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Interpretation of Male Rat Renal Tubule Tumors

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Based on an analysis of recent scientific studies, a Technical Panel of the U.S. Environmental Protection Agency's (EPA) Risk Assessment Forum recently advised EPA risk assessors against using information on certain male rat renal tubule tumors to assess human risk under conditions specified in a new Forum report. Risk assessment approaches generally assume that chemicals producing tumors in laboratory animals are a potential cancer hazard to humans. For most chemicals, including classical rodent kidney carcinogens such as *N*-ethyl-*N*-hydroxyethylnitrosamine, this extrapolation remains appropriate. Some chemicals, however, induce accumulation of α_{2u}-globulin (α_{2u}-g), a low molecular weight protein, in the male rat kidney. The α_{2u}-g accumulation initiates a sequence of events that appears to lead to renal tubule tumor formation. Female rats and other laboratory mammals administered the same chemicals do not accumulate low molecular weight protein in the kidney, and they do not develop renal tubule tumors. Because humans appear to be more like other laboratory animals than like the male rat, in this special situation, the male rat is not a good model for assessing human risk. The Forum report stresses the need for full scrutiny of a substantial set of data to determine when it is reasonable to presume that renal tumors in male rats are linked to a process involving α_{2u}-g accumulation and to select appropriate procedures for estimating human risks under such circumstances.

Introduction

EPA's Risk Assessment Forum is composed of a group of EPA scientists selected for their expertise in various areas of risk assessment. The Forum studies controversial risk assessment issues and promotes scientific consensus within EPA on these issues. For major projects, the Forum formally convenes a Technical Panel of EPA scientists to report on the issues from an Agencywide perspective.

To examine the issue of chemically induced α_{2u}-globulin (α_{2u}-g) accumulation and its relation to renal tubule tumors in the male rat, in 1987 the Forum formed a Technical Panel chaired by Karl Baetcke of the Office of Pesticides Programs. Other members of the Technical Panel were Letitia Tahan, co-chair, and Marion Copley, Julie Du, Robert McGaughy, William Pepelko, Cheryl Siegel Scott, and Lawrence Valcovic. Gordon Hard, a visiting professor at the Medical Research Council, was a consultant to the project. The Forum's Technical Panel recently completed its review of the issue and made recommendations to EPA for the evaluation of renal tubule tumors in the male rat. This article for the International Symposium on the Health Effects of Gasoline highlights the Technical Panel's findings, which are described in detail in the Risk Assessment Forum report (*1*).

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A variety of organic chemicals have produced specific renal lesions in male rats in the form of a hyaline droplet nephropathy accompanied by accumulation of the protein α_{2u}-g (*2–12*). The Technical Panel's analysis focused on compounds with both an adequate animal carcinogenesis bioassay and information on α_{2u}-g or hyaline droplet accumulation in the male rat as follows: 1,4-dichlorobenzene, dimethyl methyl phosphonate, hexachloroethane, isophorone, d-limonene, pentachloroethane, tetrachloroethylene, and unleaded gasoline. The analysis also relied on research studies on decalin and 2,2,4-trimethylpentane (a significant component of gasoline) because both chemicals have extensive information on α_{2u}-g nephropathy but no chronic bioassay data.

It is clear that not all renal tubule cancer in laboratory animals occurs through the hypothesized α_{2u}-g sequence. Other inducers of rodent renal tubule cancer are well known. These include, for example, certain nitrosamines in the rat and mouse and diethylstilbestrol in hamsters (*13,14*). Thus, any procedure that treats α_{2u}-g inducers in a manner different from classical renal carcinogens must be able to distinguish the two processes.

Recommendations of the Forum Technical Panel

The Risk Assessment Forum Technical Panel recommended that EPA's risk assessors apply the following science policy for evaluation of male rat renal effects arising as a result of the progression of lesions beginning

with chemically induced α_{2u}-g accumulation. For purposes of human risk assessment, male rat renal tumors arising as the result of other processes would continue to be evaluated by conventional procedures described in EPA's guidelines (*15*). Male rat renal tubule tumors arising as a result of a process involving α_{2u}-g accumulation would not contribute to the weight of evidence that a chemical poses a human carcinogenic hazard. Such tumors would not be included in dose–response extrapolations for the estimation of human carcinogenic risk. The associated α_{2u}-g nephropathy would not be used to determine noncarcinogenic hazard. Estimates of noncarcinogenic risk would be based on other end points.

Even when chemically induced kidney tumors in the male rat are clearly α_{2u}-g-related, other tumors in exposed laboratory animals may be important in evaluating the carcinogenic potential of the chemical. However, the role of chemically induced α_{2u}-g accumulation in the induction of renal tubule tumors in the male rat would be assessed independently of evaluations made regarding tumors at other sites.

Basis for the Technical Panel's Recommendations

The information that follows highlights critical data and outlines inferential bridges used by the Technical Panel to select the most plausible explanation for the information available on male rat kidney tumors.

Low Molecular Weight Proteins in the Rat

In rat kidneys, as in those of other mammals, naturally occurring low molecular weight proteins are transferred from the plasma into the urine by glomerular filtration. The proteins are then partially reabsorbed from the urine into the renal tubule of the kidney, where they are eventually broken down by catabolism (*16,17*). One of these low molecular weight proteins, α_{2u}-g, produced by the liver under the stimulus of testosterone, reaches very high levels in the plasma and urine of young adult male rats, gradually declining with age (*18–20*).

α_{2u}-Globulin is regarded as a member of a large superfamily of proteins thought to be carriers of lipophilic molecules (*21, 22*). Some of these proteins, e.g., retinol-binding protein and α_1-acid glycoprotein, are found in many species, including humans (*23,24*). Others, like α_{2u}-g, are species specific. The only member of the superfamily with a clearly defined physiological role is retinol-binding protein, the carrier protein for vitamin A (*22*). Although these low molecular weight proteins are believed to have similar three-dimensional structures (*25*), the alignment of amino acid residues between any pair of proteins in the superfamily is small, roughly 20% (*21*). The exception is α_{2u}-g and mouse major urinary protein(s) (MUP), which are approximately 90% homologous (*26*).

α_{2u}-Globulin derived from hepatic synthesis is not known to occur in the female rat or any other species (*27–29*), including humans. Although similar forms of α_{2u}-g are synthesized at nonhepatic sites in female rats and in the male National Cancer Institute (NCI) Black Reiter (NBR) rat (*30*), a strain whose males lack hepatic synthesis of α_{2u}-g (*31*), none of these other forms of α_{2u}-g nor MUP appears to accumulate in the renal tubule after administration of the eight model carcinogens examined by the Technical Panel.

Progression from Chemically Induced α_{2u}-Globulin Accumulation to Nephropathy and Neoplasia

The sequence of events occurring in the male rat kidney after administration of chemicals inducing α_{2u}-g accumulation (CIGA) can be portrayed on a cellular and molecular level. Initially, the test chemical appears to bind reversibly to α_{2u}-g, seemingly forming a complex more resistant to lysosomal degradation than the unreacted protein itself (*32,33*). This shifts the balance between reabsorption and catabolism and appears to result in accumulation of the protein complex in a specific area of the renal tubule, the P2 segment. Continued compound administration results in a cytotoxic response from the sustained protein overload to the renal tubule, causing single-cell necrosis of cells lining the surface of the tubule and other kidney pathology (*34,35*). The dead cells are replaced by cell division. As the cycle of cell death and cell replacement continues, with time, tubule hyperplasia and neoplasia may occur. It is presumed, but certainly not proven, that continued cell proliferation plays a role in the neoplastic process.

Morphologically, the sequence of events begins with an excessive accumulation of hyaline droplets containing α_{2u}-g in the renal proximal tubules (*36–38*). The next characteristic lesion, single-cell necrosis in the renal tubule, can be confirmed by observation of exfoliated, degenerate cells in the tubule lumen and granular casts (*2,40*). Enhanced cell replication in response to cell death can be seen as increased cell division or demonstrated by labeling techniques that measure increased DNA synthesis (*34,35,41,42*). In chronic laboratory animal bioassays, tubule hyperplasia (*43–45*), linear mineralization in the renal papilla (possibly representing remnants of debris from disintegrating granular casts) (*2,40*), and renal tubule tumors (*43–51*) are observed.

Specificity of the Sequence to the Male Rat

The consistent results from hypothesis-testing experiments conducted in various laboratories support the conclusion that accumulation of abnormal amounts of α_{2u}-g in the P2 segment of the renal tubules can cause a characteristic nephropathy in the male rat. The severity of this kidney disease is dose dependent, not only with respect to the amount of compound administered but also with respect to the concentration of α_{2u}-g in the kidney (*53–55*). This α_{2u}-g-induced nephropathy differs sufficiently from chronic progressive nephropathy commonly found spontaneously in aging male rats so that the two effects can be distinguished (*57*). In contrast, mice and female rats administered α_{2u}-g inducers under the same conditions as male rats did not develop lesions characteristic of α_{2u}-g nephropathy (*45*).

Hyaline droplets in the proximal tubule of untreated male rats contain α_{2u}-g, especially in young adults (*2,30,58*). Hyaline droplets are substantially reduced in castrated male rats, further demonstrating the dependence of this phenomenon on male hormone levels. In female rats of any age, protein droplet formation is rare, and α_{2u}-g is not present in the droplets.

Specialized studies involving hormone manipulation have shown that the development of the early features of α_{2u}-g nephropathy depends on the presence of the hepatic form of α_{2u}-g. Hyaline droplet or α_{2u}-g accumulation does not occur when α_{2u}-g inducers are administered to immature or old male rats that produce little α_{2u}-g in the liver (*2,30*). Hyaline droplet accumulation is observed from administration of α_{2u}-g inducers even in castrated rats, but the severity of the effect is diminished (*59*). Estrogen administration to male rats reduces the severity of α_{2u}-g nephropathy (*60*). Female rats administered an α_{2u}-g inducer along with α_{2u}-g purified from male rat urine clearly showed hyaline droplet formation, α_{2u}-g accumulation in the kidney, and some nephropathy even though control female rats showed no measurable effects (*61*).

The specificity of the male rat response has been tested to a limited extent in a number of other species, with no evidence of hyaline droplet nephropathy in dogs, guinea pigs, hamsters, or monkeys (*2,10,38,62–65*). Because these species (and the mouse and female rat) have proteins similar in structure to α_{2u}-g, the lack of damage to their kidney cells is consistent with the presumption that the specific α_{2u}-g produced by the liver of male rats is necessary for the expression of the renal effects.

Male rats of the NBR strain provide a unique opportunity for testing the α_{2u}-g hypothesis because this animal has no detectable levels of hepatic messenger RNA for α_{2u}-g. Under conditions of exposure that produced α_{2u}-g nephropathy in male rats of other strains, several chemicals administered to the NBR rat did not induce detectable accumulation of α_{2u}-g in the renal tubules (*66,67*).

Mice and female rats exposed to α_{2u}-g inducers in chronic bioassays did not develop an increased incidence of renal tubule tumors (*43–52*). In contrast, male rats developed a dose-dependent neoplastic response in the kidney. Additional experimentation using a nitrosamine as the initiator of cancer and an α_{2u}-g inducer as the promoter also support the observation that α_{2u}-g is involved in the process leading to renal tubule tumors in the male rat (*68,69*). In one of these studies, the promotion potential of an α_{2u}-g inducer in NBR rats was contrasted with the response in a conventional strain, the F344 rat. Consistent with the hypothesis that α_{2u}-g is necessary to induce a response, the promoter did not enhance renal tubule tumor formation in the α_{2u}-g-deficient NBR rat, but it did promote renal tubule tumor formation in the F344 rat (*68*).

Distribution studies of compounds and information on chemical binding to α_{2u}-g indicate that, of the total chemical administered to the animal, only a small portion of the metabolites (possibly the parent compound) can account for all of the α_{2u}-g accumulation (*70–73*). Considerable amounts of the chemical and other metabolites may also be present in the male rat kidney, not bound to α_{2u}-g. These other moieties may, at times, cause toxic effects in the kidney, possibly even cancer, that are unrelated to the accumulation of α_{2u}-g. Such information does not preclude a determination that the α_{2u}-g sequence is involved in some manner with the renal tumor response.

α_{2u}-Globulin Inducers and Other Renal Carcinogens

It is instructive to compare CIGA renal carcinogens with other renal carcinogens. Several genotoxic chemicals recognized as classical inducers of rodent kidney tumors have been used to study the pathogenesis of renal tubule cancer in laboratory animals (*13,14,74*). In general, these prototypic renal carcinogens produce tumors in both males and females. Although the wide range of chemicals represented suggests multiple mechanisms of action, many of the classical renal carcinogens or their active metabolites are electrophilic species able to bind covalently to macromolecules and likely to form DNA adducts in the kidney. In contrast, CIGA renal carcinogens are not known to react with DNA (*75*) and are generally negative in short-term tests for genotoxicity (*76–81*). CIGA renal carcinogens also interact with α_{2u}-g in a reversible and noncovalent manner (*71-73*).

Classical renal carcinogens, such as certain nitrosamines, induce renal tubule cancer in rats or mice with high incidence, minimal duration of exposure, and clear dose–response relationships (*82*). In contrast, the renal tumors produced by the eight model carcinogens examined by the Technical Panel tended not to be life threatening, occurred late in life, were usually found at terminal sacrifice, and were frequently microscopic. Even though the maximum-tolerated dose was exceeded for some of the eight model carcinogens, the renal tumor incidence rate, adjusted for intercurrent mortality, was never greater than 28%. An increase in renal tubule tumors was not found in mice or female rats exposed to these chemicals. Such differences in potency and species-, strain-, and sex-susceptibility suggest that CIGA renal carcinogens act via different mechanisms from classical renal carcinogens.

Renal tubule tumors produced by CIGA carcinogens also have features in common with other renal tubule tumors observed in the male rat. For renal carcinogens, in general, there is a continuum of chemically induced steps from atypical hyperplasia through microscopic adenomas to macroscopic adenocarcinomas or carcinomas (*13*). Renal tubule tumors induced by the eight model carcinogens are morphologically indistinguishable from those induced by classical carcinogens. Likewise, the sequence of development of CIGA carcinogen-induced renal tumors from tubule cell hyperplasia to carcinoma appears identical. Furthermore, none of these chemically induced tumors can be differentiated from spontaneous tumors.

Epidemiologic Studies

The Technical Panel was not aware of any epidemiologic study that has been designed or conducted specifically to examine the applicability of the CIGA hypothesis to renal

cell cancer in humans. Several epidemiologic studies were reviewed (*83–91*), but they are of limited value for this analysis because they involved exposure to complex blends, such as gasoline, or otherwise involved multiple exposures to both CIGA and nonCIGA. In addition, these studies were of limited statistical power and were not able to account for possibly confounding factors, such as smoking or obesity, which are known to influence renal cell cancer rates. In a few studies, slight increases in risk of renal cell cancer have been observed; however, the other factors described above could have easily accounted for the increased risk. These studies, therefore, are considered inadequate for purposes of exploring the relevance of the α_{2u}-g hypothesis in humans.

Conclusions

Based on the preceding analysis, the Technical Panel reached three major conclusions. First, the sequence of events proposed to link α_{2u}-g accumulation to nephropathy and renal tubule tumors in the male rat is plausible, although not totally proven.

Second, the α_{2u}-g response following chemical administration appears to be unique to the male rat. Even though closely related proteins are present in other species, there is no evidence that these species respond to α_{2u}-g inducers in a manner similar to the male rat.

Third, the male rat kidney response to chemicals that induce α_{2u}-g accumulation is probably not relevant to humans. This, of course, does not mean that all chemically induced renal tubule tumors in the male rat are α_{2u}-g associated.

Guidance for Evaluating Chemically Induced Male Rat Renal Tubule Tumors

The Risk Assessment Forum Technical Panel recommended the following guidance to EPA risk assessors for evaluation of renal tubule tumors. This guidance is based on the presumption that there are sufficient data (e.g., two-year bioassay in rats and mice, evidence to judge the genotoxicity of the chemical, information on α_{2u}-g accumulation and other toxicity, and information on the compound's potential to cause sustained cell proliferation). If these data are not available, the tumors are evaluated in accordance with standard procedures, not those specified for CIGA.

Chemicals inducing renal tubule tumors in the male rat would be placed in one of the following categories: *a*) the α_{2u}-g sequence of events accounts for the renal tumors, *b*) other potential carcinogenic processes account for the renal tumors, *c*) the α_{2u}-g-associated events occur in the presence of other potential carcinogenic processes, both of which result in renal tumors.

Evaluation of the data to determine which category applies would be a two-step process. First, the available information would be examined to determine whether the α_{2u}-g process is involved in the tumor development. If the data do not support such an association, then the male rat renal tubule tumors would be used conventionally for characterizing human risk, and no further evaluation of the role of α_{2u}-g accumulation would be possible or needed.

Renal Tubule Tumors in Male Rats and α_{2u}-Globulin Accumulation

The following information from adequately conducted studies of male rats shows that the α_{2u}-g process could be a factor in the observed renal effects; affirmation of chemically induced α_{2u}-g involvement in each of the three categories is necessary.

Increased Number and Size of Hyaline Droplets in Renal Proximal Tubule Cells of Treated Male Rats. The abnormal accumulation of hyaline droplets in the P2 segment of the renal tubule is necessary to attribute the renal tubule tumors to the α_{2u}-g sequence of events. This finding helps differentiate α_{2u}-g inducers from chemicals that produce renal tubule tumors through other means.

The Protein Accumulating in the Hyaline Droplets is α_{2u}-g. Hyaline droplet accumulation is a nonspecific response to protein overload in the renal tubule and may not be due to α_{2u}-g [e.g., as with chlorothalonil (*66*)]. Therefore, it is necessary to demonstrate that α_{2u}-g accounts for the hyaline droplet accumulation found in the male rat.

Additional Aspects of the Pathological Sequence of Lesions Associated with α_{2u}-g Nephropathy Are Present. Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, all of these lesions may not be observed; however, some elements consistent with the pathological sequence must be demonstrated to be present. In the absence of this minimum information, there is no basis for judging the applicability of the α_{2u}-g process, and it would be assumed that the male rat renal tumors are relevant for risk assessment purposes.

Additional Information Useful for the Analysis

If the α_{2u}-g process appears to be a factor in the observed renal effects, then other information is useful for determining whether the renal effects are solely α_{2u}-g-associated, a combination of the α_{2u}-g process and other potential carcinogenic processes, or due primarily to other processes. Some types of useful information are listed below.

Hypothesis-Testing Data. Data from specialized tests greatly increase confidence that the α_{2u}-g sequence is involved in the renal tubule tumor response. Such information might include: modification of the nephrotoxic response through use of the NBR rat, or manipulation of sex hormones (e.g., androgens), or α_{2u}-g levels (e.g., α_{2u}-g administration to female rats). Other information might include initiation–promotion studies comparing males of the NBR strain with males of other rat strains.

Additional Biochemical Information. Certain *in vivo* and *in vitro* data help characterize a chemical as one that

induces accumulation of α_{2u}-g. Such information might include reversible binding of the chemical (or metabolites) to α_{2u}-g, reduction in the lysosomal degradation of the α_{2u}-g complex, and disposition studies demonstrating sex- and species-specific retention of the test compound in the male rat kidney.

Sustained Cell Division in Proximal Tubule of the Male Rat. A sustained increase in cell replication in the P2 segment of the renal tubule at doses used in the cancer bioassay and a dose-related increase in atypical hyperplasia of the renal tubule is consistent with the α_{2u}-g process, especially if laboratory animals in addition to the male rat were tested and did not respond. These end points are nonspecific for α_{2u}-g inducers, however, because other renal carcinogens may also affect the P2 segment of the renal tubule.

Structure–Activity Relationship. Structure–activity relationships for chemicals that induce α_{2u}-g accumulation in the male rat kidney are not well defined, although there appear to be dimensional requirements to fit the protein pocket, a requirement for a degree of lipophilicity, and a need for an electronegative atom in the molecule or its active metabolite (*92*). Other structural features might suggest that a chemical belongs to a different class of suspected carcinogens.

Covalent Binding to Macromolecules. Some classical inducers of renal tubule cancer are known to bind covalently to DNA. Because CIGA do not appear to bind to DNA, such information may assist in distinguishing between the different processes leading to renal cancer.

Genotoxicity. α_{2u}-Globulin inducers have been nongenotoxic, and they do not depend on direct genetic injury for the production of tumors. Thus, information on potential genotoxicity in a standard battery of short-term tests relevant to the evaluation of potential carcinogenicity helps distinguish them from other renal carcinogens.

Nephrotoxicity. Chronic progressive nephropathy (CPN) in the aging male rat can complicate the analysis of other renal lesions. However, nephrotoxicity in the male rat not attributable to either CPN or α_{2u}-g accumulation, or a nephrotoxic response in the female rat or the mouse, suggests that the possibility of other processes leading to renal cancer should be considered.

Animal Bioassay Data in Other Species and Sex-Combinations. The α_{2u}-g syndrome is specific to the male rat. Positive cancer responses in the renal tubule in female rats, mice of either sex, or any other laboratory animal imply that the α_{2u}-g syndrome alone cannot account for the renal tubule tumor response in the male rats.

Confidence in determining which of the three categories applies depends on the comprehensiveness and consistency of available data. Decisions on the applicability of the three categories described above can only be made on a case-by-case basis, taking all of the information into account.

Use of the Data for Risk Assessment

Once a decision on the applicability of the three categories is made, it is possible to determine how the response in the male rat renal tubule would apply to evaluation of human hazard and to estimation of human cancer risk. The Technical Panel recommended the following guidance, recognizing that tumors occurring at other sites in laboratory animals administered compounds that induce α_{2u}-g accumulation in the male rat will be judged on their own merits.

a) Renal tubule tumors in male rats attributable solely to chemically-induced α_{2u}-g accumulation would not be used for human cancer hazard identification or for dose–response extrapolations. *b*) Renal tubule tumors that are not linked to α_{2u}-g accumulation would be appropriate for human hazard identification, and they would be considered along with other appropriate end points for quantitative risk estimation. *c*) In general, the information needed to make a quantitative determination of the relative contribution of compounds producing some renal tubule tumors in male rats attributable to the α_{2u}-g process and some attributable to other carcinogenic processes will not be available. If there is enough information, however, the non-α_{2u}-g-induced component may be used, as appropriate, for dose–response evaluation as well as hazard identification. Usually, such information is not available, in which case a meaningful dose–response estimate based on renal tubule tumors in the male rat is not possible and should not be performed; the non-α_{2u}-g-induced renal tubule tumors remain relevant for purposes of hazard identification.

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