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MEMORANDUM

SUBJECT: Review of Atrazine Hormonal Data

FROM: Ralph Cooper, Chief
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Reproductive Toxicology Branch
Developmental Toxicology Division (MD-72)

TO: Penelope Fenner-Crisp, Director
Health Effects Division
Office of Pesticide Programs (7509C)

Attached is my review of the Ciba-Geigy Atrazine documents provided to me by your office. In my responses, I have followed the outline presented in the memo of Dr. Thomas M. Crisp (ORD/OHEA). For the most part, my remarks are in agreement with those of Dr. Crisp. I have noted the few areas in which I may disagree, however these are minor points. In general, I found the studies to be of poor quality, primarily because of the way in which the vaginal smear data was obtained and presented. This fundamental flaw precludes any further valid interpretation of their results or reasonable discussion of the hypothesis presented by the Ciba-Geigy group.

I appreciate the opportunity to review this data and would be happy to provide any additional assistance on the Atrazine work should the need arise.

Attachments

cc: Robert Kavlock (MD-71)
Sally Darney (MD-72)
Mike Beringer (7509C)

1/5

Ciba Geigy Atrazine Studies

General Comments:

I have reviewed the studies examining the effects of Atrazine on the female rat's reproductive system and mammary gland tumor development. In general, I am in agreement with the review of Dr. Crisp in that I too have serious concerns about the quality of the data. The most serious of these concerns is the way the vaginal smears were examined and summarized. Since appropriate interpretation of the vaginal cytology data is critical to interpretation of the endocrine data and key to the hypothesis that this compound alters ovarian cyclicity, the conclusions about reproductive aging, tumor development or strain comparisons are without merit.

General Issues:

1. Hypothesis that Atrazine promotes precocious appearance of mammary tumors.

Although the hypothesis that Atrazine promotes an early appearance of mammary gland tumors because it induces an early reproductive senescence (i.e., constant estrus) may be valid, the quality of the vaginal smear data prevents any rigid examination of the study. It is a well-known phenomenon that female rats are prone to develop mammary gland tumors as they grow older as a result of the age-dependent disruption of ovarian cycles. This phenomenon has been well characterized in several strains of rats. However, whether or not Atrazine modified this age-dependent change in reproductive status is not supported in the present studies.

The practice of obtaining vaginal smears and allowing them to air dry before examining and classifying the ovarian status is unwise. Air-dried smears are very difficult to "read" and this approach is likely the reason for the rather bizarre patterns presented for the individual animals. For example, the day-to-day vaginal cycle data for some animals presents patterns that are just not possible in the female rat. A persistent proestrus vaginal smear has never been identified in the peer-reviewed literature. This is just one example of how the data presented in these studies would not be supported by the literature nor the normal physiology of the female rat.

I do not necessarily agree with Dr. Crisp that additional studies examining another strain of rats, or inducing "constant estrus" with estradiol or depleting oocytes in the Sprague Dawley female would strengthen the investigators hypothesis. Similar studies already exist and the results are clear. Such manipulations do alter the onset and occurrence of mammary gland tumors. I would argue that the investigator's hypothesis would be best served if they simply conducted the study properly. That is obtain an accurate characterization of the vaginal cytology, examine the endocrine status of these animals under well-defined ovarian conditions and then correlate these data with the onset of tumors.

2. Mechanism of action for Atrazine is through a threshold.

The quality of the data I have reviewed does not provide any basis for a discussion of mechanism.

3. Differences in mammary tumor response to Atrazine by Sprague-Dawley and Fischer 344 rats is attributed to differences in reproductive endocrinology between strains.

The differences in mammary gland tumor development in Sprague-Dawley vs. Fischer-344 are likely due to the differences in the age-related changes of the female's reproductive endocrinology. In fact, such tumors are considered endocrinopathies by many investigators involved in basic research of reproductive aging. Whether or not Atrazine modifies this pattern in either strain was not determined in the studies presented.

Specific Questions:

1. Were hormone assays conducted properly? Were the kits appropriate?

In general, I am in agreement with Dr. Crisp's comments concerning the prolactin assays. However, I am familiar with the steroid hormone assay kits. These do not require extraction and are in general quite accurate, although each lab should present the QC data. Whether or not atrazine (or DACT) interferes with these assays should have been considered.

More importantly, it must be kept in mind that no matter how well the hormone assays were conducted, the data in these studies is compromised because one has no idea of the ovarian status of the females when they were killed. Furthermore, prolactin is very stress sensitive and unless the female is removed from her home cage and decapitated immediately, there will be substantial stress induced increases in prolactin. Time of day variations are also considerable for prolactin with a baseline of around 5 ng/ml and peaks greater than 200 ng/ml. Baseline stressed females (i.e., killed with CO) generally average around 20 ng/ml a value curiously similar to those presented in the data.

2. Were blood sample chose at the appropriate times?

From a careful review of the data, I was unable to confidently determine the time of day or the vaginal status of the animals at the time of killing. Thus the answer to this question is an emphatic no.

Are the statistical data acceptable?

Based on the quality of the data, any statistical questions would be moot at this point.

What is the confidence that the statistically-significant increase in serum estradiol (E2) at the 400 ppm dose relative to the control at the 3 month period is real?

Without reliable data on the ovarian status of the animals (i.e., vaginal smears), this question can not be answered.

Was the estrous cycling staging appropriately conducted

No. In general I would agree with the comments of Dr. Crisp. However, contrary to current laboratory folklore, it is not that easy to induce a pseudopregnancy when obtaining a vaginal smear in the rat.

Are the group means listed in Table 6 reliable?

Again, this is a moot question because there is no reliable estimate for the stage of the estrous cycle at which these samples were collected.

Was premature senescence in the Sprague-Dawley adequately demonstrated

No.

What is the nature of the contradiction between the Ciba-Geigy study data and the data presented in the Loeb and Quimby reference?

If discrepancy exists and the Loeb and Quimby data are correct, what is the impact of the error on the integrity of the Ciba-Geigy study?

These discrepancies are apparently due to typographical errors.

Are effects of treatment over time supportable?
Do the hormonal data support the authors conclusions (presented in Tables 7-11) of the summary document?

The quality of the data do not provide sufficient grounds to support (or deny) this hypothesis.

Do the data provided support the differences in the endocrine/estrus cycle effects between the two rat strains?

No, there are several better studies in the literature documenting the age-dependent disruption of ovarian cycling in a variety of strains of rats. The data provided in these documents is based on such muddled vaginal smear results that even this very basic question can not be answered.

What is the overall quality of the hormone studies with respect to

a.	**Experimental design?**	Reasonable
b.	**Implementation?**	Poor
c.	**Working hypothesis?**	Reasonable

Do the findings in the two rat studies have applicability to the human female?

I am in concurrence with Dr. Crisp's comments.