Published in final edited form as:

Toxicol Pathol. 2008 ; 36(3): 388–396. doi:10.1177/0192623308315829.

SPONTANEOUS OCCURRENCE OF A DISTINCTIVE RENAL TUBULE TUMOR PHENOTYPE IN RAT CARCINOGENICITY STUDIES CONDUCTED BY THE NATIONAL TOXICOLOGY PROGRAM (NTP)

Gordon C Hard^a, John Curtis Seely^b, Grace E Kissling^c, and Laura J Betz^d

^aPrivate Consultant, Tairua, New Zealand ^bExperimental Pathology Laboratories Inc, P.O. Box 12766, Research Triangle Park, NC 27709 ^cNational Institutes of Environmental Health Sciences, NIH, Research Triangle Park, NC ^dConstella Health Sciences, 2605 Meridian Parkway, Durham, NC 27713

Abstract

The Toxicology Data Management System (TDMS) of the National Toxicology Program, NIEHS, NIH, was surveyed for occurrence and distribution of a distinctive renal tubule tumor type in rats. The hallmark features of this tumor included eosinophilic/amphophilic staining, large finely granular cells, and numerous vacuoles and/or minilumens. It is referred to here as the amphophilic-vacuolar (AV) variant of renal tubule tumor. Of 154 studies in which renal tubule tumors had been recorded in the standard single sections of kidney in the TDMS, there were collectively 1012 rats with renal adenomas, carcinomas or adenocarcinomas, and of these, 100 displayed the distinctive AV morphology, representing 74 studies involving mostly the F344 rat, but also the Sprague-Dawley and Wistar strains. The AV tumors (mainly adenomas but also some carcinomas) occurred usually as solitary lesions in the affected animals. However, they were multiple and bilateral in a few cases. They were equally distributed between the sexes, did not metastasize (at least to the lung), and were not associated with chronic progressive nephropathy. The distribution of this renal tumor type was random across studies and dose groups, underscoring the likelihood that it was of spontaneous origin and not chemically induced. Accordingly, it is suggested that this distinctive renal tumor phenotype be recorded as a separate category from conventional RTT when assessing the carcinogenic potential of a test compound.

Keywords

Rat; renal tubule tumors; spontaneous occurrence; amphophilic-vacuolar renal tumors; familial renal tumors

INTRODUCTION

Based on the occurrence of a specific morphologic phenotype of rat renal tubule tumor (RTT) in 90-day studies (Hard et al, 1994), as well as in one group of F344 littermates (Thurman et

Corresponding author: gordonhard@msn.com (G.C.Hard).

The opinions expressed in this paper are solely those of the authors and should not be construed as necessarily reflecting the policies or opinions of the National Toxicology Program.

al, 1995), and in the Eker rat familial renal cancer syndrome (Everitt et al, 1992), one of the authors (GCH) has posited that an eosinophilic/amphophilic variant of renal tubule tumors (RTT) encountered occasionally in carcinogenicity studies using conventional strains of rat are of spontaneous, possibly familial, origin. If spontaneous origin can be verified by supporting evidence, tumors of this specific phenotype might be regarded as a separate entity from conventional RTT when assessing the renal carcinogenic potential of test chemicals in 2-year cancer bioassays. Briefly, the morphology of this tumor type is characterized by a uniform, lobular architecture composed of large, round to polyhedral cells characterized by eosinophilic to amphophilic staining, and frequent small and large, circular vacuoles and/or minilumens. For convenience in this report, this distinctive RTT is referred to as the *amphophilic-vacuolar* (AV) tumor variant, selecting two key morphologic features that discriminate it from the predominant rat renal tumors, which characteristically have basophilic (or rarely, clear cell) staining properties.

The Archives of the National Toxicology Program (NTP) represents a vast resource of histological tissue specimens from many hundreds of long-term carcinogenicity and subchronic toxicity rodent studies. The archive was accessed to conduct a survey on the occurrence and histological characteristics of the AV phenotype of RTT. In particular, the distribution of this tumor type between dose-groups within NTP 2-year studies was analyzed to determine whether this variant was of spontaneous origin or linked to test compound treatment.

MATERIALS AND METHODS

Survey plan

In the database of the NTP, chemicals are systematically assigned a number and are logged under either the Carcinogenesis Bioassay Data System (CBDS), representing studies reported from 1971 to 1982, or the Toxicology Data Management System (TDMS), containing studies reported from 1982 to the present time. Currently, the TDMS represents 2-year bioassays that were commenced in 1980, and up to 2002. Since 1982 there have been 211 rat carcinogenicity studies reported, of which 194 were performed in the F344 rat. The TDMS was searched for studies up to July 31, 2007, in which the following terms had been diagnosed: tissue *kidney*; site *renal tubule* or *not specified*; morphology *adenoma*, *carcinoma* or *adenocarcinoma*. Each one of the kidney lesions that had been diagnosed under one of these terms by NTP in the studies reported from 1982 to 2007, was re-examined microscopically and classified into one of four morphologic categories: 1) renal tubule adenomas or carcinomas of basophilic or rarely, clear cell type (including oncocytoma), 2) the specific type of AV adenoma or carcinoma that was the subject of this survey, 3) other renal tumor types (nephroblastoma and transitional cell tumor), or 4) a metastasis not of primary renal origin. In all TDMS cases, the kidney sections examined were the standard single sections stained with hematoxylin and eosin (H & E). In order to provide additional information on the extent of occurrence of the AV tumor within studies, kidney step-sections for 6 of these chemicals were included in the histological evaluation. However, any tumors found were not included in the numerical analysis. In addition to classifying the RTT, each tumor-bearing rat of interest was scored for severity of spontaneous, age-related chronic progressive nephropathy (CPN) using a semi-quantitative system with grades ranging from 0 to 8. As described previously (Hard and Khan, 2004; Hard et al, 2007), grade 1 represented a minimal number of the earliest lesions, and grade 8, end-stage kidney signaling imminent death from chronic renal failure.

Statistical methods

As implied above, only the information generated from the standard single sections of kidney from animals identified in the TDMS database was used for statistical analysis. The Fisher's

exact tests and Cochran-Armitage trend tests were used to assess the relationship between dose of chemical/agent administered and the occurrence of AV tumors, using SAS software (SAS, 2003). Fisher's exact test is used to test the association between two categorical variables, such as diet and AV tumor occurrence. It is an alternative to the Chi-square test that is useful when one or more expected frequencies are low. The Cochran-Armitage trend test is used for a dose-related trend in the occurrence of AV tumors, that is, to determine whether the frequency of tumors increases (or decreases) with dose.

In addition to analyzing each test agent separately, studies having 4 dose groups (control, low, medium, and high) were combined and analyzed, studies having 3 dose groups (control, low, and high) were combined and analyzed, and all studies were split into two categories, control versus all treatment groups combined, and analyzed.

For control group animals only, the Fisher's exact test was used to determine if diet (NIH-07 and NTP-2000), route of exposure, or vendor source of animals were associated with the occurrence of AV tumors. The various analyses were done separately for males and females. In all tests a p-value of 0.05 was considered the limit for significance.

RESULTS

Tumor morphology

The specific renal tubule tumor type surveyed in this report was identified by characteristic morphologic features visualized in H&E-stained sections. These included: a circumscribed, non-encapsulated mass (Figures 1–2) uniformly organized into well-delineated lobules separated by a delicate network of fibrovascular stroma containing lymphoid cell infiltrates (Figure 3); lobules with central degeneration and necrosis (Figure 1); characteristically large tumor cells of rounded to polyhedral shape, with slightly eosinophilic to amphophilic staining (Figure 4); cytoplasm with finely granular or foamy texture (Figures 4–5) depending on fixation quality; prominent large circular vacuoles or spaces conferring a “moth-eaten” appearance to the tumor (Figures 1–5); and vesicular nuclei with dispersed chromatin or very enlarged central nucleolus (Figures 4–5). The enlarged, single nucleoli were often “inclusion-like” in appearance. Mitotic figures were sometimes present, but sporadic. An occasional circular space contained an apoptotic cell (Figure 2) and therefore appeared to be formed as a result of single cell degeneration. Other large circular spaces within the lobules were lined by several cells and were interpreted as minilumen formation (Figure 5). In addition, the cytoplasm of some tumor cells contained multiple, small vacuoles that could not be distinguished in the H&E-stained tissue from lipid droplets (Figures 4–5). Occasionally, the distinctive morphology of these neoplasms was compromised by tissue fixation and/or staining artifacts.

The zonal location of the lesions ranged from the outer cortex to the inner stripe of outer medulla. Typically, however, AV adenomas ran axially from a subcapsular location, where they bulged slightly from the kidney surface, through the cortex into the outer stripe of outer medulla in a wedge- or pear-shaped form (Figure 6). Often, the extension of a cortical tumor towards the medulla appeared as though following the linear course of a tubule. However, the tumors were well delineated, and appeared to grow by expansion and not by invasion, often with some compression of the adjacent parenchyma.

There were some variations on this pattern. For example, small lesions tended to lack degenerative areas, and in a few early foci, there was an association with dilated or cystic tubule profiles. In these cases, solid islands of the AV tumor were interposed between the dilated tubule profiles, or attached around the periphery of a dilated tubule (Figure 7). There were also some lesions that had been diagnosed as carcinomas based on large size and numerous areas of degeneration. The diameter of these lesions usually exceeded 1 cm, and was sometimes

greater than 2 cm. In a few cases, the larger lesions showed a transition from the typical AV morphology to solid, more conventional basophilic tumor epithelium (Figures 8 and 9). The lung tissue from all rats with carcinoma-like AV tumors was examined, but there were no metastatic deposits in this organ.

There were multiple AV neoplasms and foci of AV atypical tubule hyperplasia (ATH) in 5 female rats. These involved 3 F344 females (benzophenone high-dose group, cobalt sulfate heptahydrate control group, and 3,4-dihydrocoumarin low-dose group) and 2 Sprague-Dawley females (TEF evaluation of dioxin mixture vehicle control group, and TEF evaluation of PCB 153 high-dose group). In the benzophenone and PCB 153 examples, the multiplicity of foci included basophilic hyperplastic lesions, which were extremely numerous (Figure 10) in both kidneys.

AV neoplasms were found in kidneys in which the severity of CPN ranged from grade 2 (mild) to grade 8 (end-stage). Except in kidneys with advanced grades of CPN (grades 6 to 8), there was no apparent physical connection of the lesions with CPN-affected tissue.

Survey results

Some aspects of the data related to the distribution of the AV tumor type in the TDMS series of 2-year rat studies conducted by NTP are summarized in Table 1. In the period 1982 to July 31, 2007, the NTP database recorded 211 chronic studies (duration longer than 1 year) conducted in rats, involving over 90,000 animals (NTP, 2007). The F344 strain comprised 92% of the rats used in these studies, Sprague-Dawley, 7%, and the remainder included Wistar and Osborne-Mendel rats. The terms *kidney*, *adenoma*, *carcinoma*, *adenocarcinoma* or *not specified* were identified in 154 of the TDMS chronic studies. Altogether, the kidneys of 1012 rats with renal tumors were evaluated, and 150 studies were confirmed as having one or more RTT (excluding studies with either a single nephroblastoma or transitional cell tumor, and the cases considered as being not of primary renal origin). In the 150 studies, *adenoma* represented 78% of the recorded diagnoses, *carcinoma* 21% and *adenocarcinoma* less than 1%. *Adenocarcinoma* was an older term used in this database, and, for the rat kidney, considered to be synonymous with *carcinoma*. Just over one-third of the diagnoses of *carcinoma* were associated with 3 chemicals generally accepted as being genuine rat renal carcinogens, namely decalin, fumonisin B1, and ochratoxin A. In the majority of the 150 studies, the number of animals with RTT was small and not dose-related, and most of the test chemicals had not been identified by NTP as renal carcinogens.

Almost all of the RTT in the 150 studies were of the conventional basophilic type, but the specific AV tumor was observed in 74 of these studies in low numbers (listed in Table 2). In 57 studies, the AV tumor occurred in a single animal; in 12 studies, 2 animals; in 3 studies, 3 animals; and in 2 studies, 5 animals (the benzophenone and butyl benzyl phthalate bioassays). Altogether, across studies and dose groups, the AV lesion was diagnosed in 100 rats (Table 2). Although the vast majority of these lesions conformed to adenomas, there were also 23 rats with lesions that were judged, mainly on the basis of size and extent of degeneration, to warrant the diagnosis of carcinoma, in accordance with the NTP diagnosis. The sex distribution of the AV tumor-bearing rats was 47 males and 53 females, that is, 35 males and 39 females for adenomas, and 12 males and 14 females for carcinomas. The spontaneous nature of the AV tumor was underscored by its distribution among studies. For example, there were 35 studies in which only a single animal with an RTT had been diagnosed by NTP; 26% of these tumors were of the AV phenotype. There were 33 studies with 2 RTT, and 18 studies with 3 RTT. At least one of the tumors was of the AV phenotype in 46% and 67% of these studies, respectively. Furthermore, there were 130 control rats across the 74 studies possessing an RTT. Of these control rat tumors, which presumably were of spontaneous origin, 24 (18.5%) were of the unique AV type (Table 1).

The AV phenotype was encountered in studies commenced in December 1980 through to 2002, in animals from at least 4 different breeding sources, and in 3 strains of rat, predominantly the F344, but also in the Sprague-Dawley, and Wistar strains.

Statistical analysis

No statistically significant differences were found between dose groups and the occurrence of AV tumors for any of the test agents, when analyzed separately (all p values for both males and females were > 0.15). No significant differences between control and dosed groups were found when combining studies that had 4 dose groups (males $p = 0.23$, females $p = 0.21$), 3 dose groups (males $p = 0.59$, females $p = 0.10$), or when combining all test agents into 2 categories of control versus all dosed groups (males $p = 0.11$, females $p = 0.10$).

No statistically significant associations were evident in control group animals between the occurrence of the AV tumor and diet ($p > 0.99$ for both males and females), route of exposure (males $p = 0.32$, females $p = 0.32$), or vendor source of animals (males $p = 0.35$, females $p = 0.07$).

Step-sectioned studies

In 6 of the studies, step-section diagnoses of RTT from surveys previously conducted by two of the authors (GCH and JCS) were included as an extended check for the AV tumor type. The chemicals involved were benzophenone, coumarin, ethyl benzene, oxazepam, primidone and quercetin. Except for primidone, which had none of the tumors of interest in either the standard single or step sections of kidney, the number of AV tumors was increased by step-section analysis in the remaining 5 selected studies. One additional AV tumor was found for coumarin (making a total of 2 for this study) and benzophenone (total 6), and 3 for ethyl benzene (total 4) and quercetin (total 5). In the case of oxazepam, none had been observed in the standard single sections, but 3 were found in the step-sections.

DISCUSSION

This survey of renal tumors in all NTP 2-year rat carcinogenicity bioassays reported since early 1982 up to mid 2007, demonstrates the sporadic occurrence of a distinctive morphological phenotype of RTT that appears to occur independently of test agent exposure. The tumor type can be easily recognized in H&E-stained sections with the hallmark features of uniform lobular organization often with central degeneration/necrosis, eosinophilic to amphophilic staining, large finely granular/foamy cells, and the presence of numerous rounded vacuoles or minilumens conferring a moth-eaten appearance to the lesion. Most of the tumors occurred in the cortex, and extended towards the medulla as though following the course of a tubule. However, an origin from proximal or distal tubule could not be ascertained with any certainty. The random distribution between studies and groups suggests that it is of spontaneous origin, and the remarkable multiplicity of these and associated lesions (see Figure 10) in several animals adds further support to the possibility that a genetic abnormality may be involved in their initiation. The studies surveyed represented a pool of over 90,000 rats, the vast majority being of the F344 strain. However, in this survey, the lesion was also observed in one NTP study using Wistar rats (pyridine) and two with Sprague-Dawley rats (dioxin mixture and PCB 153). In addition, the personal experience of two of the authors (GCH and JCS), who have observed a number of examples of the phenotype in Wistar and Sprague-Dawley rats used in industrial toxicology studies, indicates that this distinctive renal neoplasm is not exclusive to the F344 rat. Considering the approximate total of 90,000 rats covered by the survey and the 100 tumors identified, the incidence of this phenotype is around 0.1%. Thus, the tumor is a rare lesion, but as shown in Table 2, it is encountered in about one-third of NTP 2-year carcinogenicity studies.

The AV tumor morphology was very different from the RTT induced by chemicals considered to be *bona fide* rat renal carcinogens in the NTP TDMS database, such as anthraquinone, 1-amino-2,4-dibromo-anthraquinone, decalin, fumonisin B1, ochratoxin A, 1,2,3-trichloropropane, and tris(2-chloroethyl) phosphate, which were basophilic (or rarely clear cell) lesions and not amphophilic or vacuolar. It is also remarkable that, of the studies with the above renal carcinogens, the AV phenotype was observed in only a few of the bioassays. Notably, among a total of 68 RTT in the bioassay of ochratoxin A, the only AV tumor present occurred in the only control animal in the study (an F344 male). The rare occurrence among the chemically-induced RTT underscores further the lack of relationship with chemical exposure. In the authors' (GCH and JCS) long experience with chemical induction of renal carcinogenesis, including numerous RTT resulting from exposure to the genotoxic carcinogen, N-nitrosodimethylamine (Hard, 1984; 1987), the AV phenotype of RTT has not been chemically induced. It also appears to have no strict morphological counterpart in humans (Storkel and van den Berg, 1995).

To date, two familial RTT syndromes have been described in the laboratory rat, in the Long-Evans derived Eker rat (Eker and Mossige, 1964; Everitt et al, 1995), and in the Sprague-Dawley from Japan, termed the Nihon rat, with the Birt-Hogg-Dubé (*Bhd*) syndrome (Hino et al, 1991; Okimoto et al, 2000; Kouchi et al, 2006). The Eker rat is a model of dominantly inherited renal cancer, which has been shown to be associated with a germline insertional mutation in the tuberous sclerosis complex 2 gene (*Tsc-2*), regarded as a tumor suppressor gene (Yeung et al, 1994; Kobayashi et al, 1995; Hino et al, 2003). The tumors are bilateral and multicentric, and develop from preneoplastic lesions at age 2 months into RTT in 100% of heterozygotes by one year (Everitt et al, 1995; Hino et al, 1999). Morphologically, the lesions consist of both basophilic and eosinophilic types, the latter having large cells and a vacuolar appearance (Hard et al 1994). In contrast, the Nihon rat has a germline mutation in the *Bhd* gene, and heterozygotes develop preneoplastic lesions by 3 weeks of age, and adenomas by 2 months (Kouchi et al, 2006). These tumors consist predominantly of clear cells, but can be admixed with eosinophilic cells, and sometimes appear as papillary projections into cystic tubules (Kouchi et al, 2006; Okimoto et al, 2000). In addition, occasional cases of similar morphology to the tumor surveyed in this present report have been described by others. Thurman et al (1995) reported two F344 rats with tumors of this phenotype, which were shown by identity records to be littermates. The tumors reported by Hard et al (1994) and Savard et al (2005) in 90-day studies with Sprague-Dawley or F344 rats had the same morphology. More recently, Lanzoni et al (2007) described tumors of similar type in toxicity studies of short duration with Sprague-Dawley rats ranging from only 12 to 18 weeks of age. The lesions included atypical tubule hyperplasia, adenoma and carcinoma, and some of the illustrations showed tumors similar to the phenotype described in this paper. On the other hand, spontaneous renal tubule hyperplasias and tumors in three Sprague-Dawley rats from a 90-day toxicity study reported by Hall et al (2007), were histologically and molecularly suggestive of the Birt-Hogg-Dubé syndrome. Based on morphology alone therefore, it appears that the tumors described here are not similar to those of the Nihon rat, i.e. rat tumors associated with the Birt-Hogg-Dubé syndrome. However, the AV tumor variant appears very similar to one of the two morphological phenotypes of the Eker rat (Hard et al, 1994)). The occasional transition of the amphophilic to a basophilic component in large carcinomas or kidneys with a multiplicity of lesions, underscores the similarity between the spontaneous neoplasm described here and the Eker rat tumor.

Although the predominant number of rats with AV tumors per NTP study recorded in the TDMS was only one, when the evaluation was extended to step-sections in the few cases followed up, the incidence of this lesion per study increased up to 6 animals, as in the benzophenone study. As mentioned above, the report of Thurman et al (1995) describing 2 tumors of the same AV morphology in a study with Fischer 344 rats, traced the lesions back

to littermates. It could be speculated that the presence of the tumor in up to 6 rats in the NTP studies surveyed here, is compatible with the lesion being expressed in siblings of a single litter within each affected study, possibly as a result of a germ-line mutation.

Most previous reports of this specific RTT phenotype have been in association with their occurrence in 90-day toxicity studies (Hard et al, 1994; Savard et al 2005), or at even earlier time-points (Lanzoni et al, 2007). Ours is the first study to attempt to document the distribution of this unique renal tumor in a very large database of 2-year studies. The information from our survey of the NTP bioassay reports, which date back to 1982, suggests that, if the phenotype does represent a genetic abnormality, it has been in the gene pool of at least the F344 rat for some decades. As the NTP rats had been obtained over the years from various rodent suppliers, it appears not to be associated with one specific breeding source.

There are additional features apart from the distinctive morphology that distinguish this tumor type from conventional basophilic RTT. Across carcinogenicity studies, there is a preponderance of basophilic tumors in male rats compared to females (Lock and Hard, 2004). In contrast, the frequency of the AV tumor was equal between males and females in this survey. Basophilic adenomas, particularly incipient ones, can show an association with advanced stages of severity of CPN in situations where CPN has been exacerbated by chemical exposure (Hard, 2002; Hard and Khan, 2004; Hard and Seely, 2005; Hard et al, 2007). In this survey there was no relationship of the specific tumor type to the severity grade of CPN. In cases of chemically-induced carcinogenesis, such as with N-nitrosodimethylamine (Hard 1984, 1986), fumonisin B1 (Hard et al, 2001), and ochratoxin A (Hard, 2000), basophilic carcinomas have a significant tendency to metastasize to the lungs when tumor dimensions approximate 2 cm or more. Although some of the AV tumors in this survey were considered to be carcinomas, some larger than 2 cm diameter, secondary deposits were not found when the lungs were examined. Thus, like the familial RTT of the Eker and Nihon rats, metastasis does not appear to be part of the biological behavior of the AV tumor. A further aspect of difference is that this neoplasm can sometimes occur at a much earlier age than conventional, basophilic RTT, as discussed above. The perceived differences between the AV tumor and conventional basophilic RTT are summarized in Table 3.

In conclusion, the random distribution of the AV renal tumor type in the NTP carcinogenicity studies recorded since 1981, the equal male to female ratio, and the significant involvement of studies with a very low incidence of renal tubule tumors (1–3 tumors only) as well as untreated control rats, all suggest that the lesion is of spontaneous origin. Accordingly, the results support the suggestion that this easily recognizable lesion might be recorded as a separate category from conventional (basophilic) RTT when assessing the numbers of compound-associated tumors in 2-year carcinogenicity bioassays.

Abbreviations

ATH	atypical tubule hyperplasia
AV	amphophilic-vacuolar
CPN	chronic progressive nephropathy
H & E	hematoxylin and eosin
RTT	renal tubule tumor(s)
TEF	Toxic Equivalency Factor
TDMS	Toxicology Data Management System

Acknowledgments

This work was supported by Federal funds from the National Institute of Environmental Health Sciences (NIEHS), NIH, under contract NO1-ES-95435 to Experimental Pathology Laboratories (EPL) Inc, Research Triangle Park, NC, and, in part, by the Intramural Research Program of NIEHS, NIH. The authors are grateful to Drs. J. Hardisty and M. Hamlin of EPL, Research Triangle Park, NC for their encouragement in this project. The authors are also indebted to Keith Connelly and Errol Parker of EPL Inc, who were responsible for accessing the slides from the Archives. Without their contribution, the survey would not have been achievable. In addition, we acknowledge Maureen Puccini and Emily Singletary of EPL Inc for assistance with photography.

REFERENCES

- Eker R, Mossige J. A dominant gene for renal adenomas in the rat. *Nature (London)* 1961;189:858–859. [PubMed: 13780066]
- Everitt JI, Goldsworthy TL, Wolf DC, Walker CL. Hereditary renal cell carcinoma in the Eker rat: a rodent familial cancer syndrome. *J Urol* 1992;148:1932–1936. [PubMed: 1433648]
- Hall WC, Elder B, Walker CL, Cai S, Peters DG, Goodman DG, Ulland BM, Borzelleca JF. Spontaneous renal tubular hyperplastic and neoplastic lesions in three Sprague-Dawley rats from a 90-day toxicity study. *Toxicol Pathol* 2007;35:233–241. [PubMed: 17366317]
- Hard GC. High frequency single-dose model of renal adenoma/carcinoma induction using dimethylnitrosamine in Crl:(W)BR rats. *Carcinogenesis* 1984;5:1047–1050. [PubMed: 6744513]
- Hard GC. Experimental models for the sequential analysis of chemically-induced renal carcinogenesis. *Toxicol Pathol* 1986;14:112–122. [PubMed: 3012758]
- Hard, GC. Chemically induced epithelial tumours and carcinogenesis of the renal parenchyma. In: Bach, PH.; Lock, EA., editors. *Nephrotoxicity in the Experimental and Clinical Situation*. Dordrecht: Martinus Nijhoff Publishers; 1987. p. 211–250.
- Hard, GC. Histopathologic Evaluation of Rat Kidney from Toxicity and Carcinogenicity Studies with Ochratoxin A. Washington DC, USA: Expert report submitted to JECFA by the International Life Sciences Institute; 2000.
- Hard GC. Significance of the renal effects of ethyl benzene in rodents for assessing human carcinogenic risk. *Toxicol. Sci* 2002;69:30–41. [PubMed: 12215658]
- Hard GC, Howard PC, Kovatch RM, Bucci TJ. Rat kidney pathology induced by chronic exposure to fumonisin B1 includes rare variants of renal tubule tumor. *Toxicol Pathol* 2001;29:379–386. [PubMed: 11442024]
- Hard GC, Long PH, Crissman JW, Everitt JI, Yano BL, Bertram TA. Atypical tubule hyperplasia and renal tubule tumors in conventional rats on 90-day toxicity studies. *Toxicol Pathol* 1994;22:489–496. [PubMed: 7899777]
- Hard GC, Khan KN. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *Toxicol. Pathol* 2004;32:171–180. [PubMed: 15200155]
- Hard GC, Seely JC. Recommendations for the interpretation of renal tubule proliferative lesions occurring in rat kidneys with advanced chronic progressive nephropathy (CPN). *Toxicol. Pathol* 2005;33:641–649. [PubMed: 16207638]
- Hard GC, Seely JC, Betz LJ, Hayashi SM. Re-evaluation of the kidney tumors and renal histopathology occurring in a 2-year rat carcinogenicity bioassay of quercetin. *Food Chem. Toxicol* 2007;45:600–608. [PubMed: 17156907]
- Hino O, Okimoto K, Kouchi M, Sakurai J. A novel renal carcinoma predisposing gene in the Nihon rat maps on chromosome 10. *Jpn J cancer Res* 1991;92:1147–1149. [PubMed: 11714437]
- Hino O, Fukuda T, Satake N, Kobayashi T, Honda S, Orimoto K, Yamashita Y, Kikuchi Y. TSC2 gene mutant (Eker) rat model of a Mendelian dominantly inherited cancer. *Prog Exp Tumor Res* 1999;35:95–108. [PubMed: 10377754]
- Hino O, Kobayashi T, Momose S, Kikuchi Y, Adachi H, Okimoto K. Renal carcinogenesis: genotype, phenotype and dramatype. *Cancer Sci* 2003;94:142–147. [PubMed: 12708488]

- Kobayashi T, Hirayama Y, Kobayashi E, Kubo Y, Hino O. A germline insertion in the tuberous sclerosis (Tsc2) gene gives rise to the Eker rat model of dominantly inherited cancer. *Nature Gen* 1995;9:70–74.
- Kouchi M, Okimoto K, Matsumoto I, Tanaka K, Yasuba M, Hino O. Natural history of the Nihon (Bhd gene mutant) rat, a novel model for Birt-Hogge-Dube syndrome. *Virchows Arch* 2006;448:463–471. [PubMed: 16447066]
- Lanzoni A, Piaia A, Everitt J, Faustinelli I, Defazio R, Cavaliere L, Cristofori P. Early onset of spontaneous renal preneoplastic and neoplastic lesions in young conventional rats in toxicity studies. *Toxicol Pathol* 2007;35:589–593. [PubMed: 17654399]
- Lock EA, Hard GC. Chemically induced renal tubule tumors in the laboratory rat and mouse: review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information. *Crit Rev Toxicol* 2004;34:211–299. [PubMed: 15239388]
- NTP. National Toxicology Program, Database Search Application. 2007. <https://ntp-apps.niehs.nih.gov/iacf/demo/denominator.cfm>
- Okimoto, k; Kouchi, M.; Kikawa, E.; Toyosawa, K.; Koujitani, T.; Tanaka, K.; Matsuoka, N.; Sakurai, J.; Hino, O. A novel “Nihon” rat model of a Mendelian dominantly inherited renal cell carcinoma. *Jpn J Cancer Res* 2000;91:1096–1099. [PubMed: 11092972]
- SAS. SAS version 9.1. Cary, NC: SAS Institute; 2003.
- Savard C, Anderson WI, Walker DB, Lambert JA. Early onset of spontaneous renal tubular adenoma and mammary gland adenocarcinoma in young Sprague-Dawley rats (*Rattus norvegicus*). *Vet Pathol* 2005;42:727. (abstract).
- Storkel S, van den Berg E. Morphological classification of renal cancer. *World J Urol* 1995;13:153–158. [PubMed: 7550386]
- Thurman JD, Hailey JR, Turturro A, Gaylor DW. Spontaneous renal tubule carcinoma in Fischer-344 rat littermates. *Vet Pathol* 1995;32:419–422. [PubMed: 7483218]
- Yeung RS, Xiao GH, Jin F, Lee WC, Testa JR, Knudson AG. Predisposition to renal carcinoma in the Eker rat is determined by germ-line mutation of the tuberous sclerosis (TSC2) gene. *Proc Natl Acad Sci* 1994;91:11413–11416. [PubMed: 7972075]

Figure 1. Amphiphilic-vacuolar (AV) tumor, approximately 1 cm in diameter, projecting from the kidney surface. It consists of multiple solid lobules, some with central degeneration, separated by thin stromal tracts. H & E.

Figure 2.

A small adenoma located in the inner stripe of outer medulla displays the typical amphophilic staining and highly vacuolated appearance. Two of the vacuoles in this image appear to be associated with single cell degeneration (arrows). This lesion probably was an extension from a tumor arising in the cortex but outside the plane of section. H & E.

Figure 3.
Higher power of an AV tumor type shows the solid lobules with intervening delicate tracts of fibrovascular stroma, including lymphoid cell infiltration. H & E.

Figure 4. Amphophilic tumor cells are large, with finely vesicular cytoplasm, and some nuclei have very enlarged, single nucleoli (arrows). H & E.

Figure 5. Some cells contain multiple small vacuoles, which are not unlike lipid droplets. However, larger vacuoles (asterisks) appear to be ringed by more than one cell, and take the form of minilumens. H & E.

Figure 6.
The typical growth form of this tumor type was a tapered or wedge-shaped extension from the surface projection, narrowing into the outer medulla. H & E.

Figure 7. Occasionally, there was an association of an early AV tumor type with cortical tubule dilatation. Note the small solid patches (arrows) of typical tumor tissue between, and external to, the dilated tubule profiles. H & E.

Figure 8.

A tumor diagnosed as carcinoma showing the AV tumor type peripherally, with a transition to basophilic tumor tissue centrally. H & E.

Figure 9.
At higher power, the basophilic component of the carcinoma in the preceding figure has tubular differentiation, and prominent lymphoid cell infiltration in the stromal tracts. H & E.

Figure 10.

Several kidneys showed numerous foci of hyperplasia and neoplasia. A small AV lesion (yellow arrows), one of several tumors of this type in the kidneys of this rat, was also accompanied by multiple foci of basophilic tubule cell hyperplasia (black arrows) that was distinct from the hyperplastic profiles of rat CPN. H & E.

Table 1

Distribution of the AV-type RTT in the TDMS series of 2-year rat studies conducted by NTP

Rat strain	No. of studies	Studies with RTT*	Studies with AV-type RTT
F344	194	145	71
Sprague-Dawley	15	4	2
Wistar	1	1	1
Osborne-Mendel	1	0	0
Total	211	150 (71.1%)	74 (35.1%)
	No. of rats	Rats with RTT	Rats with AV-type RTT
Total incidence	~90000	1012 (~1.1%)	100 (~0.1%)
	Adenoma	78%	77 (77.0%)
	Carcinoma	22%	23 (23.0%)
	Males	77%	47 (47.0%)
	Females	23%	53 (53.0%)
	Controls (~25% of rats)	130 (12.8%)	24 (24.0%)
	Treated (~75% of rats)	882 (87.2%)**	76 (76.0%)

* Excludes 4 studies with either diagnoses of nephroblastoma, transitional cell carcinoma, or metastatic lesions to the kidney (2 studies)

** Reflects the contribution of a number of genuine renal carcinogens

Table 2

Carcinogenicity bioassays in which the specific amphophilic phenotype of RTT was identified in standard single sections*.

<u>Agent tested</u>	<u>Total rats in study diagnosed with RTT by NTP</u>	<u>Number of rats with the AV tumor type</u>	
		Males	Females
Acetaminophen	10	1	1
Anthraquinone	58		1
Benzophenone	12	2	3
<i>o</i> -Benzo- <i>p</i> -chlorophenol	3		1
2,2-Bis(bromomethyl)-1,3-propanediol	6	1	
<i>tert</i> -Butyl alcohol	11	1	
Butyl benzyl phthalate	8	2	3
<i>tert</i> -Butylhydroquinone	5		1
Chloraminated water - chloramine	2		1
Chloraminated water – chlorine	4		1
Chloroprene	6	1	
1-Chloro-2-propanol, technical	3	1	
Chromium picolinate	1	1	
C.I. acid red 114	2		1
<i>trans</i> -Cinnamaldehyde	2	1	1
Citral	3		1
Cobalt sulfate heptahydrate	3		1
Coconut oil diethanolamine condensate	15	1	1
Codeine	3	2	
Coumarin	12	1	
Cumene	22	1	
4,4'-Diamino-2,2'-stilbenedisulfonic acid (disodium salt)	3		1
1,2-Dibromo-2,4-dicyanobutane	2	1	
2,4-Dichlorophenol	3		1
Diethyl phthalate	3	1	
3,4-Dihydrocoumarin	7		1
1,2-Dihydro-2,2,4-trimethylquinoline	28		1
Diisopropylcarbodiimide	1		1
3,3'-Dimethoxybenzidine dihydrochloride	2		1
5,5-Diphenylhydantoin (phenytoin)	1	1	
Dipropylene glycol	2	1	
Ethyl benzene	14	1	
Ethylene thiourea	14	1	2
Formamide	2		1
Fumonisin B1	26		1
Furfural	1		1

<u>Agent tested</u>	<u>Total rats in study diagnosed with RTT by NTP</u>	<u>Number of rats with the AV tumor type</u>	
		Males	Females
Furfuryl alcohol	7		1
2,4-Hexadienal	2	1	
Hydroquinone	12	1	
Isobutene	8	1	
Isobutyraldehyde	1		1
Isoprene	11	1	1
Lauric acid diethanolamine condensate	1		1
Manganese sulfate monohydrate	5	1	1
Mercuric chloride	3		1
Methacrylonitrile	2	2	
4-Methylimidazole	1		1
Methylphenidate hydrochloride	4	1	2
Molybdenum trioxide	9		1
Naphthalene	2		1
<i>o</i> -Nitroanisole	5	1	
Nitromethane	7	1	
Ochratoxin A	68	1	
Oxymethalone	5		1
Ozone (lifetime study))	10		1
Pentachlorophenol (purified)	6	1	
Polybrominated biphenyl mixture	5	1	
Promethazine hydrochloride	4	1	
Propargyl alcohol	1		1
Pyridine (Wistar rat)	11	2	
Quercetin	5	1	1
Rhodamine 6g	3		1
Safflower oil	1	1	
Salicylazosulfapyridine	3	2	1
Salicylazosulfapyridine (lifetime study)	2	1	
Scopolamine hydrobromide trihydrate	3	1	
Sodium azide	5	1	1
Succinic anhydride	2	1	
TEF evaluation - dioxin mixture (Sprague-Dawley rat)	2		1
TEF evaluation - PCB 153 (Sprague-Dawley rat)	2		1
Toluene	4		1
Triethanolamine	9		2
Tris(2-chloroethyl) phosphate	39	1	1
Water disinfection byproducts (dibromoacetic acid)	3		1

<u>Agent tested</u>	<u>Total rats in study diagnosed with RTT by NTP</u>	<u>Number of rats with the AV tumor type</u>	
		Males	Females
Totals 74	573	47	53

* Except where indicated in parentheses, all studies were conducted in the F344 rat and were of 2 year's duration.

Table 3

Comparison of the AV tumor type with conventional RTT in rats

<u>AV tumor type</u>	<u>Conventional RTT</u>
Morphology	
Uniform lobular appearance	Variable growth pattern, occasionally cystic
Eosinophilic/amphophilic staining	Basophilic staining
Variably-sized cytoplasmic vacuoles frequent	Vacuoles infrequent
Minilumens frequent	Minilumens occasional
Nucleoli prominent and sometimes inclusion-like	Nucleoli prominent but not inclusion-like
Low mitotic rate	Variable mitotic rate, frequent in carcinomas
Biology	
Equal sex distribution	Males predominate
Can occur in very young rats	Occurs mainly in older rats
No association with CPN	Often linked with CPN
Spontaneous in origin	Spontaneous or chemically induced
Metastases not reported	Metastases frequent if carcinoma exceeds 2 cm