrominated biphenyl mixture (Firemaster FF-1) (CASRN 36355-01-8), TCDD (CASRN 1746-01-6), 1,2-dibromoethane (CASRN 106-93-4), 1,2-dichloroethane (CASRN 107-06-2), DBCP (CASRN 96-12-8), glycidamide (CASRN 5694-00-8), bromodichloroacetic acid (CASRN 71133-14-7), acrylamide (CASRN 79-06-1), bromochloroacetic acid (CASRN 5589-96-8), MDA

Table 4. A total of 179 chemicals tested by way of gavage, drinking water, dermal, or intraperitoneal.

	Compounds that produced clear, clear, clear, clear[a] neoplastic evidence	Compounds that produced 3/4 clear[b] neoplastic evidence	Compounds that produced no, no, no, no[c] neoplastic evidence
Number of compounds	29	8	37
Compounds without SAs (120)	1	1	13
Compounds with SAs (59)	28	7	24
Percentage	97% (28/29)	87% (7/8)	65% (24/37)[d]

SA: structural alert.
[a]Clear evidence of neoplasticity for male and female rats and male and female mice.
[b]Clear evidence of neoplasticity for 3 of 4 sex–species (male and female rats and male and female mice).
[c]No evidence of neoplasticity for male and female rats and male and female mice.
[d]This represents the false positive rate.

dihydrochloride (CASRN 13552-44-8), 2,3-dibromo-1-propanol (CASRN 96-13-9), 4-vinyl-1-cyclohexene diepoxide (CASRN 106-87-6), phenoxybenzamine hydrochloride (CASRN 63-92-3), thio-TEPA (CASRN 52-24-4) *N*,*N*′,*N*″-triethylenethiophosphoramide, sodium dichromate dihydrate (CASRN 7789-12-0), and procarbazine (CASRN 366-70-1). The only outlier not possessing an SA for carcinogenicity was methyleugenol (CASRN 93-15-2). Methyleugenol is a phenolic tumor promoter and was found to be carcinogenic in experimental animals via oral gavage.

Eight of the 179 chemicals tested by way of gavage, drinking water, dermal administration, or intraperitoneal injection displayed clear evidence of neoplasia in three of the four categories of male rats, female rats, male mice, and female mice (Table 4). Seven of these eight chemicals either possessed an SA or was a phenolic tumor promotor. Therefore, these results were consistent with the structural predictions. The only outlier not possessing an SA for carcinogenicity was pulegone. Pulegone is highly hydrophobic with a calculated $\log p = 3.08$. Whether this high $\log p$ value adversely affects the predictability of the SAs is unknown. The genotoxicity associated with pulegone may be the result of reactive metabolites formed from pulegone via epoxidation.

Thirty-seven of the 179 chemicals tested by way of gavage, drinking water, dermal administration, or intraperitoneal injection were negative in male and female rats and in male and female mice. Twenty-four of these 37 chemicals ubiquitously negative for neoplasia nonetheless contained an SA representing a false positive rate of 65% (24/37; Table 4). An additional 2 of these 37 chemicals could be categorized as phenolic tumor promoters. In contrast with the high degree of association between possession of an SA and development of a tumor for the ubiquitously neoplastic chemicals and the chemicals neoplastic in the three of four species/sex categories (low false negative rate), only 13/37 (35%) of the completely non-neoplastic chemicals did not possess structural characteristics frequently associated with neoplasia (high false positive rate).

The remaining 120 (179 (total)—59 (with SAs)) ubiquitously non-neoplastic chemicals did not contain SAs and were classifiable as selective primary and secondary alcohols, primary and secondary amines, ketones, amides, acetates, phosphates, sulfates, sulphones, lactams, anhydrides, amino acids, gums, natural products, phytochemicals, sugars, acids, unsubstituted aromatics, phenolics, thiocarbamates, industrial chemicals, azo dyes, and similar compounds.[11]

The selective chemicals mentioned above are those that are not likely to form electrophiles and react with biological nucleophiles. Additionally, these chemicals are not expected to undergo S_N, S_N2, acylation, Schiff base formation, Michael addition, and S_NAr reaction mechanisms.[11]

Discussion

The overall results from this analysis of the NTP studies by the gavage, drinking water, dermal and intraperitoneal injection routes of administration support the previous findings from the evaluations of the NTP inhalation[2] and feed[3] studies. First, the results show that tumor site concordance is higher within male rats and female rats, and within male mice and female mice, than is concordance across rodent species. Second, historical Ames test results do not accurately predict the development of rodent tumors in 2-year bioassays, although a negative Ames test results appears to possess more predictive capability than does a positive Ames test result. The poor prediction accuracy of historical Ames test results suggests that decisions regarding the potential mutagenicity of test articles should be based on recent results from batteries of genotoxicity tests conducted following the relevant Organization for Economic Cooperation and Development (OECD) protocols and under Good Laboratory Practices (Table 2).

In contrast with the poor predictive accuracy of historical positive Ames test results, in this study, we found that the presence of "structural alerts" correlated well with the development of rodent tumors although the false positive rate for non-neoplastic chemicals is quite high at 65%. Benigni and Bossa first reported the use of SAs to predict genotoxicity in 1985.[12] These authors described SAs as follows:

> The Structural Alerts are molecular substructures or reactive groups that are related to the carcinogenic and mutagenic properties of the chemicals, and represent a sort of

"codification" of a long series of studies aimed at highlighting the mechanisms of action of the mutagenic and carcinogenic chemicals.

The use of structural alerts can be helpful in the classification of potential carcinogens and are important to understanding the mechanisms of genotoxicity.[10,13–17]

Several studies have examined or reexamined the carcinogenicity of chemicals, groups of chemicals, and exposure circumstances in animals (and in certain circumstances humans) by the International Agency for Research on Cancer and the NTP.[17–20] Previous studies have found that the level of concordance in chemicals tested causing cancer in each of the four sex/species groups used for testing, that is, female rats, male rats, female mice, and male mice, was between 14% and 20%.).[17–20]

Ghanayem et al. tested a number of chemicals that have been shown to cause malignant neoplasms in the forestomach of Fischer 344 rats when administered chronically by gavage (2-week repeated gavage).[21] Histopathologic examination of forestomach of rats killed 24 h after the last dose indicated no significant difference in the incidence or severity of epithelial cell proliferation in the rat forestomach between the vehicle control group and the two negative control groups. In contrast, the incidence and severity of epithelial cell proliferation of the rat forestomach in every group treated with a forestomach carcinogen was significantly higher than the incidence in the vehicle or negative control groups. These results suggest that early epithelial cell proliferation of the forestomach may be associated with at least some chemicals that induce forestomach neoplasia following chronic administration by gavage. Some have argued that forestomach tumors associated with chronic irritation of the forestomach epithelium, particularly those induced by repeated oral gavage dosing, should not form the basis for carcinogenic determination.[22]

The results of this evaluation are consistent with those of past studies in that there is a low level of tumor site concordance in chemicals tested for causing cancer in each of the four sex/species groups used for testing, that is, male rats, female rats, male mice, and female mice. The results indicated that only 16% (29/179) of all chemicals tested by NTP were concordant across all species/sex categories (14% (18/127) in the NTP gavage studies, 26% (6/23) in the NTP drinking water studies, 11% (2/18) in the NTP dermal studies, and 27% (3/11) in the NTP intraperitoneal studies).

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Supplementary material

Supplementary material for this article is available online.

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