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Gender differences in chemical carcinogenesis in National Toxicology Program two-year bioassays

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Abstract

Differences in cancer incidences between men and women are often explained by either differences in environmental exposures or by influences of sex hormones. However, there are few studies on intrinsic gender differences in susceptibility to chemical carcinogens. We have analyzed the National Toxicology Program (NTP) database for sex differences in rat responses to chemical carcinogens. We find that the odds that male rat bioassays were assigned a higher level of evidence than female rat bioassays was 1.69 ($p < 0.001$). Of 278 carcinogenic chemicals in the database, 201 (72%) exhibited statistical gender differences ($p = 0.05$) in at least one non-reproductive organ. 130 of these 201 chemicals induced gender-specific tumors in male rats and 59 in female rats. 68 chemicals induced tumors in males but no tumors in females. Less than one third, i.e. 19 chemicals, induced tumors in females but not males. Male-specific tumors included pancreatic tumor and skin tumor, and female-specific tumors included lung tumors. For some tumor types these differences in gender susceptibility can be associated with literature data on sex hormone receptor expression. In conclusion, gender-specific tumors were common. The male dominance is in line with human data and the male susceptibility to carcinogens should be further studied.

Keywords

NTP; gender; two-year bioassay; tumor; carcinogens

Introduction

Excluding reproductive organs, cancer incidences are often different in men and women (Cook *et al.*, 2009; Edgren *et al.*, 2012). The fact that mammary and prostate cancers are among the most common human cancer types suggests a fundamental influence of sex hormones (c.f. (Lin and Giovannucci, 2010)). The role of sex hormones in cancer development has been extensively studied (Anderson, 2005; Folkard and Dowsett, 2010)

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Conflict of interest statement

None

along with the role of hormonal replacement therapy (Santen *et al.*, 2010). However, gender differences in response to xenobiotic carcinogens may also contribute. Such differences can often be related to gender differences in exposure to carcinogens in e.g. work places. Lifestyle or nutritional factors may also influence gender-specific responses, as discussed previously (Kirsch-Volders *et al.*, 2010). Of importance for the present work is that it can be assumed that there are intrinsic differences in susceptibility to xenobiotic carcinogens, as, for example discussed for kidney tumors in male rats (Leavens and Borghoff, 2009), or for human lung tumors (Uppstad *et al.*, 2011; Edgren *et al.*, 2012). This aspect of carcinogenesis has been surprisingly little studied (Gochfeld, 2007; Cook *et al.*, 2009; Kirsch-Volders *et al.*, 2010; Edgren *et al.*, 2012).

In an effort to get an overview of a possible gender difference in susceptibility to chemical carcinogens we have analyzed the results obtained in two-year bioassays published in the National Toxicology Program (NTP) database.

The relevance of rodent carcinogenesis for humans has been discussed in numerous publications (Maronpot *et al.*, 2004; Anisimov *et al.*, 2005; Hoenerhoff *et al.*, 2009). Although relevance has been demonstrated in some cases but not in others, animal models remain important in predicting human risk for carcinogens (Huff, 2010). A recent example is animal data influencing formaldehyde risk assessment (Andersen *et al.*, 2010). Here, we present rat (mainly Fisher 344) data, which generally are considered more comparable for human cancer than mouse data (Uppstad *et al.*, 2011). Thus, there is little similarity in the spectrum of spontaneous tumors developed by humans compared with mice and rats, but in contrast to mice and similar to humans, rats develop more frequently epithelial tumors (Anisimov *et al.*, 2005). Furthermore, at ages above the female reproductive period, overall cancer risk is higher in males than in females in both rats and humans (Anisimov *et al.*, 2005; IARC, 2010). It is also crucial to emphasize that cancer epidemiology has inherent weaknesses. These include problems associated with decades of latency periods, problems associated with exposure assessments and the complexity of interacting exposures, dietary differences, and the relative rarity of tumors at a specific site. These weaknesses indicate that e.g. negative epidemiological data for a given chemical and a given tumor site does not necessarily exclude human risks (Huff *et al.*, 2008).

Cancer tests typically employ male and female rats, mimicking lifetime exposure to carcinogens (National Toxicology Program, 2009). In contrast to practically all human observational studies, exposures, diets and environmental factors are very well controlled. For example, in NTP tests male and female rats are treated identically, and although doses are not adjusted to give e.g. equal internal dose, doses are adjusted to weight for each sex. Thus, it can be expected that the NTP database is informative concerning fundamental intrinsic aspects of gender differences in chemical carcinogenesis.

We here present an evaluation of NTP chronic bioassays published between 1976 and 2009. Our hypothesis is that carcinogens might interact with sex hormones, and that such interactions should be reflected in the NTP data base. Such interactions has been suggested by Edgren *et al.* (Edgren *et al.*, 2012) and studied e.g. in bladder cancer (Miyamoto *et al.*, 2007). It was assumed that even weak carcinogenic responses might be informative when

analyzing gender specific trends among several bioassays, so we included not only assays with *clear evidence* and *some evidence* but also assays with *equivocal evidence* in most analysis. Of 278 carcinogenic chemicals identified by NTP, 201 (72%) showed gender differences, and we found that males had more tumors. In this presentation we will focus commonly affected tumor sites in non-reproductive organs, not on individual chemicals.

Methods

The NTP database and 2-year cancer bioassays

This list of NTP Peer Reviewed Technical Reports (<http://ntp.niehs.nih.gov/>) is arranged by Report Number and includes carcinogenicity conclusions and links to the Technical Report abstracts, Levels of evidence, and Results & Status Reports. Levels of evidences include *Clear evidence*, *Some evidence*, *Equivocal evidence*, and *No evidence*. *Clear evidence* is conferred on chemicals which cause dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy. *Some evidence* indicates a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence. *Equivocal evidence* indicates a marginal increase of neoplasms that may be chemically related. *No evidence* is defined as any chemically-related increase in tumors. All assays with *Clear evidence*, *Some evidence* and *Equivocal evidence* and with both male and female rats were included. Bioassays performed in one gender, classified by NTP as “inadequate experiment” (“IS”), or with *No evidence* were excluded. Also excluded were results without statistically significant effects at a specific site.

Tumors in the following male and female reproductive organs were not included in the analysis: penis, testis, epididymis, vas deferense, seminal vesicles, ejaculatory ducts, prostate and bulbourethral glands, vulva, clitoral glands, clitoris, vagina, uterus, ovary, fallopian tubes and mammary glands. Tumors in *tunica vaginalis* in scrotum were included as mesotheliomas originating in this tissue may spread to abdominal mesothelium and site of origin was not reported.

Statistical analysis

We compared the tumor rates of males and females by each organ separately using standard analysis of variance (ANOVA). Since there were several potential differences among various studies, such as, year of the study, number of doses used in the study, route of exposure, feed, etc., we compared the two sexes after adjusting for these study differences. This was done by nesting sex within study (i.e. chemical). Note that a large number of comparisons are being made when investigating the differences between the sexes with respect to each chemical (i.e. each NTP study) for a given tumor. To deal with multiple comparison issues, we performed the pairwise comparisons only in those cases where the overall “sex within study” effect was significant at $p < 0.05$. Consistent with the NTP’s standard protocol, all dose groups were compared with the untreated vehicle control group and we did not perform multiple testing adjustments for different tumor types. Lastly we did

not use historical control data but only used the data from concurrent vehicle control group in our analysis. Note that control animals in the historical control database change over time. To avoid any misinterpretation of data, it is critical to match historical controls with the current study. For this reason, typically the NTP uses a 5 year moving window of historical controls. However, since in our analysis we considered data from multiple studies over a large time span, there is no convenient database that would match data with each study used in this investigation with its suitable historical control data. Thus it was not practically feasible to include historical control data in the present investigation.

Results

To identify gender differences in chemical carcinogenesis two-year rat NTP bioassay data was analyzed. This was done using the evidence level as indicator of carcinogenic potential for the chemicals. A subset of 477 bioassays included both genders. Among these, 278 showed *clear*, *some* or *equivocal* evidence for carcinogenicity and were identified as carcinogens. Of these 278 carcinogens 13 exhibited tumors only in mammary or reproductive organs and were excluded from further analysis. 200 exhibited some type of gender difference in non-reproductive organs as defined in Methods (Table 1). 87 (31%) gave tumors in either males or females (68 in males only and 19 in females only). 102 gave tumors in at least one organ in one sex but showed no evidence in the same organ in the other sex (62 in males and 40 in females). A third category was 12 chemicals that gave tumors in both sexes, but significantly more in one sex (8 in males and 4 in females) (Table 1). Thus, out of 278 agents 87 were gender-specific and the rest, 191 agents, induced tumors in both sexes. It is also apparent from Table 1 that there was a male dominance with e.g. 68 assays with tumors in males only and 19 in females only. In further analysis we excluded equivocal test results. We found that assays with *clear* and *some evidence* for carcinogenicity exhibited even stronger dominance for males. Thus, 40 male-specific and seven female-specific chemicals were identified. In other words, male-specific chemicals were 5.7 times more common than female-specific chemicals when these more stringent criteria for carcinogenic activity (see Methods) were used. All these data indicate that male rats were more susceptible for chemical carcinogenesis in non-reproductive organs.

We also performed an overall analysis of data including all non-reproductive and reproductive organs. It revealed that male rats were 1.69 ($p < 0.001$) times more often classified to a higher level of evidence for carcinogenic activity than female rats.

Most bioassays (95 %) employed Fischer 344 rats (King-Herbert *et al.*, 2010) and we did not observe any bias for other strains among bioassays showing gender differences. There was a change in diet 1995, which have been reported to affected tumor incidences of e.g. pituitary and testis tumors (Haseman *et al.*, 2003). However most bioassays used in these studies were performed prior to the food change, and we did not observe trend changes in later studies. The publication years for bioassays used in this study are given in supplementary Table 1. As can be seen assays exhibiting gender dimorphisms were evenly spread over the years, and we did not observe trends that can be explained by e.g. genetic drift among test animals. Likewise, we did not observe any bias for route of exposure.

Table 2 shows the fraction of rat carcinogens inducing tumors in an organ or tissue in a gender-specific way, i.e. there were tumors in one organ/site in one sex but no tumors in the same organ/site in the other sex. Table 2 is based on results from 188 (87 + 102) bioassays (or carcinogens) shown in Table 1, and lists 170 positive male organs and tissue sites and 70 positive female sites. It should be noted that some chemicals were multi-site carcinogens. The results are presented as fractions and percent of the total number of chemicals inducing tumors at the same site. Overall, males were more affected and more often tumors were induced in male rats than in female rats. The four sites that exhibited the highest fractions of male-specific tumors were mesothelium (mesotheliomas) (83% of all mesothelioma-inducing carcinogens), pancreas (79%), kidney (64%) and skin (58%). The four sites that exhibited the highest fractions of female-specific tumors were pituitary (80%), bone marrow and lymphoid tissues (leukemias and lymphomas) (47%), lung (33%) and urinary bladder (29%). 15 of 18 chemicals inducing mesotheliomas induced this tumor in males only. Of importance is that the majority, if not all, of mesotheliomas likely derived from the male-specific *tunica vaginalis* in scrotum (Haber *et al.*, 2009) and may thus be regarded as a male-specific reproductive organ tumor.

Another conspicuous finding was that 11 chemicals out of 14 pancreatic carcinogens induced tumors in males only and none in females. Eight chemicals induced exocrine pancreatic cancer in males only, but none in females only. In other organs such as lung both female- and male-specific carcinogens were identified. Female-specific carcinogens dominated among chemicals inducing leukemia and lymphoma. Interestingly, for some organ/tissues a high proportion of carcinogens gave gender-specific responses. For example, 78% (31% in males and 47% in females) of chemicals induced leukemia/lymphoma in one gender only. Also for lung carcinogens the majority (58%) induced tumors in one gender only (25% and 33% respectively). All gender-specific carcinogens (and their technical report numbers) are listed in supplementary Table 1. As expected, male-specific carcinogens causing tumors in the kidney were common (64%) whereas female-specific carcinogens causing tumors in the kidney were uncommon (8%). When chemicals with *equivocal* evidence were excluded a male dominance was still evident. However the isolated female dominance regarding pituitary tumors was lost. The difference in weighted averages was strengthened (Table 2).

Weighted averages for all listed organs affected by gender-specific carcinogens were 39% for males and 16% for females (Table 2). If mesotheliomas were excluded the weighted averages were 38% for males and 18% for females. Many kidney tumors and splenic leukemia have previously been discussed as rat-specific, and if these two tumor types were excluded the weighted averages were 35% for males and 18% for females. If all these three tumor types (mesothelioma, kidney and splenic leukemia) were excluded, the weighted averages were 32% for males and 19% for females. If also chemicals with *equivocal* evidence weighted averages were 28% for males and 14% for females.

Table 3 shows the number of chemicals in the NTP database inducing tumors in both genders, but statistically significantly more in one gender. Table 3 is based on results from 12 bioassays, but 3 of these were also included in Table 2 as they also exhibited gender-specific tumor sites. Only three organs were affected in this way, and again males were more

affected. The list of individual chemicals and technical report numbers are shown in supplementary Table 2.

Next we studied how often the 188 gender-specific carcinogens, analyzed in Table 2, induced tumors at other sites in the same gender. Table 4 shows that weighted average for both male-specific and female-specific carcinogens inducing additional tumors in the same gender was high, 54% and 59% respectively. We also analyzed whether the gender-specific carcinogens induced tumors in other non-reproductive organs in the opposite gender. As shown in Table 5, both gender/organ-specific carcinogens in males and females commonly induced tumors in other organs in the opposite gender. For skin-inducing carcinogens 11 of 14 induced additional tumors in females. The weighted average of male organ-specific carcinogens inducing other tumors in females was 45%, and the weighted average of female organ-specific carcinogens inducing other tumors in males was 66% (Table 5).

Male- and female-specific carcinogens may have different modes of action (MOA) and we investigated whether gender-specific chemicals (analyzed in Table 2, 4 and 5) could be classified as genotoxic or non-genotoxic. For this classification we used test results given by NTP and classified chemicals as genotoxic if the Salmonella assay was positive in at least one test (for more comprehensive results see supplementary Table 1). Weakly positive results according to NTP were classified as negative. Overall, genotoxicity was indicated for 38% (105) of 278 carcinogenic chemicals in the NTP database. Table 6 shows the fraction of gender-specific carcinogens that were classified as genotoxic. The weighted average for male-specific carcinogens was 43%, and for female-specific carcinogens the same value was 31%. These data indicate an overall difference in genotoxicity between male and female specific carcinogens. Ten (71%) of male-specific skin carcinogens were genotoxic and five (45%) of pancreatic carcinogens. Also among leukemia- and lymphoma-inducing male-specific carcinogens genotoxic chemicals dominated. Thus, 60% of male-specific chemicals were genotoxic while the corresponding value for female-specific carcinogens was 13%. However, the male dominance among genotoxic chemicals was not true for all tumor sites. For example, 63% (5/8) of female specific lung carcinogens were genotoxic, while 33% (2/6) of male specific lung carcinogens were genotoxic (Table 6). Together, these data indicate differences in genotoxicity between male- and female-specific carcinogens and that genotoxicity was more common among male- than among female-specific carcinogens. An exception was lung carcinogens.

Discussion

There are numerous publications showing gender differences in carcinogenicity, but the contribution of sex-specific susceptibility for carcinogens is to a large extent unknown. We find that out of 278 rat carcinogens in the NTP database 201 exhibit gender differences in at least one tumor site. 68 of these chemicals induced tumors only in males and 19 induced tumor in females only. The male sensitivity is further supported by the fact that male rats were 1.69 ($p < 0.001$) times more often classified to a higher level of evidence for carcinogenicity than female rats. We thus conclude that gender-specific responses to carcinogens are common, and that males are more often affected. In the site-specific analysis we found that mesothelium, pancreas, kidney and skin exhibited the highest frequencies of

male-specific tumors. The male dominance is quite strong, and to our knowledge this observation has not previously been discussed.

We do not have an overarching explanation for the male dominance, but our rat data are in line with conclusions in two recent human studies. The conclusions were that there might be i) “universal mechanisms that increase male susceptibility to cancer” (Cook *et al.*, 2009), or ii) “intrinsic factors” that e.g. “modify the susceptibility to extrinsic oncogenic factors” (Edgren *et al.*, 2012).

The intention with our study was to perform a statistical analysis of the NTP database in order to document gender differences in response to chemical carcinogens, and to get support for future mechanistic studies. Such future studies may also include a more detailed analysis of different tumor types at a single site. Here we will discuss our site-specific findings in connection to already published data.

Male-specific mesotheliomas originating in *tunica vaginalis* might be regarded as a reproductive organ-derived tumor and will not be comprehensively discussed here. However, it is of interest that mesotheliomas in pleura or peritoneum in humans are far more common among men than among women (Wolf *et al.*, 2010), and that it has been suggested that estrogen receptor (ER) β expression may confer resistance among women (Pinton *et al.*, 2010).

Male rats express a small protein, α 2u-globulin, in liver and plasma, and its expression is androgen dependent (Chatterjee *et al.*, 1989). It is excreted in primary urine and is absorbed in kidney tubular cells. Xenobiotics, including many NTP test chemicals, may bind α 2u-globulin and inhibit its degradation so that it accumulates in tubular cells and becomes toxic. The cytotoxicity may promote renal tubular tumor development (Hard *et al.*, 2009; Leavens and Borghoff, 2009), which may work in concert with a chronic progressive nephropathy (CPN), in male rats (Hard *et al.*, 2009). Both these modes of action have been discussed as non-relevant for humans (Hard *et al.*, 2009). Challenging is however that kidney cancer is more common among men (Cook *et al.*, 2009; Knox and Colli, 2010; Edgren *et al.*, 2012), and a significant proportion of renal cell carcinomas in humans express androgen receptor (AR) (Brown *et al.*, 1998; Langner *et al.*, 2004). Further MOA analysis that can ascertain that rat male kidney carcinogens do not cause cancer via other mechanisms than α 2u-globulin toxicity or CPN is thus warranted.

The male dominance regarding exocrine pancreatic cancer was discussed 20 years ago (Longnecker, 1990; Longnecker and Sumi, 1990), and our data confirm and extend those earlier observations. Rats (and mice) develop an acinar type of exocrine tumor, whereas humans usually develop a ductal type, and this phenotypic difference has raised questions about the relevance for humans of pancreatic carcinogenesis in rodents (Maronpot *et al.*, 2004). However, pancreatic cells exhibit great plasticity (Puri and Hebrok, 2010) and uncertainty still remains regarding cells of origin for ductal cancer (Kong *et al.*, 2011). Early work shows that caerulein promotes cancer development in rats by inducing growth of azaserine-induced acinar cell nodules (Lhoste and Longnecker, 1987), whereas caerulein-induced chronic pancreatitis leads to ductal carcinoma in mice harboring mutated K-ras in

acinar or centroacinar cells (Guerra *et al.*, 2007). These data suggest that long-term treatments resulting in pancreatitis may promote ductal cancer development provided key oncogenic mutations are expressed in progenitor cells. Xenobiotic-induced ductal carcinomas in rats have also been reported (Rivera *et al.*, 1997), and it can be suggested that the induction of acinar carcinomas in the NTP tests reflect critical carcinogenic stimuli that may result in either acinar or ductal carcinomas depending on whether cells of origin harbor e.g. K-ras mutations. Further studies are thus needed to understand the relevance, if any, of acinar rat pancreatic tumors for humans. It should also be noted that pancreatic cancer is more common among males (Cook *et al.*, 2009; Edgren *et al.*, 2012), and that young men (< 60 years of age) are 1.6 times more likely to develop this neoplasm than young women (Zhou *et al.*, 2010). This difference might be explained by e.g. differences in alcohol consumption, but AR signaling has been reported in pancreatic cancer (Okitsu *et al.*, 2010). Furthermore, there are epidemiological studies supporting a male-specific susceptibility to e.g. crystalline silica dust (Birk *et al.*, 2009) and to red meat (Larsson and Wolk, 2012).

We also evaluated data on genotoxicity in the NTP database. Genotoxicity was 1.4 times more common among the male-specific carcinogens than among the female-specific carcinogens. Considering e.g. the high numbers of kidney carcinogens in male rats, and the fact that they often are non-genotoxic, this figure is surprisingly high. A site-specific analysis is hampered by low numbers in many cases but it is interesting to note that 63% of female-specific lung carcinogens were genotoxic, whereas only 33% of male-specific lung carcinogens were genotoxic. This suggests a greater susceptibility among female rats for genotoxic lung carcinogens, and is in line with epidemiology suggesting that women are more susceptible to tobacco smoking (Kiyohara and Ohno, 2010). Further support is provided by biomonitoring data on susceptibility to tobacco mutagens among women (Kirsch-Volders *et al.*, 2010), and by lung epithelial cell studies suggesting that xenobiotic metabolism in lung cells is tuned to give more DNA adducts in women (Mollerup *et al.*, 2006; Uppstad *et al.*, 2011). It has also been observed that expression of high levels of ER α receptors is associated with poor prognosis in men or women with lung cancer (Olivo-Marston *et al.*, 2010). This observation may reflect estrogen influences on lung carcinogenesis, but may also reflect influences on cancer therapy.

Site-specific carcinogens in both males and females commonly induced tumors at other sites in both sexes. This suggests that the majority of site-specific effects was not a consequence of well-studied gender dimorphisms in xenobiotic metabolism (Oertelt-Prigione and Regitz-Zagrosek, 2009; Kirsch-Volders *et al.*, 2010), altering e.g. blood levels or elimination rates. A possible exception is urinary bladder tumors, which might be heavily influenced by excreted metabolites (Zenser *et al.*, 1998). This tumor type did not exhibit marked differences between females and males in the NTP assays, so toxicokinetic differences may have concealed an influence of AR expression as reported by (Miyamoto *et al.*, 2007).

As mentioned above, recent studies show that incidence rates often are higher for men than for women (Cook *et al.*, 2009; Edgren *et al.*, 2012). These human rates include causative or modulating factors supposedly not affecting NTP data, such as viruses, food and other factors that make them not directly comparable to our data. However, interesting is that Cook *et al.* found that an overall male-to-female incidence rate ratio (IRR) for cancers in

non-sex organs is 1.77 (Cook *et al.*, 2009), and that this ratio is strikingly similar to our overall male/female ratio of 1.69 for NTP evidence levels. It remains to be clarified whether this similarity is a sheer coincidence, or strengthens the notion that there are “universal mechanisms that increase male susceptibility to cancer” (Cook *et al.*, 2009).

There is ample literature support for an influence of hormones in rats and humans. It may e.g. involve of oncogenes and suppressor genes and their signaling pathways. Thus hormonal- and age-related decline in p53 function, occurring later in male than in female mice, has been reported (Feng *et al.*, 2007). If this also occurs in rats and humans, it may explain the overrepresentation of genotoxic lung carcinogens among female rats (Table 6) and the susceptibility to tobacco smoke among women (Kirsch-Volders *et al.*, 2010). Other influences may include cancer promoting effects of AR (Yu *et al.*, 2000; Yoon *et al.*, 2006; Ma *et al.*, 2008; Zender and Kubicka, 2008), upstream AR regulators (Tsuei *et al.*, 2011) or estrogen inhibition of IL-6 production (Naugler *et al.*, 2007) on hepatocellular carcinoma. Of further interest is that shorter CAG repeats in the AR gene associate with hepatocellular carcinoma (Yu *et al.*, 2000) and with pulmonary, esophageal or bladder cancer (Teng *et al.*, 2010), and that the expression of a transporter of hormones, including testosterone and estrogens, correlated to the differentiation of liver, colon and other cancer types (Pressler *et al.*, 2011). Yet another example is that estrogen may have a protective effect in human stomach cancer (Chandanos and Lagergren, 2008). These studies support the notion that sex hormones may have influenced not only human cancers in non-reproductive organs, but also our NTP data.

In summary, we show that gender-specific tumor responses in rats are common. We also show that male rats are more often affected than female rats by chemical carcinogens. The male dominance in rats is in line with human incidence rates being higher in males than in females. Thus, although some affected rat tumor sites are regarded as non-relevant to humans, and e.g. male rat kidney tumors have been explained in molecular terms, the male dominance should be further studied with the aim to understand this susceptibility among male rats and men. Literature data on sex hormone receptor expression in non-reproductive organs might provide a base for one of several possible explanations. Further experimental and epidemiological studies may disclose interactions between chemical carcinogen and hormones overlooked in the past.

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Abbreviations

NTP	National Toxicology Program
MOA	Mode of action
AR	Androgen receptor

ER Estrogen receptor**References**

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Table 1Gender differences in NTP rat cancer bioassays^a

Gender difference categories	Number of gender specific carcinogens		
	Male	Female	Total
	<i>Clear, Some, Equivocal evidence</i>	<i>Clear, Some, Equivocal evidence</i>	
Tumors in one sex only	68*	19	87
Tumors in both sexes, but organ-specific tumors in one sex ^b	62*	40	102
Tumors in the same organ in males and females, but with significant difference ^b	8	4	12
	<i>Clear, Some evidence</i>	<i>Clear, Some evidence</i>	
Tumors in one sex only	40*	7	47
Tumors in both sexes, but organ-specific tumors in one sex ^b	53*	26	78
Tumors in the same organ in males and females, but with significant difference ^b	8	4	12

^a 477 rat bioassays were analyzed (<http://ntp.niehs.nih.gov/>) and results are given for assays with *Clear, Some* and *Equivocal evidence* in the upper part of the table. In the lower part assays with *Equivocal evidence* for both sexes, or with *Equivocal evidence* for one sex and *No evidence* for the other sex were excluded).

^b three carcinogens were included in both these categories

* significantly greater than the opposite gender, p = 0.05.

Table 2

Target organs/tissue for gender-specific carcinogenic responses in NTP rat bioassays^a.

Target organ/tissue	Fraction and percentage of gender-specific carcinogens			
	Clear, Some, Equivocal ^b		Clear, Some ^c	
	Male ^d	Female ^d	Male ^d	Female ^d
Liver ^e	16/63 25%	8/63 13%	11/55 20%	6/55 11%
Thyroid	9/32 28%	5/32 16%	5/23 22%	3/23 13%
Kidney	42/66 64%*	5/66 8%	35/57 61%*	3/57 5%
Intestine	5/16 31%*	1/16 6%	4/14 29%	1/14 7%
Forestomach	3/21 14%	0/21 0%	3/20 15%*	0/20 0%
Leukemia, Lymphoma	10/32 31%	15/32 47%	9/23 39%	8/23 35%
Adrenal gland	12/31 39%	8/31 26%	7/20 35%	4/20 20%
Lung	6/24 25%	8/24 33%	4/21 19%	7/21 33%
Vascular system	5/10 50%*	2/10 20%	4/8 50%*	1/8 13%
Urinary bladder	4/21 19%	6/21 29%	4/18 22%	2/18 17%
Mesothelium	15/18 83%*	0/18 0%	13/15 87%*	0/15 0%
Brain	3/8 38%	2/8 25%	0/2 0%	0/2 0%
Nasal cavity	4/15 27%*	0/15 0%	3/13 23%*	0/13 0%
Spleen ^f	4/8 50%	1/8 13%	3/7 43%	1/7 14%
Pancreas	11/14 79%*	0/14 0%	6/9 67%*	0/9 0%
Pituitary	1/5 20%	4/5 80%*	0/1 0%	0/1 0%
Skin	14/24 58%*	2/24 8%	10/20 50%*	2/20 10%
Subcutaneous tissue ^g	4/8 50%*	1/8 13%	4/7 57%*	0/7 0%
Oral cavity	2/15 13%	2/15 13%	2/15 13%	2/15 13%
Ureter	1/2 50%	0/2 0%	1/2 50%	0/2 0%
Weighted average	39%	16%	37%	12%

^a 477 rat bioassays were analyzed in the NTP database (<http://ntp.niehs.nih.gov/>).

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^b assays with *Clear*, *Some* and *Equivocal evidence*.

^c assays with *Clear* and *Some evidence* (see Table 1).

^d the first column gives the fraction of gender-specific chemicals per total number of chemicals inducing tumors at respective target site, the second column gives the % of gender-specific chemicals.

^e mainly hepatocellular tumors

^f designated as sarcomas

^g epithelial tumors

* significantly greater than the opposite gender, p = 0.05.

Table 3

Number of chemicals in NTP rat bioassays^a inducing tumors in both genders, but statistically significantly more in one gender^b.

Fraction and percentage ^c of gender specific carcinogens				
Target organ/tissue	Male		Female	
Liver	4/63	6%	3/63	5%
Lung	3/24	13%	1/24	4%
Adrenal gland	1/31	3%	–	–

^a statistically significant difference between genders in respective organ.

^b 477 rat bioassays were analyzed (<http://ntp.niehs.nih.gov/>).

^c the first column gives the fraction of chemicals giving more tumors in respective gender/total number of chemicals inducing tumors at the same site. The second column gives the %.

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Table 4Gender-specific rat carcinogens inducing tumors in other organs in the same gender^a.

TARGET ORGAN/TISSUE	Male-specific carcinogens inducing tumors in other organs in males ^b	Female-specific carcinogens inducing tumors in other organs in females ^b
Liver	9/16 (56%)	6/8 (75%)
Thyroid	4/9 (44%)	2/5 (40%)
Kidney	17/42 (40%)	2/5 (40%)
Intestine	3/5 (60%)	1/1 (100%)
Forestomach	2/3 (67%)	0/0
Leukemia,Lymphoma	7/10 (70%)	7/15 (47%)
Adrenal gland	4/12 (30%)	6/8 (75%)
Lung	5/6 (83%)	7/8 (87%)
Vascular system	3/5 (60%)	1/2 (50%)
Urinary bladder	2/4 (50%)	4/6 (67%)
Mesothelium	12/15 (67%)	0/0
Brain	3/3 (100%)	1/2 (50%)
Nasal cavity	2/4 (50%)	0/0
Spleen	1/4 (25%)	0/1 (0%)
Pancreas	7/11 (64%)	0/0
Pituitary	0/1 (0%)	1/4 (25%)
Skin	9/14 (64%)	1/2 (50%)
Subcutaneous tissue	3/4 (75%)	0/1
Oral cavity	1/2 (50%)	2/2 (100%)
Ureter	1/1 (100%)	0/0
Weighted average	55%	59%

^a gender-specific target site responses (<http://ntp.niehs.nih.gov/>) were analyzed for additional targets in the same gender (and the same bioassay).

^b first column gives the fraction of gender-specific carcinogens (see Table 1) inducing tumors at additional sites in the same gender per total number of gender-specific targets (%). The second column gives the % carcinogens inducing tumors at additional sites.

Table 5Gender-specific rat carcinogens inducing tumors in other organs in the other gender^a.

Target organ/tissue	Male-specific carcinogens inducing tumors in females ^b		Female-specific carcinogens inducing tumors in males	
Liver	7/16	44%	7/8	88%
Thyroid	2/9	22%	2/5	40%
Kidney	14/42	33%	1/5	20%
Intestine	3/5	60%	1/1	100%
Forestomach	1/3	33%	0/0	–
Leukemia,Lymphoma	4/10	40%	9/15	60%
Adrenal gland	4/12	33%	5/8	63%
Lung	5/6	83%	6/8	75%
Vascular system	4/5	80%	1/2	50%
Urinary bladder	2/4	50%	4/6	67%
Mesothelium	11/15	73%	0/0	–
Brain	1/3	33%	2/2	100%
Nasal cavity	0/4	0%	0/0	–
Spleen	1/4	25%	1/1	100%
Pancreas	3/11	27%	0/0	–
Pituitary	0/1	0%	3/4	75%
Skin	11/14	79%	1/2	50%
Subcutaneous tissue	3/4	75%	1/1	100%
Oral cavity	1/2	50%	2/2	100%
Ureter	1/1	100%	0/0	–
Weighted average		46%		66%

^a Gender-specific target site responses (<http://ntp.niehs.nih.gov/>) were analyzed for additional targets in the same gender and the same bioassay.

^b first column the fraction of gender-specific carcinogens (see Table 1) inducing tumors at additional sites in the other gender per total number of gender-specific targets. The second column gives the % carcinogens inducing tumors at additional sites.

Table 6Genotoxicity^a among rat carcinogens inducing gender-specific tumors^b.

Fraction and percentage of genotoxic and gender-specific carcinogens ^c				
Target organ/tissue	Male		Female	
Liver	8/16	50%	3/8	38%
Thyroid	4/9	44%	2/5	40%
Kidney	8/42	19%	1/5	20%
Intestine	3/5	60%	1/1	100%
Forestomach	3/3	100%	0/0	–
Leukemia,Lymphoma	6/10	60%	2/15	13%
Adrenal gland	3/12	25%	1/8	13%
Lung	2/6	33%	5/8	63%
Vascular system	4/5	80%	0/2	0%
Urinary bladder	1/4	25%	2/6	33%
Mesothelium	8/15	53%	0/0	–
Brain	0/3	0%	2/2	100%
Nasal cavity	2/4	50%	0/0	–
Spleen	1/4	25%	0/1	0%
Pancreas	5/11	45%	0/0	–
Pituitary	1/1	100%	0/4	0%
Skin	10/14	71%	1/2	50%
Subcutaneous tissue	4/4	100%	0/1	0%
Oral cavity	0/2	0%	2/2	100%
Ureter	0/1	0%	0/0	–
Weighted average		43%		31%

^a all positive results except “weakly positive” results given by NTP (<http://ntp.niehs.nih.gov/>) generated a positive classification.

^b carcinogens inducing gender-specific carcinogenic responses were analyzed.

^c number and fraction of gender-specific responses induced by genotoxic chemicals/total number of responses with gender-specific tumors (see Table 1).