UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
THE ADMINISTRATOR

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August 20, 1991

Honorable William K. Reilly
Administrator
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Subject: Science Advisory Board review of the draft document *Alpha-2u Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in The Male Rat (EPA/625/3-91/019A)*

Dear Mr. Reilly:

There has been growing interest in recent years in the possibility that renal tubule tumors produced in the male rat by exposure to various toxicants, and associated with the accumulation of the low-molecular-weight protein, alpha-2u-globulin, might be a process occurring only in the male rat. According to the alpha-2u-globulin hypothesis, the renal tubule tumors in the male rat arise as the end stage in a sequence of events beginning with the reversible binding of an exogenous compound to alpha-2u-globulin. The resultant complex appears to be more resistant to lysosomal degradation than the unreacted protein, causing a shift in balance between reabsorption and hydrolysis. As a consequence, the protein complex begins to accumulate in the P2 segment of the renal tubule. If exposure to the exogenous compound continues over a period of time, single cell necrosis of the tubule epithelium occurs as a cytotoxic response. The regenerative process that occurs to compensate for the necrosis increases the probability that a process leading to renal tubule cancer will be initiated.

Because of the implications of such a mechanism for characterizing risk to humans, the Risk Assessment Forum (RAF) established a Technical Panel in 1988 to examine the available evidence on this hypothesized mechanism and to relate their findings to Agency policy on using such data for human risk assessment. The Technical Panel found this proposed sequence of events linking alpha-2u-globulin accumulation to renal tubule tumors in the male rat to be credible and capable of providing key histopathologically observable changes.

Following a Peer Review Group study on the Technical Panel findings, the RAF developed a draft report on alpha-2u-globulin and chemically induced effects in the male rat kidney. The RAF draft report advises that EPA risk assessors not use evidence of renal tubule tumors and nephrotoxicity in male rats to assess human risk when these endpoints in the male rat are associated with accumulation of alpha-2u-globulin. The Forum then requested that the Science Advisory Board (SAB) review the draft report. The SAB's Environmental Health Committee (EHC), joined by a Member of the Agency's Science Advisory Panel (SAP), met on March 27, 1991, in Bethesda Maryland to receive briefings on the topic by staff of the RAF, and to initiate development of this report. Dr. Bernard Weiss served as Chair, in the absence of Dr. Arthur Upton. A Committee roster is enclosed

The EHC was asked to review the Forum's analysis of the following three issues (listed as a, b, and c, below) and to comment on the fundamental tenets (enumerated as points (1) through (5), below) underlying their conclusions :

a. The specificity of the alpha-2u-globulin nephropathy for the male rat

Exposure of male rats to either classic "genotoxic" chemical carcinogens or to chemicals of the CIGA (Chemicals Inducing alpha-2u-globulin Accumulation) class yield renal tumors, adenomas and carcinomas of clear cell or granular type, that are histologically identical. However, the cytologic and histologic changes and the tissue changes that occur during the process of tumor development (carcinogenesis) preceding the occurrence of renal tumors differ distinctively for the two types of carcinogenic agents. CIGA compounds elicit a reproducible pattern of tissue changes in the kidneys of male rats sufficiently unique to be pathognomonic for identifying the action of chemicals of this class. The pathognomonic tissue pattern consists of the accumulation of alpha-2u-globulin in lysosomes of epithelial cells of the P2 segment of the proximal tubules, followed by necrosis of these cells, and hyperplasia (proliferation) of surrounding cells. Chronic exposure to CIGA compounds leads to further changes in the proximal tubules, including atypical hyperplasia and dysplasia of tubular epithelium, formation of adenomas and, eventually, carcinomas. Two other histologic changes in kidneys of male rats are also characteristic of exposure to CIGA compounds: casts of cell debris which fill lumens of proximal tubules and linear mineralization of the papilla. These pathognomonic preneoplastic tissue changes have not been demonstrated to occur in the kidneys of animals incapable of hepatic synthesis of alpha-2u-globulin. Such animals include female rats, genetically defective male rats of the NBR strain, and mice. Less extensive studies also extend this list to dogs, monkeys, hamsters and guinea pigs. Given the uniqueness of the tissue reaction, and the fact that only male rats of genetically sufficient strains synthesize alpha-2u-globulin, this tissue reaction is conclusively linked to this metabolic quirk of the male rat. This opinion is substantiated by the fact that alpha-2u-globulin uniquely binds CIGA compounds, and that such binding impairs the catabolism of the

protein, which apparently causes it to accumulate in the P2 segment of proximal tubules where it is catabolized normally.

b. The linkage of alpha-2u-globulin nephropathy in the male rat to neoplasia of the renal tubules

Linkage of alpha-2u-globulin nephropathy to renal neoplasia can only be inferred, since the chain of continuity between tubular necrosis, hyperplasia, and cancer has not been demonstrated directly. Nevertheless, the reproducible occurrence of both alpha-2u-globulin nephropathy and renal tumors in rats chronically exposed to CIGA compounds, and the reproducible failure of both of these lesions to develop after exposure to CIGA compounds in animals that do not produce alpha-2u-globulin provides strong circumstantial evidence that alpha-2u-globulin nephropathy is mechanistically linked to renal tumors.

c. The recommendation advising against the use of male rat renal tubule tumors associated with the alpha-2u-globulin syndrome for human risk assessment.

The Committee concurs with this recommendation, based on the comments preceding, and following, this issue.

Comments on the five fundamental elements in the Technical Panel's rationale follow below:

1. Since renal tubule toxicity induced in the male rat by the alpha-2u-globulin mechanism is unlikely to occur in humans, renal tumors in humans are not likely to occur via this mechanism.

Given the persuasive evidence of linkage between CIGA-induced alpha-2u-globulin nephropathy and alpha-2u-globulin in the male rat, the strongly inferred linkage between alpha-2u-globulin nephropathy and renal tumors in the male rat, the failure of this typical nephropathy to develop in female rats and other laboratory animals which do not produce alpha-2u-globulin, and the absence of production of alpha-2u-globulin in humans, this rationale appears to be well founded. This line of reasoning is supported by the experimentally demonstrated binding of CIGA compounds to alpha-2u-globulin and the conclusion, sustained experimentally, that binding of CIGA to alpha-2u-globulin is necessary for its accumulation in tubular epithelial cells of the male rat, coupled with the failure to demonstrate proteins in human urine which bind CIGA.

2. The applicability of the alpha-2u-globulin mechanism to the male rat renal tubule response can be determined even when there are other forms of chemically-induced nephrotoxicity or increased incidence of cancer at other sites in the rat or other species.

The evidence shows that alpha-2u-globulin nephropathy is a pathognomonic tissue response signalling exposure of male rats to CIGA compounds. When animal bioassays are appropriately structured (i.e., with the sampling of renal tissue soon after cessation of chemical exposure, before the hyaline droplets become undetectable) alpha-2u-globulin nephropathy should be readily separable from renal tissue reactions induced by chemicals that act through other mechanisms.

3. *If a chemical induces alpha-2u-globulin accumulation in hyaline droplets [intracellular vacuoles containing amorphous material] the associated nephropathy observed in male rats may not be an appropriate endpoint for assessing non-cancer risk in humans.*

It follows from the comments to item 1 and 2 above that this is a reasonable and conservative rationale, provided (as noted under item 2) that tissue is sampled under appropriate circumstances so that the lesions of alpha-2u-globulin nephropathy are distinct.

4. *Renal tubule tumors in the male rat that appear following administration of clearly mutagenic agents may be appropriate for the characterization of human risk on a case-by-case basis even when alpha-2u-globulin accumulation has been observed.*

This position appears to be reasonable, based on the current understanding of alpha-2u-globulin nephropathy and other nephropathic patterns preceding the development of carcinogen-induced renal tumors. Some studies suggest that a few chemicals may act as both CIGA-type and classic "genotoxic" carcinogens in the male rat, that is, they work both through the alpha-2u globulin tumorigenic mechanism, and more "conventional" mechanisms of carcinogenicity. Furthermore, in some situations, humans might be exposed to mixtures of toxicants in which both classes of agents are present.

5. *The information on other laboratory species tested to date supports the conclusion that the protein-induced renal tubule toxicity occurs only in the male rat. The Technical Panel expressed a low level of concern for the possibility that functionally analogous human proteins exist.*

Male and female rats and mice have been studied extensively, as have been the genetically defective NBR rats. In addition, dogs, monkeys, hamsters, and guinea pigs have been studied less extensively. Alpha-2u-globulin nephropathy, and its consequences for tumor induction, have been found only in normal male rats, which synthesize large amounts of alpha-2u-globulin in the liver. Furthermore, alpha-2u-globulin has been shown to bind CIGA compounds with a resultant prolongation of renal half-life of the protein. Because of these observations, extensive studies of the urinary proteins of other species

(including man), have been undertaken to determine the affinities of these proteins to bind CIGA compounds and other chemicals. No instances of the occurrence of major urinary proteins that bind CIGA compounds in either man or other species have been reported. Therefore, it appears highly likely that alpha-2u-globulin nephropathy is unique to the male rat. No comparable nephropathy in man has been described.

The EHC unanimously approved this report following their March meeting. The Executive Committee (EC) approved the report at their July 23, 1991 meeting, although one Member of the EC encouraged greater attention to the uncertainties concerning hypothesis. In summary, the SAB concurs with the current position put forth by the Risk Assessment Forum in the draft report. We find the report itself to be well organized, well written, and fully substantiated in its statements, and wish to commend the authors for an excellent piece of work.

We look forward to receiving your response to our comments

Raymond C. Loehr

Dr. Raymond Loehr, Chairman
Science Advisory Board

Bernard Weiss

Dr. Bernard Weiss, Acting Chairman
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