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NOTE TO READERS

Part 4 - Science Policy of the document titled **ALPHA-2u-GLOBULIN: ASSOCIATION WITH CHEMICALLY INDUCED RENAL TOXICITY AND NEOPLASIA IN THE MALE RAT** has recently been completed. A copy is attached for your information.

With the addition of Part 4 to the document, please note that the page numbering in the sections that follow Part 4 is no longer consecutive.

PART 4. SCIENCE POLICY

I. Background and Introduction

An increased incidence of neoplasms in chemically tested animals is customarily viewed by scientists as an indication of carcinogenicity in animals and as some signal that humans may be similarly affected. From this line of reasoning, EPA generally presumes that animal tumor findings indicate there may be a cancer hazard to humans, although a final judgment as to human carcinogenic potential can be made only in relation to all other relevant information. There has been growing interest in recent years in the possibility that tumors produced in the tubule of the male rat kidney subsequent to the accumulation of the low-molecular-weight protein alpha-2u-globulin (α_{2u}-g), might involve a process that occurs only in the male rat. Because of the implications to cancer risk assessment, the Risk Assessment Forum established a Technical Panel to examine the available information on α_{2u}-g accumulation in the kidney, associated renal disease, and kidney cancer. These findings are reported in parts 1-3 of this report.

This part 4 provides guidance to EPA risk assessors regarding evaluation of renal tubule tumors in the male rat and presents Forum conclusions regarding potential human hazard and risk for a special subset of renal cancer, those renal tubule tumors appearing to arise as a result of chemically induced α_{2u}-g accumulation in the male rat kidney.

II. Basis for Science Policy on Male Rat Kidney Tumors

The scientific data supporting the Technical Panel's conclusions regarding the α_{2u}-g sequence of lesions[1] are covered in depth in the preceding sections (parts 1-3) of this document. The information below highlights critical data and outlines inferential bridges used to select the most plausible explanation for the information available on male rat kidney tumors.

A. Low-molecular-weight proteins in the rat

In the rat kidney, as in those of other mammals, low-molecular-weight proteins are removed from the plasma to the urine by glomerular filtration followed by partial reabsorption from the urine into the renal tubule of the kidney with subsequent catabolism (destructive metabolism). 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.

Alpha-2u-globulin is regarded as a member of a large superfamily of proteins thought to be carriers of lipophilic (affinity for fat) molecules. Some of these proteins, e.g. retinol-binding protein and lactoglobulins, are found in many species, including humans. Others, like α_{2u}-g, are found only in selective species. The only member of the protein superfamily with a clearly defined physiological role is retinol-binding protein, the carrier protein for vitamin A. Although these low-molecular-

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[1] Alteration is structural or functional, due to disease; commonly limited to morphological alterations.

weight proteins are believed to have a similar three-dimensional structure, the alignment of amino acid residues (sequence homology) between any pair of proteins in the superfamily is small, roughly 20 percent. The exception is α_{2u}-g and mouse major urinary protein(s) (MUP) which share approximately 90 percent sequence homology.

Alpha-2u-globulin derived from hepatic synthesis is not known to occur in the female rat or any other species, including humans. Although similar forms of α_{2u}-g are synthesized at nonhepatic sites in female rats and in the male NCI Black Reiter rat (NBR), a strain whose males lack hepatic synthesis of α_{2u}-g, none of these other forms of α_{2u}-g nor MUP accumulate in the renal tubule following administration of the compounds discussed in the Technical Panel report.

B. Description of the progression from chemically induced alpha-2u-globulin accumulation to nephropathy and neoplasia

1. Overview

The information available provides a plausible, although probably incomplete picture of a sequence of events occurring in the male rat kidney following chemical administration. This sequence can be portrayed on a molecular and cellular level. Initially, the test chemical appears to bind reversibly to α_{2u}-g, seemingly forming a complex more resistant to lysosomal degradation[2] than the unreacted protein itself. This shifts the

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[2]Lysosomes are intracellular bodies present mostly in the liver and kidney. They contain hydrolytic enzymes responsible for the catabolism of α_{2u}-g and other proteins.

balance between reabsorption and catabolism and appears to result in accumulation of the protein complex in a specific segment (P2) of the renal tubule. Continued compound administration results in a cytotoxic response from the sustained protein overload to the renal tubule, causing single cell necrosis (death) of cells lining the surface of the tubule and other kidney pathology. Dead cells are replaced by cell division. As the cycle of cell death and cell replacement continues, tubule cells increase in number (hyperplasia), and with time neoplasia may occur. It is presumed, but certainly not proven, that continued cell proliferation plays a role in the neoplastic process.

The morphological basis for the sequence of events beginning with an increase in number and size of hyaline droplets[3] containing α_{2u}-g is the demonstration of a progression of characteristic lesions. Single cell necrosis in the renal tubule can be confirmed by observation of granular casts.[4] Enhanced cell replication in response to cell death can be seen as increased cell division or can be demonstrated by labeling techniques that measure increased DNA synthesis. In chronic laboratory animal bioassays involving administration of compounds that induce α_{2u}-g accumulation, tubule hyperplasia, linear mineralization in the renal papilla (possibly

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[3]Spherical inclusions in the cytoplasm that may contain various proteins.

[4]The granular casts are composed of sloughed cell debris from the dead cells. They accumulate at the junction between the P3 segment of the proximal tubule and the descending thin loop of Henle where diameter is constricted.

representing remnants of debris from disintegrating granular casts), and renal tubule tumors are observed.

2. Evidence supporting the specificity of the sequence to the male rat

Hypothesis-testing experiments conducted over the last decade in various laboratories have shown a remarkable consistency of results indicating that chemically induced accumulation of α_{2u}-g in the male rat kidney can lead to renal tubule tumors. This information establishes an association between exposure of the male rat to compounds that induce α_{2u}-g accumulation and a specific form of nephropathy (kidney disease), and it supports an association between this nephropathic response and renal tubule tumors.

The male rat has consistently responded to administration of compounds that cause the accumulation of abnormal amounts of α_{2u}-g in the P2 segment of the renal tubules with a characteristic nephropathy. The severity of the nephropathy 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. Alpha-2u-globulin nephropathy also differs sufficiently from chronic progressive nephropathy commonly found in aging male rats so that the two effects can be differentiated.

In contrast to the response of male rats to compound administration, mice and female rats administered α_{2u}-g-inducers under the same conditions did not develop lesions characteristic of α_{2u}-g nephropathy. When exposed to α_{2u}-g-inducers in chronic bioassays, these latter animals did not develop an increased incidence of renal tubule tumors, even though male rats developed

a dose-dependent neoplastic response in the kidney. The specificity of the male rat response has also been tested to a limited extent in a number of other species, with no evidence of protein droplet nephropathy in dogs, guinea pigs, hamsters, or monkeys. Since these species (and the mouse and female rat) have proteins similar in structure to α_{2u}-g, their lack of nephrotoxic (damage to kidney cells) response is consistent with the presumption that the unique α_{2u}-g produced by the liver of male rats is the necessary determinant for the expression of the renal effects.

Hyaline droplets in the proximal tubule of untreated male rats contain α_{2u}-g, especially in young adults. Hyaline droplets are substantially reduced in castrated male rats, further indicting the dependence of this phenomenon on male hormone levels. In female rats of any age, an observation of protein droplets is rare and α_{2u}-g is not involved.

Specialized studies involving hormone manipulation have shown that the development of the early features of α_{2u}-g nephropathy is dependent on the presence of the hepatic form of α_{2u}-g. (1) 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) Hyaline droplet accumulation is observed from administration of α_{2u}-g-inducers even in castrated rats, but the severity of the effect is diminished. (3) Estrogen administration to male rats reduces the severity of α_{2u}-g nephropathy. (4) 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 show no measurable effects.

Male rats of the NBR strain provide a unique opportunity for testing the α_{2u}-g hypothesis since 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.

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. In one initiation-promotion study, the promotion potential of the α_{2u}-g-inducer was compared and contrasted in male Fischer and NBR rats. 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 Fischer rat.

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. In general, these prototypic renal carcinogens are active in both males and females. The acute

nephrotoxic changes in the renal tubules include mild lipid droplet accumulation and scattered single cell necrosis, but hyaline droplet accumulation and its specific associated nephropathy are not characteristic.

Based on the information available to date about α_{2u}-g-inducers, these compounds may have additional features that help to distinguish them from some other chemicals, such as the nitrosamines, that are also inducers of rodent kidney tumors. Alpha-2u-globulin inducers identified to date appear to be non-genotoxic, or only marginally so, suggesting that the mechanism for tumor induction may not depend on direct genetic injury. So far, the incidence of renal tumors produced in the male rat has been relatively low, occurring late in life, and metastasizing rarely.

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) appear responsible for inducing α_{2u}-g accumulation. Considerable amounts of the chemical and other metabolites may be present in the male rat kidney in apparently unbound form. These other moieties can, at times, cause toxic effects in the kidney, including 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.

III. Science policy statement

Based on interpretation of current data, the Technical Panel

made the following three 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 they respond to chemical administration in a manner similar to the male rat.

Third, the response seen in the male rat kidney following chemically induced α_{2u}-g accumulation is probably not relevant to humans.

Given the Technical Panel's findings, EPA science policy regarding use of male rat renal tubule tumors attributable to individual chemicals or chemical mixtures for human risk assessment is as follows.

(1) Male rat renal tubule tumors arising as a result of a process involving α_{2u}-g accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk.

(2) If a chemical induces α_{2u}-g accumulation in male rats, the associated nephropathy is not used as an endpoint for determining non-carcinogenic hazard. Estimates of non-carcinogenic risk are based on other endpoints.

On the other hand, α_{2u}-g-related kidney tumors do not negate the consideration of tumors in other sites in the male rat or tumors in other species. Chemicals can influence cells of various organs and tissues in different ways. Thus, the determination of the relevance of tumors at other sites for hazard identification and the characterization of risk from these tumors proceeds on their own merits and is not influenced by judgments about the applicability of the α_{2u}-g process to the evaluation of renal tubule tumors in male rats. Likewise, the analysis of the role of chemically-induced α_{2u}-g accumulation proceeds on its own merits and is not influenced by determinations made regarding tumors at other sites.

To determine the applicability of EPA's science policy, chemicals inducing renal tubule tumors in the male rat are grouped into one of the following three categories.

(1) The α_{2u}-g sequence of events apply.

(2) Other potential carcinogenic processes apply.

(3) The α_{2u}-g-associated events occur in the presence of other potential carcinogenic processes.

IV. Guidance for evaluating chemically-induced male rat renal tubule tumors

To determine which of the three categories described above apply for a given chemical that has produced renal tubule tumors in the male rat two questions need to be answered. The first is whether or not α_{2u}-g-associated events are involved in the tumor development. The second, given an affirmative answer to the first, is to determine if other processes may also account for the

demonstrated tumor increase. Guidance for answering these two questions follows.

A. Renal tubule tumors in male rats and α_{2u}-g accumulation

Three kinds of information from adequately conducted studies are needed on a chemical producing renal tubule tumors in the male rat to ascertain if the α_{2u}-g process is operative or not. Affirmative responses define the minimal amount of data needed to determine that the α_{2u}-g-related sequence pertains. In their absence, it would be concluded that the α_{2u}-g process is not operative. The three components for making these determinations follow.

(1) Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats

The abnormal accumulation of hyaline droplets is a necessary but not sufficient feature of compounds that induce renal tubule tumors through the α_{2u}-g sequence of events and helps differentiate them from chemicals that induce renal tubule tumors through other means.

(2) Accumulating protein in the hyaline droplets is α_{2u}-g

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

(3) 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. Some elements may not be visibly present if the response is mild. Nevertheless, some endpoints should always be observable.

B. Additional information useful for the analysis

If the preceding analysis (section IV-A) indicates that the α_{2u}-g process is a determinant in the observed renal effects, then other types of information are reviewed to decide if the renal effects are most likely due solely to the α_{2u}-g-associated phenomenon or whether this process in combination with other potential carcinogenic processes is more likely. Many kinds of information are available to assist in strengthening the determination that chemically-induced α_{2u}-g accumulation appears to be involved in the renal tumor response or that other processes cannot be ruled out. Some of these findings are listed below; the information should not be considered exhaustive.

(1) Hypothesis-testing data

The determination that the α_{2u}-g sequence is involved in the renal tubule tumor response would be greatly enhanced by the availability of specialized test results. Such information might include: modification of the nephrotoxic response through manipulation of sex hormones (e.g., androgens), α_{2u}-g levels (e.g., α_{2u}-g administration to female rats), or use of the NBR rat. Other information might include initiation-promotion studies comparing males of the NBR strain with males of other rat strains.

(2) Additional biochemical information

Certain in vivo and in vitro data help characterize a chemical as one that would induce accumulation of α_{2u}-g. Such information might include: demonstration of reversible binding of the chemical (or metabolites) to α_{2u}-g, demonstration of a 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.

(3) Sustained cell division in proximal tubule of the male rat.

Demonstration in the male rat of a sustained increase in cell replication in the P2 segment of the renal tubule and a dose-related increase in atypical hyperplasia of the renal tubule is consistent with a conclusion that the α_{2u}-g process is operative, especially if other laboratory animals were tested and did not show similar responses. These endpoints may be non-specific for α_{2u}-g-inducers, however, since there are other renal carcinogens also thought to affect the P2 segment of the renal tubule.

(4) Structure-activity relationships

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. Other structural features may suggest that a chemical may also belong to a different class of suspected carcinogens.

(5) Covalent binding to macromolecules

Some inducers of renal tubule cancer in rodents (e.g., nitrosamines) are known to bind covalently to DNA or other macromolecules. Others do not appear to bind to DNA (e.g., isophorone) suggesting that such information may assist in distinguishing different processes leading to renal cancer.

(6) Genotoxicity

. Although renal tubule neoplasia associated with clearly genotoxic chemicals is a well known response, information to date supports a conclusion that α_{2u}-g inducers are essentially nongenotoxic and 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 provides a possible device for helping to distinguish between these processes.

(7) Nephrotoxicity

Although the presence of chronic progressive nephropathy (CPN) in the aging male rat can complicate the analysis of other renal lesions, if there is additional nephrotoxicity seen in the male rat not attributable to either CPN or α_{2u}-g accumulation, or if there is a nephrotoxic response in the female rat or the mouse, then the possibility of other processes leading to renal cancer should be considered.

(8) The availability of animal bioassay data in other species-, sex- combinations.

The α_{2u}-g-syndrome is a male rat-specific event. Positive cancer responses in the renal tubule in female rats, mice of either sex, or any other laboratory animal immediately suggest that the

α_{2u}-g syndrome, alone, is unable to account for the renal tubule tumor response in the male rats.

The overall confidence in determining which of the three categories applies in any given set of compound-induced renal tubule tumors depends on the comprehensiveness of available data. If these data all support a role for the involvement of chemically induced α_{2u}-g, there is a high degree of confidence in assuming that the α_{2u}-g syndrome, alone, accounts for the renal tubule tumors. In contrast, if information from adequate testing leads to serious doubt about α_{2u}-g involvement (see sections IV-A and IV-B), there is a high degree of confidence that other carcinogenic processes account for the renal tumors. Sometimes, the information will suggest that more than one carcinogenic process probably occurs; in these cases, as a minimum, the criteria in support of α_{2u}-g involvement (section IV-A) are present, but there is also evidence supporting involvement of other mechanisms (section IV-B). Decisions on the applicability of the three categories can only be made on a case-by-case basis, taking all of the information into account. Whatever the finding, the risk assessor should clearly delineate and thoroughly document the basis for any decisions made.

Once a decision on the applicability of the three categories is made, it becomes possible to determine whether to use renal tubule tumors in male rats to evaluate human hazard and to estimate human cancer risk. In general, the following guidance applies, 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.

1. For compounds producing renal tubule tumors in male rats attributable solely to chemically-induced α_{2u}-g accumulation, the renal neoplasms will not be used for human cancer hazard identification or for dose-response extrapolations.

2. For compounds producing renal tubule tumors that do not appear to be linked to the accumulation of α_{2u}-g, those tumors will be used both for human hazard identification and for quantitative risk assessment.

3. For compounds producing renal tubule tumors in male rats attributable to the α_{2u}-g syndrome and to some other carcinogenic process, recognizing that some portion of the renal tubule tumors in the male rat remain relevant to hazard identification, a preference is generally given for not basing the risk estimation on the renal tubule tumors.

If there is enough information available to determine the relative contribution of each process to the overall renal tubule cancer response in male rats (e.g., from new mechanistic approaches or hormone manipulation studies), this information may be used accordingly both for hazard identification and dose-response analysis. Clearly, case-by-case determinations are needed, and the rationale for any position should be thoroughly presented.

V. Guidance for evaluating nephropathy as a toxic endpoint

If a compound induces α_{2u}-g accumulation in hyaline droplets, the associated nephropathy in male rats is not used as an endpoint

to determine noncancer (systemic) effects potentially occurring in humans. Likewise, quantitative estimates of noncancer risk (e.g., reference doses and margin-of-exposure determinations) are based on other endpoints wherever possible.

It should not be anticipated that a compound that produces nephropathy in the male rat through the sequence of events beginning with the accumulation of α_{2u}-g will always be found to induce renal tubule tumors in the male rat. The ability to detect neoplasia depends on many features that may not be present in any individual experiment, eg. sufficient dose to induce effect without early deaths of the animals, competing toxicity from other moieties not bound to α_{2u}-g, insufficient length of exposure or followup, and incomplete histopathology. Even in the absence of renal tubule neoplasia in the male rat, if the sequence of lesions characteristic of the α_{2u}-g syndrome are present, the associated nephropathy in the male rat does not contribute to determinations of noncarcinogenic hazard or risk.