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FIFRA Scientific Advisory Panel Meeting
June 27- 29, 2000
Held at the Sheraton Crystal City Hotel, Arlington, Virginia

Atrazine :
Hazard and Dose-Response Assessment and Characterization

Mr. Larry Dorsey
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date:_____

Christopher Portier, Ph.D.
FIFRA SAP Session Chair
FIFRA/Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
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Atrazine: Dose -Response Assessment and Characterization

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding issues pertaining to Atrazine's Hazard and Dose-Response Assessment and Characterization. Advance notice of the meeting was published in the *Federal Register* on June 7, 2000. The review was conducted in an open Panel meeting held in Arlington, Virginia, on June 27, 28, and 29, 2000. The meeting was chaired by Christopher Portier, Ph.D. Mr. Larry Dorsey served as the Designated Federal Official.

More than 10 years ago atrazine was found to induce mammary tumors in female Sprague-Dawley (SD) rats and was classified by the EPA as a possible human carcinogen. In 1998, the FIFRA Scientific Advisory Panel agreed with the EPA's classification of atrazine and raised the issue that atrazine exposure may result in an endocrine imbalance. EPA, therefore, encouraged the registrant of atrazine to pursue studies on a potential endocrine mechanism. Since that time the registrant has completed numerous studies concerning atrazine's mode of carcinogenic action to explain the mammary gland and pituitary tumor responses in female Sprague-Dawley rats. EPA's National Health and Environmental Effects Laboratory has also generated information on atrazine's neuroendocrine site of action, as well as its effects on reproductive development. After evaluating this information, EPA's Office of Pesticide Programs (OPP) submitted to the SAP a preliminary assessment of atrazine's postulated mode of action and relevance to human hazard potential. The draft document presents an integrative approach that uses a common neuroendocrine mode of action to evaluate potential for both cancer and noncancer health effects.

CHARGE

The specific issues to be addressed by the Panel are keyed to the background document, "EPA's Preliminary Draft Document on Atrazine: Hazard and Dose-Response Assessment and Characterization," dated May 2000, and are presented as follows.

Issue 1. Rodent Tumor Findings

The focus of atrazine induced tumor responses has been on mammary gland neoplasia in female Sprague Dawley rats. The December 1999 draft assessment document concludes, as in previous assessments, that treatment of female Sprague Dawley (SD) rats with atrazine, but not male SD rats or Fischer 344 rats or CD-1 mice of either sex, results in neoplastic responses expressed as an increased incidence and/or an early onset of mammary gland tumors. This preliminary hazard assessment further concludes that the mammary gland adenomas/carcinomas and the fibroadenomas should not be combined because they are of a different cell origin. The current assessment also points out that there is an early onset of pituitary adenomas. These preliminary conclusions regarding the rodent tumor findings form the basis of the mode of action analysis.

1.1 Please comment on the EPA preliminary findings that atrazine treatment induces an increased incidence and early onset of (a) mammary gland adenomas/carcinomas and (b) fibroadenomas. Please comment on the importance and significance of the data showing histomorphologic changes in mammary tissues (*e.g.*, increased incidences or increased severity of alveolar development, acinar development, dilated ducts, increased secretory activity, and galactoceles) and their relevance to data showing an early onset and increased incidence of mammary fibroadenomas and pituitary adenomas in female SD rats following atrazine exposure. Also, are these histologic effects valid indicators of increased or prolonged exposure to estrogen and prolactin.

1.2 Please comment on the overall EPA preliminary conclusion that atrazine treatment does not lead to tumor formation in male SD rats, and F344 rats and CD-1 mice of either sex. Please comment specifically on OPP's evaluation of the study (Pinter *et al.*, 1990), which reported neoplastic findings in the male F344 rat strain.

Issue 2. Mode of Action Analysis

The draft OPP cancer assessment on atrazine includes evaluation of toxicological and mechanistic information to explain the rodent tumor responses and to identify what is believed to be the important elements of the carcinogenic process.

2.1 Sufficiency of Evidence: Does the draft assessment adequately describe the data used to identify the key events in atrazine's mode of carcinogenic action? Are the available data sufficient to describe these events? Have the uncertainties and limitations of these data been adequately and clearly characterized? Which event(s) is viewed as critical to the carcinogenic process? Are the preliminary conclusions as to atrazine's mode of action supported by the analyses presented in the draft EPA document and consistent with the mode of action framework analysis described in EPA's July 1999 Draft *Guidelines for Carcinogen Risk Assessment?*

2.2 Have alternative modes of carcinogenic action been sufficiently discussed and ruled out?

Issue 3. Dose Response

The attenuation of the LH surge is viewed by EPA as the necessary step that eventually leads to neoplasia in rodents. EPA concluded that this critical event is consistent with a nonlinear phenomenon, and thus dose-response assessment should proceed by a margin of exposure analysis.

3.1 Because it is the attenuation of the preovulatory LH surge that results in disruption of the estrous cycle and is a necessary step in the mode of carcinogenic action, the Draft EPA document proposes that the cancer dose-response assessment should proceed by nonlinear dose-response extrapolation. The document further proposes that the NOAEL for the attenuation of the LH surge be used as the point of departure for a margin of exposure analysis. We would like the

Panel to comment on this proposed dose-response approach and the consistency of other effects going along with the LH suppression effect.

3.2 Is there a common endpoint in the mode of action that could be used as a point of departure that would be protective of both cancer and reproductive developmental effects? The EPA draft document proposes the LH data as such.

Issue 4. Human Relevance

It is EPA science policy that animal tumor responses are presumed to be indicative of human cancer potential unless there is substantive information to the contrary. When there is information on an agent's mode of action in laboratory animals, it is important to address whether or not it would be anticipated to be operative in humans. For atrazine, two questions need answering to determine the relevance of the animal model to humans. One deals with the possibility that atrazine produces neuroendocrine disruption in humans as it does in rats; the second is whether potential adverse human consequences including carcinogenicity may ensue if these neuroendocrine effects develop from atrazine exposure.

4.1 The EPA draft document concludes that there is suggestive evidence of a possible association of triazine exposure and cancer occurrence for three hormone-responsive cancers in humans - ovary, breast and prostate cancer. However, these associations should not be considered as conclusive evidence of an association of triazine exposure with these tumor types, and triazine exposure should not be interpreted as a causal factor in these tumor types. Please comment on EPA's evaluation of the epidemiologic studies and how much weight it should be given in the hazard and mode of action assessment.

4.2 Does the document provide a thorough and adequate discussion of the similarities and differences in Sprague-Dawley (and Long Evans) rats compared to humans with respect to this hypothalamic-pituitary-ovarian axis perturbation? Given the steps outlined in Figure 2-1 contained in Part A, Chapter 2, what events are conserved and show homology between rats and humans, and when does the process diverge? Currently the document indicates that CNS-GnRH control of pituitary function is conserved and similar.

4.3 Given the neuroendocrine associated effects (*i.e.*, decreased secretion of hypothalamic catecholamine levels and gonadotropin releasing hormone, attenuation of the LH pituitary surge, prolonged estrogen and prolactin exposure) found with atrazine treatment in rats, please comment on the conclusions drawn in the Draft EPA document regarding relevance and the possible ramifications in humans. What are commonalities between humans and rats in the endocrine effects found that would raise a concern for human health consequences including carcinogenicity? The document considers human conditions of anovulation as a means to judge the potential human health risks. Please comment on the appropriateness of these models in evaluating atrazine.

4.4 Given the toxicological and mechanistic information available on atrazine, OPP has proposed that atrazine be classified as a *likely human carcinogen* (see EPA's July 1999 draft revisions to the guidelines for carcinogen risk assessment). Please comment on this proposal.

Issue 5. Children's Health Concern

Risks to infants and children from environmental exposure of chemicals may differ qualitatively or quantitatively from those of adults due to biological, physiological, and metabolic differences. As stated in the July 1999 Draft revisions to the EPA's cancer risk assessment guidelines, when information is developed to show a mode of carcinogenic action that is expected to be relevant to adults, an evaluation also needs to be made as to whether this mode of action is relevant to children. Because of the absence of direct relevant information on atrazine, the draft document develops a "cogent biological rationale" on whether the postulated mode of carcinogenic action is applicable to children by considering what is understood about the observed endocrine effects in children versus adults and by considering animal data on developmental and reproductive effects. The document also emphasizes the concern for developmental effects.

5.1 Please comment on the applicability of the neuroendocrine mode of carcinogenic action in adult rats to the fetus, infants, and peripubertal children. Also, please comment on the adequacy of the EPA document in addressing children's cancer concern.

5.2 Does the Panel agree that the reproductive developmental findings in rats (e.g., delayed puberty, prostatitis in young animals) are a result of atrazine primary action on the hypothalamic-pituitary-gonadal axis. And if so, would the Panel also provide their view of the commonality between the cancer mode of action and the underlying basis leading to adverse reproductive/developmental outcomes and how well the EPA document addresses this commonality.

5.3 Do the rodent studies showing delayed puberty in both female and male rats raise a concern for children from a clinical perspective? What does it mean to have a delay in puberty in females and males caused by a compound that does not bind to an estrogen receptor?

5.4. Given that atrazine treatment of dams during lactation results in a decrease in prolactin, which may lead to altered TIDA neuron development and eventually prostatitis in young animals, what kind of concern does this finding mean to humans, especially as an early life stage susceptibility?

SUMMARY OF PANEL RECOMMENDATIONS

Issue 1. Rodent Tumor Findings

The members of the Panel were in agreement that high doses of atrazine cause an increased incidence and earlier appearance of spontaneously occurring mammary adenomas and carcinomas in the female Sprague-Dawley (SD) rat. Although the results obtained suggest that atrazine also causes fibroadenomas in the females of this strain of rats, the conclusions are not supported by statistically significant data, as shown in Table 1-8 (page 19) of the December 15, 1999 Draft.

Atrazine is a selective endocrine modulator, because it accelerates a normal process during the aging of Sprague-Dawley rats. The fact that it does not induce tumors in males of the same species or in mice indicates that it needs a basic substrate that is intrinsic to the host.

There was a consensus of the Panel that the mode of action for atrazine was supported by the available data; however, additional information on the levels of circulating estrogen and prolactin levels or information on potential non-receptor mediated effects would be useful.

The available studies do not show any evidence that atrazine causes tumors in either F344 rats or CD-1 mice of either sex, or male SD rats (See IARC analysis-Thakur). The only finding of significance in F344 rats (Pinter et al., 1990) was the increase in mammary tumors in males at 750 ppm (37.5 mg/kg); 17% versus 2% in the controls. However, the interpretation of this study is complicated by the design of the study (lifetime versus standard 24 months), lack of adequate histopathology and increased survival in the high dose compared to the controls. The Agency's preliminary conclusion that the mammary tumors in male rats are not treatment-related seems justified. There was general agreement among the Panel that the tumors were related to increased survival of the treated F344 rats and not a treatment related effect of atrazine.

Pinter et al. (1990) found that mammary tumors were increased in male F344 rats treated with atrazine (Table 5-10 page 31, Part B). A close look at the study revealed that the animals in the high dose group in which tumors were reported to increase lived longer than the controls by several months. Since this strain shows a significant increase in the incidence of mammary tumors with age, the Panel concluded that the observed increase in tumors is due to the increased age of the high dose group and not due to atrazine. The conclusions drawn in the Pinter publication are not supported by the data presented. Major flaws were found with the study design and data analysis. Since there are no data on number of tumors in atrazine treated animals at 113 weeks, it cannot be assessed whether the excess of tumors observed in experimental animals was due to tumor development occurring during the additional 10 and 13 weeks lived by these animals. A proper age-adjusted analysis of these tumor data agrees with the EPA conclusion that those tumors appear to be due to aging.

It was postulated that failure to observe mammary tumors in female F-344 rats may be due to reduced body weight at the higher doses. In the serial sacrifice study conducted by Thakur

(1991b) on female F344 rats, it is indicated on page 27, Part B, that there was a significant decrease in body weight due to atrazine. This may have resulted in a decrease of mammary tumors. Proper statistical tests accounting for differences in body weights, as provided by Gaylor and Kodell (1999), need to be employed. Rather than relying on historical data to adjust for body weight effects, Gaylor and Kodell (1999) stratified the animals by body weight and tested for dose effects within body weight strata using more homogeneous animals.

Issue 2. Mode of Action Analysis

Overall, the Agency's draft assessment document does an excellent job assembling and describing the data used for the proposed mode of action for atrazine induced mammary tumors in female Sprague Dawley rats. A non-genotoxic, epigenetic mode of action has been proposed for the increased incidence of mammary tumors observed in atrazine treated female Sprague Dawley rats under the conditions of the carcinogenicity studies.

The draft document provides an adequate summary of the postulated mode of action for atrazine induced mammary gland tumors. The release of gonadotropin releasing hormone (GnRH) from the hypothalamus is reduced resulting in an attenuation of the afternoon pituitary LH surge. This lengthens the estrus cycle resulting in increased estrogen levels which is the proximate cause or key event leading to an increased incidence of mammary tumors in Sprague Dawley female rats.

There is evidence that estradiol and prolactin are increased in atrazine treated Sprague Dawley female rats and this has been identified as the critical event.

The Panel identified the following uncertainties and limitations concerning the proposed mode of action of atrazine:
1) the effects on the hypothalamus are not clearly defined, 2) stop studies were not performed, 3) the lack of robust data on hormones, and 4) more comparative data between the Sprague Dawley and Fisher 344 rats would be useful, since the strain and species differences in the mammary tumor response are important.

The data strongly support the hypothesis that prolonged exposure to estrogen produced by the ovary is requisite for development of mammary tumors observed in these studies and discussed in this report. Alternative modes of action were considered including: genotoxicity (nitroso-atrazine), altered levels of hydroxylases, aromatase, or estrogen receptors. Changes in these enzymes or receptors could alter mammary gland responses. Although the data are limited, this does not detract from the Panel's conclusion that the neuroendocrine actions of atrazine are the primary and requisite mode of action for the induction of mammary tumors.

Alternative modes of action have been thoroughly discussed and ruled out. The increased level of hormones and the increased level of hormones alone, can account for the increased incidence of mammary tumors in Sprague Dawley female rats. The proposed mode of action is plausible and

each step in the pathway has been shown to be affected in atrazine treated rats. None of the effects are based on speculation.

The Panel made the following recommendations: 1) additional information on the effects of estrogen in the induction of mammary tumors in rats should be included, 2) additional information of the metabolism and pharmacokinetics of atrazine should be included in the document, and 3) similarities and differences in metabolism between the strains of rats tested (Sprague Dawley vs. Fisher 344) and humans should be indicated.

One Panel Member suggested the anorexic effects of atrazine as an alternative mechanism to atrazine effects: *Atrazine* ➜ *Brain (Appetite Systems)* ➜ *Appetite Suppression* ➜ *Weight Loss* ➜ *Reduced Adiposity* ➜ *Reduced Leptin (or other signal)* ➜ *Reduced LH* ➜ *Reduced Ovulation* ➜ *Elevated Estrogens (in rodents only)*. This Panel Member indicated that this view does not preclude a role for hypothalamic norepinephrine or other transmitters since the mechanism through which atrazine reduces appetite and food intake may still be somehow mediated by the hypothalamus. In this view, the originally proposed reduced LH-elevated estrogen mode of atrazine-induced mammary tumors in SD rats is still valid. The major difference here is in the sequence of events that triggers the initial LH reduction. Instead of a direct toxic hypothalamic-pituitary action of atrazine, it is suggested that atrazine acts to reduce food intake and weight as a potentially benign appetite suppressant, resulting in the reduced LH surge.

Issue 3. Dose Response

Most Panel Members felt that non-linear extrapolation below the point of departure was appropriate. Arguments in support of non-linear extrapolation include 1) The peak height of the LH surge can be diminished to some extent (at least in young animals) without affecting estrous cyclicity and 2) endocrine homeostasis is maintained by numerous feedback loops that can compensate, up to a point, for environmental effects.

One Panel Member stated concern with the non-linear extrapolation, making the point that the data are not analyzed in such a way that linear or non-linear behaviors are demonstrated in a statistically robust manner.

Deficiencies in the data set available for dose-response evaluation were noted. Development of a physiologically based pharmacokinetic (PBPK) model that would facilitate estimation of brain levels of atrazine and its metabolites in rodents and humans is needed. A PBPK model would be useful for identification of nonlinearities in the dose-response curve and for extrapolation between rodents and humans. Specification of the relative activity of the parent compound and its metabolites in causing adverse effects is needed. Finally, the depletion of the LH surge in the Sprague-Dawley rat is treated qualitatively. A quantitative treatment of this endpoint is needed.

Most Panel Members felt that the LH surge was the appropriate point of departure for the

evaluation of the carcinogenic effect of atrazine in the female Sprague-Dawley rats. The Panel recommended that the point of departure for a margin of exposure approach should be based on a LED_{10}, as proposed in the US EPA draft carcinogen risk assessment guidelines, and not on a NOAEL as proposed in the EPA background document. The Panel recommended that an LED_{10} of approximately 2 mg/kg/day (obtained for LH effects in female SD rats (Seilken, 2000)) be used as the point of departure for the health risk assessment using the margin of exposure approach. Since effects may be produced at this dose, possibly with a 10% incidence, this dose cannot be considered a NOAEL.

Most Panel Members agreed that the LH surge was an appropriate point of departure for both cancer and reproductive endpoints, but several Panel Members suggested that the LH surge might not be an appropriate point of departure for developmental effects of atrazine.

Some Panel Members suggested that there is a lack of data that would support identification of an alternative point of departure specific to reproductive endpoints. Data needs include identification of metabolites in urine and hormonal assays in children and adults.

Issue 4. Human Relevance

The Panel agreed that the epidemiologic data did not provide conclusive evidence of an association between atrazine exposure and cancer but it did provide some evidence. Several Panel Members commented that inadequate attention was given in the report to the studies that are available and that the epidemiological data should be discussed as extensively as the animal data. The report should include an evaluation of epidemiological data on a cancer by cancer basis with a review of the major investigations and an overall summary.

The Panel was concerned that several reports on non-Hodgkin's lymphoma (NHL) were discounted even though they suggested that atrazine could produce adverse health effects in humans unrelated to the postulated mode of action in Sprague Dawley rats.

There was some disagreement about the importance of the breast cancer study in Kentucky (Kettles et al**.,** 1997). It was suggested that the document should indicate the lack of useful studies on breast cancer rather than saying it does not provide conclusive evidence. Another Panel member noted that although the study had serious limitations it was "hypotheses generating" and should be followed up.

Several Panel Members agreed that the reported ovarian cancer study was more relevant than presented in the draft Agency report and at least raises the question as to whether a chemical acting on the pituitary-gonadal axis might be relevant to ovarian cancer in humans. Because of the lack of a specific focus on atrazine and small numbers from a single study, the appropriate interpretation of this study is that it provides a suspicion of human carcinogenicity, but not conclusive evidence.

The issue of atrazine and prostate cancer was briefly discussed but limited data were available for review. It was strongly recommended that the EPA should review the data when it becomes available.

The epidemiologic evidence on atrazine and cancer is insufficient to rule in or out an association with childhood cancer - this has not been studied. However, it should be considered whether hormonal effects in childhood or adolescence may have an impact on cancer occurrence in later years.

The summary paragraph on page 27 of the Agency's background document should be reworded as follows: *To summarize, there are a few epidemiologic studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies showing an association and internal inconsistencies in the studies available indicates the human studies by themselves do not make a strong case for an association.*

The Panel noted that there are certain similarities in the control of the hypothalamic-pituitary-ovarian axis between humans and rats but that there are important differences in the details of the control mechanism. Furthermore, there are a number of areas where sufficient data does not exist to compare the two species.

It was strongly emphasized that the effects of age on reproductive and endocrine function are much different between rats and humans. These differences have important implications in regard to the human relevance of the proposed mechanism of action for atrazine in inducing mammary tumors in the female Sprague Dawley rat. In addition to species differences, there also exist significant strain differences in the aging process. Thus, the mechanism of atrazine action in the Sprague Dawley rat is unique to that strain in that a similar process is not operable in Long-Evans or F-344 rats.

The Panel noted that there are no human data suggesting that atrazine interferes with hypothalamic-pituitary-ovarian function as demonstrated in the SD rat.

The conclusion that there could be a potential for human cancer from elevated estrogen levels if atrazine had effects on conserved hypothalamic mechanisms in humans similar to those in SD rats is at odds with the arguments and other conclusions of the Agency's Report itself. Even if atrazine did reduce LH and induce hypothalamic ammenorhea (HA), this condition is associated with reduced estrogen levels and therefore would not promote the development of mammary tumors. Further, if this effect occurred in humans it could not result in polycystic ovarian syndrome (PCOS), since this condition is associated with elevated LH.

There is compelling evidence to support the conclusion that the mode of action of atrazine is not relevant to humans on the basis of both the species-specific nature of atrazine's effect on mammary gland tumors and on the basis that the effects observed at high doses do not occur at

some lower dose.

The Panel concluded that it is unlikely that the mechanism by which atrazine induces mammary tumors in female SD rats could be operational in man. Nevertheless, it is not unreasonable to expect that atrazine might cause adverse effects on hypothalamic-pituitary function in man if exposures were high enough.

Specifically, the effect of atrazine on the hypothalamus, suppression of circulating LH levels, and induction of persistent estrous in Sprague Dawley rats which ultimately develop mammary tumors was, in the opinion of the Panel Members to be of low relevance to human health due to differences in reproductive physiology and the species specific nature of the mode of action of atrazine. Parallels with human conditions such as hypothalamic amenorrhea and polycystic ovarian syndrome are found to have little in common with the toxic phenomena induced by atrazine exposure in rodents. Hence, it is the conclusion of the Panel that the data have little relevance to human health.

The conclusion of the Panel was unanimous that atrazine should not be classified as a “likely human carcinogen”: 1) a positive response in only one species, strain and sex (female SD rats) with a negative response in male SD rats, F344 male and female rats and CD-1 male and female mice, 2) the mode of action for the mammary tumor response in SD rats is considered not relevant to humans, and 3) exposure levels and dose limited response makes any effect in humans unlikely.

Despite the unanimous conclusion that atrazine should not be classified as a “likely human carcinogen,” there were different recommendations for the classification of atrazine.

Based on the criteria included in the information presented, the Panel was of the opinion that it would be more appropriate to classify atrazine as either "unlikely to be a human carcinogen" or "not enough information to classify."

One Panel member suggested that atrazine be classified as a "possible human carcinogen" given the positive evidence from the occupational epidemiologic studies. However, when taken together, the epidemiological evidence is inconclusive and the evidence from occupational studies could be outweighed by the fact that environmental exposures to atrazine would be lower than the exposures occurring in an occupational setting.

The Panel concluded that there is no established or even likely neuroendocrine path through which atrazine could induce cancer in humans by a mode similar to that occurring in female SD rats or by alternative modes of action such as mutagenicity or direct estrogenic activity.

Issue 5. Children's Health Concern

The EPA document does not address adequately the risks of atrazine to children, primarily because the data are not available. The applicability of the neuroendocrine model of carcinogenetic action may be relevant to infant and childhood exposure, but the effects may have a long latency and may not become apparent until puberty or even later. There may be toxic mechanisms for children other than carcinogenesis.

The discussion of atrazine and its mode of action and other toxicities was informative but these issues should have been discussed in greater depth. The following are among the issues that should be addressed: 1) the pharmacokinetics and pharmacodynamics of atrazine in the adult animal and throughout development; 2) detailed discussion of metabolism including organ, age, and gender specific details; 3) end organ effect/toxicities including which metabolites may be more or less toxic than the parent chemical; 4) the effect of age on organ susceptibility or unique disease states such as functional birth defects, pregnancy losses etc; 5) in regards to mode of action, the effect of age and animal vs. human must be detailed.

It appears that a common hypothalamic-pituitary mode underlies the actions of atrazine on mammary tumors and developmental toxicity in rats, namely, reduced LH secretion, possibly induced by decreased food consumption.

The Panel agreed that the reproductive developmental findings in rats are a result of exposure to atrazine at relatively high doses. Data presented support the statement that the hypothalamic-pituitary-gonadal axis is affected in the SD rat. A potential exists for reproductive and developmental disruption to exist as a consequence of HPG disturbance. There are no apparent cancer consequences.

The same endocrine perturbations that induce tumors also appear to play a role in at least some reproductive developmental effects. However, as with the cancer mode of action, the experiments and draft do not adequately address the possibility that atrazine may alter peripheral target metabolism and sensitivities to steroids.

The basic hypothalamic disturbance reported was attenuation of the preovulatory spike in LH. This was presumably due to decreased pulses of GnRH leading to attenuation of the preovulatory spike of LH. This was accompanied by a corresponding decrease in hypothalamic catecholamines, but in the opinion of some neurophysiologists, hypothalamic norepinephrine levels reflect GnRH secretion rather than are causative thereof.

The neuroendocrine mechanisms regulating gonadotropin release and thus puberty are clearly influenced by the environment. Thus, alterations in neuroendocrine function by atrazine could have influence on onset of puberty. In the cases involving social stress in humans the puberty is accelerated whereas apparently, from the rat studies, puberty is delayed as a result of atrazine consumption.

The proposed mode of action of atrazine suggests that sustained exposure to very high levels of this substance might be responsible for delaying the onset of puberty in either sex. Epidemiologists would be hard pressed to detect such an occurrence in young males because no clear markers of puberty exist in the male. In females the onset of menses is a clear event signaling puberty.

The long-term effects of decreased LH secretion in childhood are difficult to assess. Experience with both young males and females with hypogonadotropic hypogonadism suggests that such individuals function well when given appropriate sex steroids and do not suffer from behavioral or cognitive problems or from other unusual health problems.

The finding that inhibition of prolactin transmission by atrazine exposure in milk early in life leads to increased incidence of prostatitis in young adult rats is clear from the Agency report. Previous studies have shown the importance of early post-natal availability of prolactin from milk, affect the TIDA neurons in the hypothalamus to reduce dopamine (DA) production. DA is a known neuromodulator of several functions including pituitary release of hormones and behavior.

DETAILED RESPONSES TO THE CHARGE

Issue 1. Rodent Tumor Findings

1.1 ***Please comment on the EPA preliminary findings that atrazine treatment induces an increased incidence and early onset of (a) mammary gland adenomas/carcinomas and (b) fibroadenomas. Please comment on the importance and significance of the data showing histomorphologic changes in mammary tissues (e.g., increased incidences or increased severity of alveolar development, acinar development, dilated ducts, increased secretory activity, and galactoceles) and their relevance to data showing an early onset and increased incidence of mammary fibroadenomas and pituitary adenomas in female SD rats following atrazine exposure. Also, are these histologic effects valid indicators of increased or prolonged exposure to estrogen and prolactin***.

The members of the Panel were in agreement that high doses of atrazine cause an increased incidence and earlier appearance of spontaneously occurring mammary adenomas and carcinomas in the female Sprague-Dawley (SD) rat. Although the results obtained suggest that atrazine also causes fibroadenomas in the females of this strain of rats, the conclusions are not supported by statistically significant data, as shown in Table 1-8 (page 19) of the December 15, 1999 Draft. The increased tumor incidence occurred only at the highest dose, thus raising the issue of the NOAEL for these tumors. At 12 months there is a clear evidence of carcinogenic activity, as indicated by the increased incidence of both benign and malignant tumors, but only at the highest dose (50 mg/kg). At 24 months the increase in fibroadenomas is apparent at 25 mg/kg and equivocal at 3.5 and 0.5 mg/kg. The lack of a dose increase between 0.5 and 3.5 mg/kg argues

against this being a biologically relevant effect. However, there is a definite increase in malignant tumors at 3.5 mg/kg. Therefore, the NOAEL in this study is 0.5 mg/kg. This response has been found in several studies by different investigators and with similar triazines. However, only the SD strain of rat showed significant tumor (adenoma/carcinoma) development in response to atrazine. This strain of rats is prone to the spontaneous development of a high incidence (>50%) of mammary tumors as a consequence of aging. The SD rat is quite unique in the mode of aging, since it does not suffer ovarian failure and atrophy, as it occurs with menopause in women. In older rats the ovaries become cystic, resulting in hyperestrogenism, hyperprolactinemia, and pituitary adenomas, thus creating an endocrinologic milieu conducive to mammary tumor development. These changes are not observed in other strains of rats. Three studies in Fischer 344 rats were all negative for the development of mammary tumors.

The earliest study (Mayhew et al., 1986) shows a dose related increase in both benign (fibroadenomas) and malignant (various types of mammary carcinomas). The four Thakur studies are particularly important for evaluating the carcinogenic potential of atrazine because of their robustness, especially concerning the inclusion of interim sacrifice points. The results of the study suggest that there was a mild decrease in the latency time for the induction of pituitary adenomas at 26.63 but not at 4.23 mg/kg. Although there was no treatment effect at the end of the study, this would be expected since a high percentage of this strain of rat have such tumors, and they are often the cause of death. While there was no increase in the number of benign or malignant tumors in this study, there appeared to be a decreased latency period at 26.63 mg/kg.

The Morseth (1998) study is important in answering the question of hormonal influence in the development of mammary tumors in response to atrazine. These authors used ovariectomized (OVX) rats as well as gonadally intact ones. There was a dose-related increase in the incidence of benign and malignant mammary tumors in intact females. While the increase in fibroadenomas was statistically significant at all doses, the incidence in the controls, 26%, was abnormally low for SD rats, in which most studies report approximately 50% or higher. In addition, there was no apparent dose-response. The incidence of carcinomas was increased in the high-dose (24.4 mg/kg) only. The most important finding in this study was that not a single mammary tumor occurred in any of the OVX rats, suggesting that there is a direct hormonal etiology to the induction of mammary tumors in this strain of rats. The Patterson and Turnier (1995) study also employed the use of interim sacrifices but only went to 12 months. The results of the study suggest that there is a reduced latency for mammary carcinomas but not adenomas or fibroadenomas. These effects were only observed at 23.9 mg/kg. The results in SD rats are supported by studies of simazine and propazine in female SD rats, which show similar changes in the mammary gland. There was consensus that the data are solid for implicating atrazine in both types of mammary tumor development in SD female rat. It appears that the Long-Evans (LE) rat may also be susceptible to atrazine induced mammary tumors since atrazine blocks the LH surge similar to that of the SD rat. It was postulated that the discovery that atrazine induces mammary tumors in a specific strain of rats makes it likely to occur in some other rodent species. The reasons for the susceptibility to atrazine appear to be on solid ground but there are some problems. For example, it is proposed that the continuously elevated levels of estradiol and

prolactin in circulation, and in combination with low serum concentrations of progesterone, account for the mammary tumors in the SD females treated with atrazine. In the draft document, however, estradiol is only slightly higher and progesterone is lower than appropriate controls, but there was no difference in prolactin levels. Several Panel Members questioned the validity of the levels of estradiol reported in the SD rats, which ranged from ~3 to 16 pg/ml at 3 months, as well as the levels reported at the other times (Table 15 Appendix). It was noted that most of the blood for the estradiol assays should have been collected on proestrus in cyclic animals; when the animals were not cyclic the samples should have been collected on another day. Proestrous estradiol values in serum are not 3-16 pg/ml in rats, as indicated by numerous studies, such as that of Butcher and others, that report serum proestrus estradiol levels in the 40-70 pg/ml range. A value of 3 pg/ml on proestrus in controls, as reported in Table 15 (Appendix), is unusually low. This may provide an explanation of some of the variations observed in table 15; possibly, a large number of the animals were not in proestrus. Since there is a large standard error an attempt to correlate vaginal cytology with the level of estradiol in all of those animals should have been made. Obviously elevated estradiol levels are critical to the central hypothesis, and no one to date has shown convincing evidence of increased serum estradiol in atrazine treated females. An unpublished study by Stoker et al (from Cooper group at EPA) in the attachment reveals a slight increase in serum estradiol at the highest dose of atrazine in male rats. How this observation correlates with mammary and pituitary tumors was not provided.

There was consensus among the Panel Members in the observation that the histological appearance of the mammary gland is indicative of a xenobiotic whose effects are mediated by the pituitary gland and its hormones. The presence of non-neoplastic changes in the mammary gland were noted in the Mayhew study. Although there was a slight increase in the incidence in all treatment groups compared to the controls at 24 months, they did not appear to be treatment-related. For this to be biologically important there should have been increasing incidence with dose. However, there was a treatment-related increase in the number of animals showing lactation and galactocele formation at 12 months at 50 mg/kg. Importantly, the dose response for these changes are similar to those for neoplastic change. The histomorphologic changes in the mammary tissues (e.g., increased incidences or increased severity of alveolar development, acinar development, dilated ducts, increased secretory activity, and galactoceles) are consistent with an effect of high doses of estrogen, progesterone, and prolactin on the mammary gland. Similar histomorphological changes are induced in the mammary gland by pregnancy, pseudopregnancy induced by vaginal stimulation, exogenously administered steroid, pituitary, or placental hormones, i.e., human chorionic gonadotropin (hCG), all conditions characterized by elevated circulating hormonal levels. Environmental conditions, such as lengthening of the light period, or constant light, that inhibit the melatonin peak and increase prolactin secretion, induce similar changes. If prolactin and estradiol are indeed not higher, and progesterone levels are lower in the serum of atrazine-treated SD rats, then possibly atrazine promotes the action of these hormones on the mammary gland. Several questions should be answered in this respect, i.e., whether prolactin, estradiol, and progesterone receptors in the mammary tissue are increased by atrazine. In addition, possibly atrazine enhances the action of prolactin and steroid hormones at the signal transduction level on the mammary cells. This possibility has not been considered and has not

been discussed in the document. The data provided do not allow a firm conclusion regarding prolactin, since prolactin levels are not increased by atrazine in SD females. The data on histology of the mammary gland under the influence of atrazine is critical and very well presented. Several tables clearly show prolactin-like and estrogenic effects in the draft document.

The development of mammary adenomas/fibroadenomas/carcinomas in response to endocrine disruption, even in the absence of a classical carcinogen, indicates that cancer is initiated by endogenous hormones. Estrogen is the most widely accepted mammary carcinogen. Classical models of estrogen-induced carcinogenesis are the rodent mammary tumor and the male hamster kidney tumor models, both of which require the presence of an intact pituitary and the development of prolactin-secreting pituitary tumors as a necessary precursor of target organ tumor development. Although these observations strongly suggest that prolactin plays a key role in estrogen-induced carcinogenesis, the role of this hormone in human breast cancer has not been well characterized. Estrogens act on the cell by binding to its specific receptors (ER) alpha and beta, which are present in the normal mammary gland as well as in greater than 50% of human breast carcinomas. They also act through metabolic activation to carcinogenic catechol estrogens. However, they do not induce mammary gland proliferation directly but induce the synthesis of growth factors that stimulate cells capable of proliferating. Progesterone, which induces differentiation in the endometrium and is a mitogen in the breast, increases breast cancer incidence in women receiving hormone replacement therapy. Since the data presented indicate that atrazine reduces circulating levels of progesterone, it would be necessary to clarify whether atrazine upregulates the progesterone receptor content in specific target cell populations. The effects of atrazine on the mammary tissues appear to be reproducible including early onset and increased number of animals bearing tumors. However, the higher tumor incidence in the hormonally-differentiated mammary gland indicates that the mechanism(s) of tumor development are different from those operating in chemically-induced mammary carcinogenesis, being in this sense more similar to the virally induced tumors in mice, which are pregnancy dependent. Mammary carcinomas induced in rats or mice with the polycyclic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA), or N-methyl-N-nitrosourea (NMU) are eliminated or markedly inhibited by the hormonal changes described above. The initiation of cancer requires that the carcinogen binds undifferentiated and highly proliferating structures present in the mammary gland of young virgin animals. Even though the cause of breast cancer in women is not known, environmental chemical carcinogens have been postulated to be implicated in its initiation. The rodent model mimics the conditions of cancer initiation in the human female population, in which a higher breast cancer risk is observed when the undifferentiated condition of the breast is prolonged, such as with early menarche, late menopause, and nulliparity. Exposure to radiation before age 19 induces mammary cancer, whereas radiation of the chest at older ages or after pregnancy fails to induce cancer, but it induces fibroadenomas and other benign conditions. Both human and rodent data indicate that the appearance of benign lesions, i.e., adenomas, fibroadenomas, or cystic conditions, i.e., galactocele, are indicative of an exposure of the breast tissues to genotoxic or non-genotoxic agents. The damage caused by these agents in differentiated mammary structures, however, does not necessarily lead to malignancy. Thus, these lesions might represent pathological entities with a different biological significance than that of

carcinomas.

Atrazine is a selective endocrine modulator, because it accelerates a normal process during the aging of Sprague-Dawley rats. The fact that it does not induce tumors in males of the same species or in mice indicates that it needs a basic substrate that is intrinsic to the host.

1.2 ***Please comment on the overall EPA preliminary conclusion that atrazine treatment does not lead to tumor formation in male SD rats, and F344 rats and CD-1 mice of either sex. Please comment specifically on OPP's evaluation of the study (Pinter et al., 1990), which reported neoplastic findings in the male F344 rat strain.***

The available studies do not show any evidence that atrazine causes tumors in either F344 rats or CD-1 mice of either sex, or in male SD rats (See IARC analysis-Thakur). The reasons for the inability of atrazine to induce tumor formation in male F344 rats and CD-1 mice are unknown. However, it is clear from several studies that tumors do not form in these rats and mice under very similar, if not identical, protocols as SD female rats. The only finding of significance in F344 rats (Pinter et al., 1990) was the increase in mammary tumors in males at 750 ppm (37.5 mg/kg); 17% versus 2% in the controls. However, the interpretation of this study is complicated by the design of the study (lifetime versus standard 24 months), lack of adequate histopathology, and increased survival in the high dose compared to the controls. The authors note that the tumor incidence is in line with the spontaneous rate of this tumor in other studies of F344 rats that are allowed to survive past 24 months (Solleveld et al., 1985). Life table analysis shows that this result is probably not treatment related. Supporting evidence is the lack of a response in female F344 rats. Therefore, the Agency's preliminary conclusion that the mammary tumors in male rats are not treatment-related seems justified. There was general agreement among the Panel that the tumors were related to increased survival of the treated F344 rats and not a treatment related effect of atrazine.

Pinter et al. (1990) found that mammary tumors were increased in male F344 rats treated with atrazine (Table 5-10 page 31, Part B). A close look at the study revealed that the animals in the high dose group in which tumors were reported to increase lived longer than the controls by several months. Since this strain shows a significant increase in the incidence of mammary tumors with age, the Panel concluded that the observed increase in tumors is due to the increased age of the high dose group and not due to atrazine. The conclusions drawn in the Pinter publication are not supported by the data presented. Major flaws were found with the study design and data analysis. Since there are no data on number of tumors in atrazine treated animals at 113 weeks, it cannot be assessed whether the excess of tumors observed in experimental animals was due to tumor development occurring during the additional 10 and 13 weeks lived by these animals. A proper age-adjusted analysis of these tumor data agrees with the EPA conclusion that those tumors appear to be due to aging.

It was postulated that failure to observe mammary tumors in female F-344 rats may be due to reduced body weight at the higher doses. In a large study of historical data, Seilkop (1995) shows a significant decrease in the incidence of mammary tumors in female F-344 rats with decreased body weights in controls. Unfortunately, attempts to increase the sensitivity of cancer tests in rodents by testing at the maximum tolerated dose, as evidenced by moderate lowering of body weight, may actually reduce the sensitivity for detecting some types of tumors. The study in F-344 female rats by Thakur (1992b), summarized in Table 5-9, page 29 Part B, indicated that there was a 6% decrease in body weight at 20.2 mg/Kg/d of atrazine. Based on Seilkop (1995), an expected decrease of 8% incidence of mammary tumors would result from the decreased body weight. If the tumor incidence in the high dose group is adjusted by this amount, a significant ($p<0.01$) dose-response trend is achieved. That is, tumorigenesis in female F-344 rats may be obscured by the decrease in body weight.

Rather than relying on historical data to adjust for body weight effects, Gaylor and Kodell (1999) stratified the animals by body weight and tested for dose effects within body weight strata using more homogeneous animals. It is recommended that the body weights of rats at 12 months be obtained for the Thakur (1992b) study. Then, body weight strata may be formed and tests for tumor incidence from atrazine exposure, adjusted for body weight, can be conducted by the procedure given by Gaylor and Kodell (1999).

It is indicated on page 26, Part B of the Agency's document, that there was increased mortality and decreased body weights at the higher doses in the study on CD-1 mice conducted by Hazelette and Green (1987). Both of these factors could have resulted in a failure to detect effects due to atrazine. A proper statistical analysis of these data needs to be conducted that adjusts for differences in mortality and body weight across dose groups.

In the serial sacrifice study conducted by Thakur (1991b) on female F344 rats, it is indicated on page 27, Part B of the Agency's document, that there was a significant decrease in body weight due to atrazine. Again, this may have resulted in a decrease of mammary tumors. Proper statistical tests accounting for differences in body weights, as provided by Gaylor and Kodell (1999), need to be employed.

Mammary tumors were increased in male F344 rats due to atrazine, as displayed in Table 5-10 (page 31, Part B) in a study conducted by Pinter et al. (1990). The conclusions drawn in this publication are not supported by the data presented. Major flaws were found with the study design and data analysis, i.e., control animals survived 113 weeks, whereas males treated with 375 and 750 ppm atrazine survived 123 and 126 weeks, respectively (Table 1, page 535). Since there are no data on number of tumors in atrazine treated animals at 113 weeks, it cannot be assessed whether the excess of tumors observed in experimental animals was due to tumor development occurring during the additional 10 and 13 weeks lived by these animals. Errors were found in the percentage of tumors reported, i.e., 16.9% of the high dose treated males were reported to have developed benign tumors. However, there were 10 tumors in 8 animals (15.0%). This percentage differs little from historical lifetime (>116 weeks) studies, that report a tumor

incidence of 13.4%. Weak arguments are presented that the tumors were due to aging and not due to atrazine. Statistical tests that consider time-to-tumor and adjust for different survival across dose groups have been widely used for 20 years. There is no need to present speculative arguments about the effects of aging. A proper age-adjusted analysis of these tumor data agrees with the EPA conclusion that those tumors appear to be due to aging.

Issue 2. Mode of Action Analysis

2.1 *Sufficiency of Evidence: Does the draft assessment adequately describe the data used to identify the key events in atrazine's mode of carcinogenic action? Are the available data sufficient to describe these events? Have the uncertainties and limitations of these data been adequately and clearly characterized? Which event(s) is viewed as critical to the carcinogenic process? Are the preliminary conclusions as to atrazine's mode of action supported by the analyses presented in the draft EPA document and consistent with the mode of action framework analysis described in EPA's July 1999 Draft Guidelines for Carcinogen Risk Assessment?*

Overall, the Agency's draft assessment document does an excellent job compiling and describing the data used for the proposed mode of action for atrazine induced mammary tumors in the female Sprague Dawley rats. A non-genotoxic, epigenetic mode of action has been proposed for the increased incidence of mammary tumors observed in atrazine treated female Sprague Dawley rats under the conditions of the carcinogenicity studies.

The proposed mode of action for atrazine involves an altered secretory activity of the hypothalamic-pituitary-ovary axis beginning with a decrease in the release of GnRH by the hypothalamus, which attenuates the afternoon LH surge. As a result, ovulation does not occur, which prolongs the estrus cycle increasing the exposure to estrogen. Increased estrogen also stimulates prolactin secretion from the pituitary, both of which can stimulate mammary gland tumor formation in rodents.

Mode of Action - The proposed mode of action for atrazine involves a sequence of events in neuro-endocrine function in female Sprague Dawley rats.

***Hypothalamic and pituitary effects* -** Studies by Cooper *et al*., in Long Evans rats have shown that atrazine decreases norepinephrine levels in the hypothalamus. This could cause a decrease in the secretion of GnRH with a corresponding diminution of the pituitary LH surge. Several additional studies have shown a marked decrease in LH levels at 40 and 200 mg/kg of atrazine after one month of treatment and at 29.4 mg/kg of atrazine administered for six months. Thus, there is evidence for an effect of atrazine in the hypothalamus and pituitary gland affecting gonadotropin and LH release.

***Estrus cycle disruption* -** With respect to estrus cycle disruption, atrazine at a dose of 29.4 mg/kg administered for six months prolongs estrus as compared to controls.

Histomorphology **-** In atrazine treated rats, there were a number of histomorphological changes indicative of stimulation with estrogen and prolactin. Experimentally, there was an increase in acinar development at 3 and 9 months indicative of increased estrogen exposure. There was an increased secretory activity at 9 months indicative of increased prolactin exposure. There was an increase in dilated ducts at 9 and 12 months indicative of increased prolactin exposure. There was an increase in galactoceles at the low and high dose at 9 and 12 months indicative of increased prolactin exposure.

Thus, there is histologic evidence in the mammary gland of stimulation by increased levels of estrogen and prolactin.

Changes in hormone levels **-** The proximate cause of tumors in Sprague Dawley female rats is increased exposure to estrogen and/or prolactin. An increase in both hormones or either hormone alone would increase the incidence of mammary tumors in Sprague Dawley rats. This is based on a large body of evidence which, for estrogen, dates back to the '40s and '50s and, for prolactin, dates back to the '60s and '70s.

The data on the effect of atrazine on the levels of estrogen and prolactin are listed in Table 15 from a two-year study by Elbridge, 1993a. After 3 months of treatment, there was a marked increase in the level of estrogen at 4.23 mg/kg (3 fold) and at 26.23 mg/kg (4 to 5 fold) as compared to controls. Estrogen levels were not changed at 9 months or thereafter in the 2-year study. Prolactin values were not available at the 3-month time point; however, the prolactin levels after 9 months of treatment tended to be increased at 4.23 mg/kg and were statistically significantly increased (2 to 3 fold) at the high dose level of 26.23 mg/kg.

Thus, there is evidence that estradiol and prolactin are increased in atrazine treated Sprague Dawley female rats and this has been identified as the critical event.

Framework Analysis for the Proposed Mode of Action

The EPA guidelines have a framework against which the proposed mode of action for atrazine was analyzed.

1. The draft document provides an adequate summary of the postulated mode of action for atrazine induced mammary gland tumors.
2. The key events have been identified: The release of gonadotropin releasing hormone (GnRH) from the hypothalamus is reduced resulting in an attenuation of the afternoon pituitary LH surge. This lengthens the estrus cycle resulting in increased estrogen levels which is the proximate cause or key event leading to an increased incidence of mammary tumors in Sprague Dawley female rats.
3. Strength, consistency, and specificity of association of the mode of action: There is consistent evidence from many studies on atrazine supporting this mode of action. Morphological changes indicative of estrogen and prolactin stimulation are observed in

the mammary gland and precede the occurrence of tumors. In addition, there is a substantial independent literature on the role of estrogen and prolactin in the pathogenesis of mammary tumors in rats. There are no significant contradictory data.

4. Dose response relationships : There is a strong correlation between dose and effect. NOELs have been defined.
5. The temporal aspects : The key events have been described and occur in the expected sequence and precede the appearance of tumors.
6. Biological plausibility and coherence of the database: There appears to be consensus on the part of EPA and the sponsor on the plausibility of the mode of action of atrazine. There is a large body of independent information that increased levels of estrogen and/or prolactin will increase mammary tumor development in Sprague Dawley female rats.

The evaluation of the mode of action for atrazine meets the criteria described in the framework. All of the key events have been demonstrated experimentally. None are based on speculation.

Uncertainties

There were a number of uncertainties and limitations that were expressed concerning the mode of action. They are:

1. Effects on the hypothalamus are not clearly defined. Although it appears that the first step is an effect of atrazine on catecholamines in the hypothalamus, the precise mechanism has not been clearly defined. Although the precise "mechanism of action " of atrazine in the hypothalamus is not clear, in terms of "mode of action," all downstream events from the point of decreased hypothalamic secretion of GnRH have been clearly defined.

2. Stop studies were not performed. Although stop studies are not performed, this is offset by the lack of a tumor response and ovariectomized rats. The lack of stop studies is not a major deficiency.

3. Perhaps the most important uncertainty is lack of robust data on hormones. In a two-year rat study there was in increased level of estradiol at six months and not at later time points. There was an increased prolactin levels at 9 months; however, the prolactin levels at an earlier time point were not available due to sampling or assay problems. Although the data were minimally adequate to support the proposed mode of action, additional hormone data would have been useful.

4. Since the strain and species differences in the mammary tumor response are important, more comparative data between the Sprague Dawley and Fisher 344 rats would be useful.

   The data strongly support the hypothesis that prolonged exposure to estrogen produced by the ovary is requisite for development of mammary tumors observed in these studies and discussed in this report. However, this does not mean that other peripheral factors

might not play facilitatory roles. For instance, atrazine may alter levels of hydroxylases, aromatase, or estrogen receptors. Changes in any of these proteins might alter mammary gland responses to circulating estrogens or estrogen precursors. There are limited data addressing these issues. This caveat should not, however, detract from the Panel's conclusion that the neuroendocrine actions of atrazine are the primary and requisite mode of action for carcinogenicity.

The weaknesses and limitations have been adequately addressed and none of these appear to be sufficient to raise doubt about the overall mode of action.

2.2 *Have alternative modes of carcinogenic action been sufficiently discussed and ruled out?*

There are no data that would suggest other plausible modes of action. The increased level of hormones and the increased level of hormones alone, can account for the increased incidence of mammary tumors in Sprague Dawley female rats. The proposed mode of action is plausible and each step in the pathway has been shown to be affected in atrazine treated rats. None of the effects are based on speculation.

Undoubtedly, the neuro-endocrine effects of atrazine will be further refined as time goes on and more will be learned about the actions of atrazine. There will always be some degree of uncertainty about a potential role of some yet to be determined action of atrazine. Although we will learn more about the action of atrazine, no new information will alter the fact that if you have an increased level of estradiol, you will increase the incidence of mammary tumors in Sprague Dawley rats.

Other potential modes of action for atrazine have been considered:

1. Genotoxicity -- The first consideration would be the possibility of a genotoxic mode of action. A large number of studies have been conducted to assess the genotoxic potential of atrazine and there is no evidence of genotoxic activity that would be considered relevant to the mammary tumor response in SD female rats.
2. Direct estrogenic activity- Several studies have assessed the potential estrogenic activity of atrazine. The compound has no direct estrogenic activity that could account for the increased incidence of mammary tumors.

Other potential modes of action discussed at the meeting included:

1. A possible cancer risk due to nitroso-atrazine. The potential here (formation under environmental conditions) would be a separate issue since it is unlikely that this would be formed under the conditions of the rodent carcinogenicity studies and this would involve a genotoxic mode of action for which there is no evidence. Furthermore, atrazine did not produce mammary tumors in ovariectomized rats, whereas directly genotoxic compounds such as DMBA or NMU will produce mammary gland tumors in ovariectomized rats. In addition, there is a well-known strain difference between Sprague Dawley and F344 rats. F344 female rats do not

have increased estrogen and do not show an increase in mammary tumors with atrazine treatment. The effect of nitroso-atrazine or any other, yet to be identified genotoxic mode of action, would be less likely to exhibit strain or species differences in response.

2. Atrazine has been postulated to stimulate aromatase activity that may increase the rate of estrogen formation from androgens.

The Panel made the following recommendations:

Estrogen induction of mammary tumors in rats: Additional information on the effects of estrogen in the induction of mammary tumors in rats should be included.

Metabolism and pharmacokinetics of atrazine in rodents and primates: Additional information of the metabolism and pharmacokinetics of atrazine should be included in the document. Similarities and differences in metabolism between the strains of rats tested (Sprague Dawley vs. Fisher 344) and humans should be indicated.

One Panel Member suggested the anorexic effects of atrazine as an alternative mechanism to atrazine effects. The Panel Member's opinion follows:

One major likely alternative mechanism of reduced LH that could account for most of the atrazine effects are the dramatic effects of atrazine in reducing food intake and body weight. Essentially every study to date has shown that at the moderate or high doses of atrazine that induce mammary tumors in SD rats (or induce any toxicity for that matter) there is also substantial weight loss associated with reduced food intake.

In humans as well as rats, reduced food intake and weight loss is a potent stimulus for reduced LH secretion. Even mild nutritional restriction results in reduced LH and functional hypothalamic amenorrhea (Cousinet et al, *Clin. Endocrinol.* 50:229-235, 1999). For example, leptin, which is produced by adipose cells, normally stimulates FSH and LH in females (Waiczewska et al, *Proc. Soc. Exp. Biol. Med.* 222:170-177, 1999). Anorexic or low body fat (e.g., from exercise) conditions directly reduce leptin and are strongly associated with human amenorrhea (Kopp et al, 1997, *Mol. Psychiatry*, 2:335-340).

Thus, the evidence is consistent with the view that appetite suppression and reduced food intake is the primary mechanism of the effect of Atrazine on LH in all rats. However, this leads to mammary tumors only in those rat strains that respond to reduced nutrition with a rapid falloff of LH and elevated estrogen. That is, it appears reduced LH and elevated estrogen may both be required for the mammary tumor effect.

This view does not preclude a role for hypothalamic norepinephrine or other transmitters since the mechanism through which atrazine reduces appetite and food intake may still be somehow mediated by the hypothalamus. However, if the body

weight interpretation is correct, it argues for a more benign mode of action on appetite suppression as follows:

Atrazine ➜ Brain (Appetite Systems) ➜ Appetite Suppression ➜ Weight Loss ➜ Reduced Adiposity ➜ Reduced Leptin (or other signal) ➜ Reduced LH ➜ Reduced Ovulation ➜ Elevated Estrogens (in rodents only)

In this view, the originally proposed reduced LH-elevated estrogen mode of atrazine-induced mammary tumors in SD rats is still valid. The major difference here is in the sequence of events that trigger the initial LH reduction.

Issue 3. Dose Response

3.1 ***Because it is the attenuation of the preovulatory LH surge that results in disruption of the estrous cycle and is a necessary step in the mode of carcinogenic action, the Draft EPA document proposes that the cancer dose-response assessment should proceed by a nonlinear dose-response extrapolation. The document further proposes that the NOAEL for the attenuation of the LH surge be used as the point of departure for a margin of exposure analysis. We would like the panel to comment on this proposed dose-response approach and the consistency of other effects going along with the LH suppression effect.***

Deficiencies in the data set available for dose-response evaluation were noted. The site and nature of the primary lesion caused by atrazine in the hypothalamus is unknown. The dose-response curve for the primary lesion is thus also unknown, so nothing can be said about the appropriate low-dose extrapolation for the primary lesion. Data are lacking on pharmacokinetics and metabolism. Development of a physiologically based pharmacokinetic (PBPK) model that would facilitate estimation of brain levels of atrazine and its metabolites in rodents and humans is needed. A PBPK model would be useful for identification of nonlinearities in the dose-response curve and for extrapolation between rodents and humans. Specification of the relative activity of parent compound and its metabolites in causing adverse effects is needed. Finally, the depletion of the LH surge in the Sprague-Dawley rat is treated qualitatively. A quantitative treatment of this endpoint is needed.

Most Panel Members felt that non-linear extrapolation below the point of departure was appropriate. Arguments in support of non-linear extrapolation include 1) The peak height of the LH surge can be diminished to some extent (at least in young animals) without affecting estrous cyclicity and 2) endocrine homeostasis is maintained by numerous feedback loops that can compensate, up to a point, for environmental effects.

The contribution to dose-response nonlinearity of the diminution of the LH surge must vary with age. In young animals the distance between the peak height of surge and the minimal height needed to maintain the cycle is relatively large. This distance decreases with age and eventually

becomes zero at about 1 year of age in the Sprague-Dawley rat. However, when the distance becomes zero, exposure to atrazine becomes a moot point with respect to the postulated mode of action. Thus, for the fraction of the lifetime of the female Sprague-Dawley rat during which concern for the mode-of-action is relevant, some reserve capacity exists, albeit a reserve capacity that is decreasing with age, and a nonlinear low-dose extrapolation is appropriate.

One Panel Member stated concern with the non-linear extrapolation, noting that the data are not analyzed in such a way that linear or non-linear behaviors are demonstrated in a statistically robust manner. This comment thus focused more on the adequacy of the data analysis in support of nonlinearity, or linearity, than on the question of whether or not non-linear extrapolation is appropriate.

Most Panel Members felt that the LH surge was the appropriate point of departure for the evaluation of the carcinogenic effect of Atrazine in the female Sprague-Dawley rats. Specific endpoints downstream from diminution of the LH surge can vary between rodent strains and species and probably between rodents and humans. The linkage between the primary effect of atrazine on the hypothalamus and diminution of the LH surge can be viewed as a common pathway that diverges subsequent to the LH surge. Use of the LH surge as the point of departure is thus economical and can serve for a number of different toxic endpoints.

Several Panel Members strongly supported use of the LED_{10} rather than the NOAEL. A NOAEL of approximately 2 mg/kg/day was observed and proposed as a point of departure for risk assessment by the USEPA. However, no biological meaning can necessarily be attached to a NOAEL. The NOAEL is primarily a statistical result that is dependent on the sample sizes employed that may not have the power to detect a biological effect. Hence, it is recommended that the point of departure for a margin of exposure approach should be based on a LED_{10}, as proposed in the US EPA draft carcinogen risk assessment guidelines.

An LED_{10} of approximately 2 mg/kg/day also was obtained for LH effects in female SD rats (Seilken, 2000). It is recommended that this dose should be used as the point of departure for the health risk assessment using the margin of exposure approach. Since effects may be produced at this dose, possibly with a 10% incidence, this dose cannot be considered a NOAEL.

3.2 *Is there a common endpoint in the mode of action that could be used as a point of departure that would be protective of both cancer and reproductive developmental effects? The EPA draft document proposes the LH data as such.*

Most Panel Members agreed that the LH surge was an appropriate point of departure for both cancer and reproductive endpoints. Several Panel Members suggested, however, that the LH surge might not be an appropriate point of departure for developmental effects of atrazine. Changes in 5-α-reductase were also suggested as being potentially important. It was noted that neuroendocrine effects in the hypothalamus could have a variety of consequences, including behavioral and neurological effects. Some Panel Members suggested that data are lacking that

would support identification of an alternative point of departure specific to reproductive endpoints. Data needs include identification of metabolites in urine and hormonal assays in children and adults.

Issue 4. Human Relevance

The EPA stated that it was policy to presume that animal tumor responses are presumed to be indicative of human cancer potential unless there is substantive information to the contrary. The Panel was asked to discuss whether atrazine acts as a neuroendocrine disrupter in humans, and if so, would this have adverse human consequences including carcinogenicity? The Panel also was asked to comment on the EPA's evaluation of epidemiologic studies and its recommendation to classify atrazine as a likely human carcinogen.

In this regard, four specific questions were presented to the panel. The questions and panel responses are as follows:

4.1 ***The EPA draft document concludes that there is suggestive evidence of a possible association of triazine exposure and cancer occurrence for three hormone-responsive cancers in humans - ovary, breast and prostate cancer. However, these associations should not be considered as conclusive evidence of an association of triazine exposure with these tumor types, and triazine exposure should not be interpreted as a causal factor in these tumor types. Please comment on EPA's evaluation of the epidemiologic studies and how much weight it should be given in the hazard and mode of action assessment.***

The Panel agreed that the epidemiologic data did not provide conclusive evidence of an association between atrazine and cancer but it did provide some evidence. Several Panel Members commented that inadequate attention was given in the report to the studies that are available and that the epidemiological data should be reviewed and discussed as extensively as the animal data . The EPA document should comment on whether the studies are just a little better than no evidence to something just less than conclusive or somewhere between these extremes. Greater detail on strengths and weaknesses of the studies should be included in the report. For example, case-control studies are stronger than ecological studies and therefore different weights need to be given to the studies. Finally, it was suggested that the report should include an evaluation of epidemiological data on a cancer by cancer basis with a review of the major investigations and an overall summary.

The Panel was concerned that several reports on non-Hodgkin's lymphoma (NHL) were discounted even though they suggested that atrazine could produce adverse health effects in humans unrelated to the postulated mode of action in Sprague Dawley rats. The association of atrazine with NHL has been evaluated in several studies with both cohort and case-control designs.

The studies in the central U.S. (details in following paragraphs) focus specifically on atrazine, include information on other risk factors for these tumors, and, with information on duration and frequency of use, provide some measure of exposure. The study of manufacturers of atrazine (details below) provides important additional information because it has a different study design (and thus different strengths and weaknesses) and may have had higher exposure levels. Several points should be made about investigations of NHL. First, they have elevated relative risks, although these are usually not statistically significant. Second, the relative risks from the manufacturer study, which are likely to have higher exposure levels, have larger relative risks than the study of farmer users. The number of deaths among the manufacturers is very small and there is considerable uncertainty surrounding the risk estimate. This also means that a clear, monotonic exposure-response gradients is unlikely to be observed. Third, the relative risks in the pooled analysis of the case-control study of NHL found that as adjustments were made to improve the clarity and precision of the comparison, the relative risk tended to decrease. This suggests confounding or bias may have contributed to the excess.

Studies of farmers in Midwestern states (NE, IA, MN, KS) have shown consistent positive associations with NHL, obtaining odds ratios in the vicinity of 1.5. The major limitation in these studies has not been confounding bias. For example, in the Iowa and Minnesota Study, the authors stated (p. 2451, Cantor et al 1992): " There was minimal evidence for confounding of results for any single pesticide by exposure to pesticide belonging to other chemical families. " In the evaluation of the pooled results for the four states, adjusting for organophosphate and 2, 4-D exposures decreased the odds ratios for atrazine in the Nebraska data by >10% but increased the odds ratios in the Iowa data by >10%. The odds ratios for the other two states were minimally affected by the adjustment. In the Nebraska data, even after adjustment for organophosphate and 2, 4-D, the odds ratios for those exposed for >20 days/year was 1.4. The key problem in the Nebraska data is the small numbers of cases and controls exposed to atrazine, which results in extremely unstable odds ratio estimates when adjusting for other pesticide exposures. An example of how small numbers of exposed cases and controls affect the adjustment of odds ratio estimates can be seen in the results for 2,4-D in the Nebraska data. When the odds ratio for 2,4-D and NHL is adjusted for organophosphate exposures, it declines sharply. On the other hand, this effect on the odds ratios is completely reversed if adjustment is also made for fungicide exposures. Perhaps the same thing would have occurred for the atrazine odds ratios.

The major limitations of these studies were biases that most likely would result in underestimates of a true exposure effect. For example, the grouping together of the different types of NHL might have resulted in an underestimate of the effect, since the diffuse type of NHL appears to be more strongly associated with atrazine exposure than the follicular type. Errors in the recall of past exposures and the use of next of kin interviews for deceased subjects could introduce exposure misclassification that most likely would bias the odds ratios towards the null. Case-response bias could also be operating and could artificially increase estimates of relative risks.

Besides the consistent findings among farmers, there is also evidence from a mortality study of atrazine production workers that links atrazine exposure to NHL. Although almost 80% of the workers with "definite or probable" exposures were followed for a period of < 20 years from the

start of their exposure, and 90% of the follow-up time(person years) represented ages under 45 years, an excess of NHL mortality was observed: three observed cases/0.8 expected, SMR= 385. In addition, two myelocytic leukemia deaths were observed, constituting between a 3 to 4 fold excess. Excesses in NHL mortality were seen for workers employed for < 1 year (SMR= 390) and ≥ 1 year (SMR=370). All of the leukemia deaths occurred among workers with less than 1 year of employment at the two plants.

These data probably reflect the tip of the iceberg for the effect of atrazine exposure in this workforce since only cancer mortality was evaluated, the cohort was very young, and the follow-up period was not sufficient to account for latency. Interestingly, about 60% of the workers with definite/probable exposures, and two of the three NHL cases, worked for < 1 year at these two plants, suggesting that relatively short term atrazine exposures may be sufficient to cause NHL.

There was some disagreement about the importance of the breast cancer study in Kentucky (Kettles et al.**,** 1997). It was agreed that although an association was reported, the study was ecologically based which is the weakest epidemiologic design. One Panel Member commented that with no analytic investigations available on which to base an evaluation, the epidemiologic data essentially provided no information on the issue of atrazine and breast cancer. It was suggested that the document should indicate the lack of useful studies on breast cancer rather than saying it does not provide conclusive evidence. Another Panel Member noted that although the study had serious limitations it was "hypotheses generating" and should be followed up.

Several reviewers thought the reported ovarian cancer study was more relevant than presented in the Agency draft report and at least raises the question as to whether a chemical acting on the pituitary-gonadal axis might be relevant to ovarian cancer in humans. The study itself is in the mid-range in terms of quality. As a case-control study, it is stronger than the ecologic design and it deals specifically with the class of chemicals to which atrazine belongs. The authors were able to adjust estimates of relative risks for most of the major ovarian cancer risk factors. The main weaknesses of the study are small numbers and relatively weak exposure assessments based on triazine exposures and not just atrazine. Comments raised by some presenters on the issue of reclassification of subjects for exposure have primarily focused on the cases, but an appropriate reclassification of exposure must include controls as well as cases. When this is done, an elevated odds ratios for ovarian cancer still remains in this study. Because of the lack of a specific focus on atrazine and small numbers from a single study, the appropriate interpretation of this study is that it provides a suspicion of human carcinogenicity, but not conclusive evidence.

The issue of atrazine and prostate cancer was briefly discussed but limited data were available for review. It was strongly recommended that the EPA should review these data when it becomes available.

Finally it was mentioned that epidemiologic evidence on atrazine and cancer is insufficient to rule in or out an association with childhood cancer -- this has not been studied. However it should be considered whether hormonal effects in childhood or adolescence may have an impact on cancer occurrence in later years.

It was suggested that the summary paragraph on page 27 of the Agency document be reworded as follows:

> "To summarize, there are a few epidemiologic studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies showing an association and internal inconsistencies in the studies available indicates that the human studies by themselves do not make a strong case for an association."

4.2 ***Does the document provide a thorough and adequate discussion of the similarities and differences in Sprague-Dawley (and Long Evans) rats compared to humans with respect to this hypothalamic-pituitary-ovarian axis perturbation? Given the steps outlined in Figure 2-1 contained in Part A, Chapter 2, what events are conserved and show homology between rats and humans, and when does the process diverge? Currently the document indicates that CNS-GnRH control of pituitary function is conserved and similar.***

The Panel noted that there are certain similarities in the control of the hypothalamic-pituitary-ovarian axis between humans and rats but that there are important differences in the details of the control mechanism. Furthermore, there are a number of areas where sufficient data do not exist to compare the two species. It was strongly emphasized that the effects of age on reproductive and endocrine function are much different between rats and humans. These differences have important implications in regard to the human relevance of the proposed mechanism of action for atrazine in inducing mammary tumors in the female Sprague Dawley rat. In addition to species differences, there also exist significant strain differences in the aging process. Thus, the mechanism of atrazine action in the Sprague Dawley rat is unique to that strain in that a similar process is not operable in Long-Evans or F-344 rats. Details of these differences are presented below.

a. Comparison of the rat and human hypothalamic-pituitary-ovarian axis:

There are both similarities and differences in the control of ovulation between the human and the rat and still many areas where there is insufficient data in either or both species to understand the process. Classical experiments by Knobile and colleagues suggested that the control of reproductive cycles in female macaques resided to a somewhat greater degree in the ovary as compared to principally CNS control in the rat. This should not be construed to mean that the hypothalamus does not play a key regulatory role in primates but to emphasize that these control mechanisms differ between species. The rat estrus cycle and ovulation seems to have a much greater entrainment to a circadian oscillator than the human or primate menstrual cycle. Gonadotropin release is under the stimulatory and inhibitory control of numerous neurotransmitters, neuropeptides, and neuromodulators and there appear to be major differences in the relative importance of these factors in rats and man. Furthermore, ovarian function, most notably CL activation also is quite different between these species. For example, the CL in the rat is initially dependent on a diurnal and nocturnal PRL surge to cause progesterone synthesis and release. The luteal phase in the human is not dependent on pituitary prolactin release. The rat is

also dependent on the ovary for progesterone production throughout pregnancy, a process that is relegated to the placenta in human pregnancies.

The authors place great importance on the observed atrazine dependant decrease in hypothalamic catecholamine levels. Figure 2-1 (page 33 of the Agency background document) suggests that atrazine initially interferes with NE stimulation of GnRH release. Although this has not proven to be the case and measurements of NE content may not accurately reflect changes in NE signaling (turnover or release studies need to be done), there is considerable evidence that NE is of lesser importance in inducing human LH release. In primates, however, catecholamines are probably not the driving force of hypothalamic GnRH secretion. Instead, as demonstrated in studies by Terasawa, a balance between glutamine-stimulatory and GABA-inhibitory mechanisms may be ultimately responsible for the control of GnRH release. It has been suggested that hypothalamic norepinephrine levels in primates reflect GnRH release rather than cause it. Nevertheless, NE has a role in many physiological and behavioral processes and changes in NE metabolism as a result of atrazine exposure might have numerous effects.

b. Reproductive aging :

Reproductive aging is quite different between humans and rats. There are also some major differences in reproductive aging across rat strains. A limited supply of ovarian follicles appears to be a major cause of the menopause while hypothalamic dysfunctions appears to lead to cessation of ovulation in rats. The menopause is characterized by low levels of estrogen and high levels of both LH and FSH. Prolactin levels are usually unchanged or slightly reduced secondary to decreased estrogen. In the old constant estrus SD rat, estrogen and Prl are elevated while LH and FSH levels are unchanged or reduced. The elevated estrogen levels in the aging female SD rat lead to an increased risk of mammary tumors. This risk is potentiated by increased levels of prolactin. In humans, the reduced levels of estrogen after the menopause possibly decreases the risk of developing mammary tumors.

Advancing age in the female Sprague Dawley rat is associated with increasing numbers of days spent in estrus with eventual entry into a constant estrus state associated with elevated estrogen levels and prolactin levels and a very high incidence of mammary tumors. Other rat strains are more likely to show age related increases in the number of days spent in diestrus followed by a constant diestrus or pseudopregnant like condition. This reproductive state is associated with the development of a number of corpora lutea which are stimulated to secrete progesterone by prolactin. Animals in this reproductive state show a much lower incidence of mammary tumors.

4.3 ***Given the neuroendocrine associated effects (i.e.,decreased secretion of hypothalamic catecholamine levels and gonadotropin releasing hormone, attenuation of the LH pituitary surge, prolonged estrogen and prolactin exposure) found with atrazine treatment in rats, please comment on the conclusions drawn in the Draft EPA document regarding relevance and the possible ramifications in humans. What are commonalities between humans and rats in the endocrine effects found that would raise a concern for human health consequences including carcinogenicity? The document considers human conditions of anovulation as a means to***

judge the potential human health risks. Please comment on the appropriateness of these models in evaluating atrazine.

Before the relevance of the mode of action data in the SD rats is discussed, it should be emphasized that there are no human data suggesting that atrazine interferes with hypothalamic-pituitary-ovarian function as demonstrated in the SD rat. There is little or no evidence that atrazine affects a species-conserved hypothalamic NE mechanism (if so, why doesn't it also alter estrous cycles in F344 rats or in mice?). *Most critically, even if atrazine did reduce LH and induce hypothalamic ammenorhea (HA), this condition is associated with reduced estrogen levels and therefore would not promote the development of mammary tumors. Further, if this effect occurred in humans it could not result in polycystic ovarian syndrome (PCOS), since this condition is associated with elevated LH. Therefore, the conclusion that there could be a potential for human cancer from elevated estrogen levels if atrazine had effects on conserved hypothalamic mechanisms in humans similar to those in SD rats is at odds with the arguments and other conclusions of the Agency draft assessment.*

a) Extrapolation from rodents to humans: In the consideration of the relevance of atrazine's mode of action to humans, one is first confronted with the strain and species specificity of atrazine induced mammary gland tumors in female Sprague Dawley rats. There is no effect in male Sprague Dawley rats; there is no effect in Fisher 344 rats of either sex or in CD-1 mice of either sex.

There are two fundamental considerations in the extrapolation of carcinogenicity data between species. First, the species-to-species extrapolation where one takes into consideration evidence for species-specificity of effect and second, the extrapolation of effects observed at high doses to low doses.

Species Extrapolation - Although there is strong evidence for species and strain specificity of atrazine's mode of action, EPA dismisses this idea and reverts to a default consideration of relevance based on speculation that "If atrazine were to act on the hypothalamus of humans as in the rat …" the mode of action is considered relevant. For this assumption there is considerable data to the contrary. In fact, if the same thing were to happen in humans there would be a decrease, not an increase in estrogen levels. Although there are similarities in hypothalamic/pituitary function, there is a fundamental difference between rats, where reproduction is under pituitary control, and humans, where reproductive senescence is controlled by the ovary.

High-to-Low Dose Extrapolation - EPA has concluded that a nonlinear extrapolation procedure is the most appropriate in view of the mode of action of atrazine. Implicit in this choice is the assumption that what happens at high doses to produce mammary gland tumors in rats does not happen at some lower dose. Thus, by choosing a nonlinear extrapolation one must conclude that at some lower dose the mode of action is not relevant to humans. If the mode of action were in fact considered relevant to humans (at any dose), then the choice of a nonlinear extrapolation procedure would not be appropriate.

In the case of atrazine, there is compelling evidence to support the conclusion that the mode of action is not relevant to humans on the basis of both the species-specific nature of atrazine's effect on mammary gland tumors and on the basis that the effects observed at high doses do not occur at some lower dose.

b) Similarities with reproductive pathologies in women: The interpretation of the human hypothalamic ammenorhea data is somewhat misleading. This condition often is associated with low serum estrogen as evidenced by direct measurement. Hypothalamic amenorrhea in humans presents a much different endocrine profile than age-related persistent or constant estrus in the female SD rat. Most notable is diminished estrogen secretion in the former and enhanced secretion in the latter condition. Thus, anovulation in women does not increase the risk factors for the development of mammary tumors. Hypothalamic amenorrhea also is usually associated with reduced prolactin levels. It should be emphasized that estrogen although present in low amounts is not opposed by the cyclic increase in progesterone. We know that prolonged elevated prolactin levels lead to mammary tumors in the rats but there is little if any such effect in man. Often, women with hyperprolactinemia are anovulatory and have low estrogen levels.

Furthermore, if as discussed in the draft Agency report (page 45), an increased number of ovarian cycles (early menarche, late onset of menopause, nulliparity) is positively associated with breast cancer, ammenorrhea might be associated with reduced cancer risk. The draft report offered this as a possible explanation for reduced breast cancer risk with exercise although it did not extend this speculation to possible effects of atrazine to reduce breast cancer. Again, all of the suggestions in this paragraph are extremely speculative.

Polycystic ovarian disease in women likewise does not increase the incidence of breast tumors, although there may be some limited association with the development of endometrial cancers. The draft report is correct in discounting the discussion of Polycystic Ovarian Disease.

c) Summary: In summary, there are considerable differences between hypothalamic-pituitary-ovarian function in rats and humans, and the effects of aging on the function of the axis also is quite dissimilar. Therefore, it is unlikely that the mechanism by which atrazine induces mammary tumors in female SD rats could be operational in humans. Nevertheless, it is not unreasonable to expect that atrazine might cause adverse effects on hypothalamic-pituitary function in humans.

4.4 ***Given the toxicological and mechanistic information available on atrazine, OPP has proposed that atrazine be classified as a likely human carcinogen (see EPA's July 1999 draft revisions to the guidelines for carcinogen risk assessment). Please comment on this proposal.***

The Panel was not convinced by the data presented in the EPA report that atrazine should be classified as a "likely human carcinogen": 1) atrazine was negative in two species of rodents tested, 2) animal tumor response not considered relevant to humans (in the Sprague Dawley rat), and 3) route and dose limited response precludes relevance to humans (with regard to exposure). Despite the overwhelming agreement that atrazine should not be classified as a "likely human

carcinogen," no consensus was reached on the classification that atrazine should receive. Comments from the Panel Members are summarized below:

The "strength of evidence" for the carcinogenicity of atrazine in the rodent bioassay is weak. The tumor type has a high spontaneous incidence and the effect is observed in a single sex, species, and strain. In the International Agency for Research on Cancer (IARC) classification scheme, this would be considered "limited evidence of carcinogenicity" in animals, which along with "inadequate evidence" in humans, would most likely end up in Group 3 "not classifiable". In a worst-case scenario, atrazine would be classified no higher than Group 2B "possible human carcinogen" in the IARC scheme. In the EPA scheme, the classification would be no higher than "possible human carcinogen" as was decided in 1988 for atrazine.

In the meantime, the registrant of atrazine was invited to provide mode of action information for atrazine and although there is not agreement on every point, there is compelling evidence that 1) the mode of action is strain and species specific and 2) the effects that occur at high doses in rats do not occur at lower doses. Even if the species specificity of atrazine effects is disregarded, the lack of effect at low doses remains.

After a review of a similar database for atrazine, IARC concluded that the mode of action of atrazine is species specific and thus not relevant to humans and downgraded the classification from Group 2B "possible human carcinogen" to Group 3 "not classifiable". In contrast, EPA has proposed to upgrade the classification of atrazine from "possible human carcinogen" to "likely human carcinogen". In view of the information available on the mode of action of atrazine, there does not appear to be any scientific justification for an upgrade in the classification of atrazine to "likely human carcinogen".

There was a consensus of the Panel that atrazine should be classified as "not likely to be carcinogenic to humans" or "not enough information to classify" based on two criteria in the EPA draft guidelines. First that "Extensive experimental evidence showing that the carcinogenic effects observed in animals are not considered relevant to humans". Second, "Evidence that carcinogenic effects are not anticipated below a defined dose range". Furthermore, the evidence is very strong to eliminate atrazine as a direct mutagen. Finally, epidemiological data doesn't support the classification of atrazine as a likely human carcinogen. The IARC classification is a better reflection of the carcinogenic potential of atrazine in humans.

While mechanisms of action of atrazine and mammary gland tumors in the Sprague Dawley rat may not be directly relevant to human breast cancer, the perturbations to the hormonal system (i.e. effects on GnRH and subsequent downstream hormonal effects) in the human could have a large impact on health outcomes- some cancers may be inhibited while others may be promoted.

Given the proposed mode of action, atrazine is likely to have an impact on health outcomes (both cancer and non-cancer outcomes). It is not clear that cancer risk will necessarily be increased. Some hormonal effects may lead to a decreased risk of some cancers while others may be increased.

One Panel Member suggested that atrazine be classified as a "possible human carcinogen" given the positive evidence from the occupational epidemiologic studies. However, when taken

together, the epidemiological evidence is inconclusive and the evidence from occupational studies could be outweighed by the fact that environmental exposures to atrazine would be lower than the exposures occurring in an occupational setting. The single species, single strain, single gender data and the lack or relevance of the SD rat to man certainly was strong evidence to rule out animal data when evaluating atrazine as a human carcinogen.

The US EPA Draft Guidelines for Carcinogen Risk Assessment encourage the fullest possible use of relevant data in assessing the carcinogenic potential of chemicals. If these data are sufficient, a plausible mode (or modes) of action is developed and this mode of action is used to inform the choice of the option for low dose extrapolation. Beyond this obvious application of the mode of action, however, is another application that has the potential to diminish the effectiveness of the draft guidelines as a framework for carcinogen risk assessment. When data on specific toxicological endpoints are lacking, the mode of action that specifies an intermediate endpoint can be used to support speculation about downstream toxicities that might occur but which have not actually been observed. This speculation can be useful in identifying what might be possible, for hypothesis generation, and for planning research. However, speculation about potential toxicities for which data are lacking is unlikely to be a suitable basis for risk assessments.

In summary, there is apparently no clear, established or even likely neuroendocrine path through which atrazine could induce cancer in humans, whether by a mode similar to that in SD rats or by mutagenicity or direct estrogenic actions. Moreover, if reduced food intake does underlie the atrazine effect in female SD rats, then it could only be relevant to humans at doses that induced anorexic conditions similar to those that induce HA in humans. However, as noted above, regardless of how HA is triggered it does not in any case increase E_2 in humans. Thus, an opposite argument is more consistent with the data, namely, that HA from anorexia might be associated with reduced overall cancer risk (based on dietary restriction studies in rodents). Therefore, the Agency's conclusion is not supported. Following this reasoning, atrazine is <u>not</u> <u>likely</u> to be a human carcinogen. However, there are enough questions on atrazine’s mechanism of inducing LH reduction that it might be worthwhile to further investigate the role of food intake vs. direct hypothalamic actions in triggering this cascade.

Issue 5. Children's Health Concern

We know very little about atrazine exposure and cancer risk to the human adult and we have almost no information on childhood cancer risk. We will begin with what we know about children and their development.

Children differ starkly from adults in many dimensions including gastrointestinal absorption, daily requirements of water on a volume to Kg body weight basis, permeability of the blood-brain barrier, metabolizing/detoxifying mechanisms, dynamic changes in morphology and central nervous system, and immune competence. These differences are in general not neutral. They tend to make the fetus, infant, and young child more vulnerable to insults, whether chemical, radiological, infectious, or physical.

The drastic effects of methyl mercury on a newborn's brain, the terrible effects of alcohol on the developing human as seen in fetal alcohol syndrome, the increase in thyroid cancer after thymus irridation all certify that when an organism is developing, it is much more vulnerable than after the developmental task has been completed. These differences are not quantitative. They are qualitative and fundamental.

No attention is given to transplacental transfer of atrazine to the fetus, or transfer in breast milk. Other toxic molecules, e.g., PCB's and dioxin, are transported through these routes, and there is no reason to believe that atrazine is not. This issue should be given high priority and should be discussed in the evaluation.

The Agency has developed a cogent if not complete model of the mechanism of carcinogenesis due to premature onset of constant estrus. The evidence is not perfect, but persuasive. The Agency should not restrict their attention only to this mechanism by which atrazine causes tumors. The evidence that atrazine is not a mutagen is persuasive but not conclusive. More data are needed to this point. The possibility of conjugation with nitrites to yield a nitrosamine requires additional investigation.

We know that neurogenesis is not limited to the intrauterine period, and may continue throughout the lifespan. Brain development goes at an explosive pace during the first few years of life. During that time, neurons and glia are migrating and dendrites are sprouting and are being pruned back. A three year old has fewer synapses than a two year old because of the pruning process. This pruning is tightly orchestrated and under the influence of the genes and the experiences of the child. A synaptic connection that is reinforced by experience at this time is more likely to persist. Any perturbation of CNS metabolism at this time may decrease the specificity and increase the randomness of these connections.

The consequences of toxic chemical exposures are particularly strong during the fetal period. The CNS and other organ systems are undergoing rapid development and minor perturbations of that development can have consequences lasting into adulthood. Considerable animal data and human observations reveal that the development of the reproductive systems is modulated by natural hormones and is particularly susceptible to environmental challenges.

The effect of atrazine on LH and Prolactin are a result of altered GnRH output, and this is mediated by neurotransmitters, NE and DA. Prolactin is regulated by DA. Because of the rapid developmental brain changes alluded to above, the influence of atrazine on neurotransmitters in the hypothalamus and on GnRH may well have a differential, permanent effect on children. This phenomenon is the basis of the relatively new field of behavioral teratology. Atrazine could influence the migration of cells and the connectivity of the CNS. This effect could be latent, and emerge later during the challenge of puberty, or during senescence. Behavioral alterations may be the most sensitive outcome. This possibility should be addressed, and behavioral protocols for atrazine-dosed animals developed. Epidemiological studies also should include behavioral assessments.

The influence of atrazine on the hypothalamus and on GnRH may have a differential effect on children. It could influence the migration of cells and the connectivity of the CNS. This possibility should be addressed.

The issue of potential carcinogenicity from ingesting atrazine by the developing fetus and children is probably the same as that for adults because the mode of action would be essentially not applicable (see 4.1- 4.4). However, the potential for other developmental effects is not as clear. To date, there are no human data to suggest that there are potential hazards. Therefore, the Agency must rely on animal data to characterize the potential hazard realizing that infants are exposed to a greater dose on a body weight basis than adults. It seems prudent at this point to consider using a mathematical model using the available data on developmental toxicity in animals and applying this information to potential human exposures.

Although there is widespread human exposure to atrazine in water, greater than 97% of the exposures are below 3 ppb in water (the EPA standard). This level would translate to ~0.1 ug/kg BW for adults 0.6 ug/kg for children. The highest environmental exposure has been reported to be ~60 ppb in drinking water. This translates into an exposure of 10-12 ug/kg BW for a child whose entire fluid intake is comprised of this level of contaminated water. In other words, the worst case drinking water exposure in humans would be at least 3 orders of magnitude lower than the NOAEL (~4 mg/kg) for cancer and hormonal perturbations in the most sensitive strain of rodents (SD rats) consuming the chemical for most of their lives. It seems reasonable that the Agency could accomplish the same type of analysis for developmental effects in rodents and apply it to children.

5.1 ***Please comment on the applicability of the neuroendocrine mode of carcinogenic action in adult rats to the fetus, infants, and peripubertal children. Also, please comment on the adequacy of the EPA document in addressing children's cancer concern.***

The thrust of the research presented related to children focuses on the effects of atrazine in early post-natal life on adult reproductive functions. Compared to the perinatal period and to reproductive adulthood, the period of " childhood " in animals and humans is relatively endocrine quiescent. It is often termed the "juvenile pause". Thus, what we find is that early exposure to atrazine in the rat may have long-term effects on the adult but no apparent effects during "childhood". These effects may have been obscured because of the low level of endocrine activity until the onset of puberty.

The primary "childhood" effect reported is a delay in the onset of puberty in male and female Wistar rats. In the male, preputial separation was delayed when the rats were fed 12.5 to 150 mg/kg atrazine from day 23 to 53 of age. At the higher doses, 50 to 200 mg/kg, there was a significant reduction in ventral prostate weight. No significant differences in circulating LH or PRL were found although there was a trend to lower concentrations after atrazine exposure.

In female rats, higher doses of atrazine, < 50mg/kg, resulted in delayed puberty in a dose dependant fashion. Vaginal opening occurred 2.3 to 7.1 days later when rats were fed 50, 100 and 200 mg/kg of atrazine. In the rodent model, these are biologically significant delays.

The EPA document does not address adequately the risks of atrazine to children, primarily because the data are not available. Prenatal hormonal perturbations due to environmental substances, such as atarzine, have consequences on reproductive function. Early exposure can then have long-term effects. The applicability of the neuroendocrine model of carcinogenetic action may be relevant to infant and childhood exposure, but the effects may have a long latency, and may not become apparent until puberty or even later. There are toxic mechanisms for children other than carcinogenesis.

The discussion of atrazine and its mode of action and other toxicities was informative but not adequately discussed in the Agency document. A number of other issues should be addressed. The Panel Members are aware that for many issues inadequate data exist to draw conclusions, but it would be beneficial for future research directions to identify where data gaps exist. Among these issues are the following:

a. The pharmacokinetics (PK) and pharmacodynamics (PD) of atrazine in the animal adult and throughout development; in the human adult and in the developing human from preconception to the end of sexual maturation; and in other susceptible populations such as individuals with pituitary-hypothalamic dysfunction. In cases where there are inadequate data, the data published in the literature should be used to study the potential altered toxicities (including birth defects, reproductive losses, etc) resulting from exposures to atrazine, similar chemicals, and herbicides involved in PK-PD or mode of action.

b. The routes and capacity of absorption and manner in which children may be exposed through pica, chewing on stuffed animals, etc., or through germ cell, transplacental and transmammilary routes.

c. Organ distribution including the CNS,and the potential for altered distribution in fetuses and children due to altered blood brain barrier and percent tissue type, i.e., percent adipose tissue in the body.

d. Metabolism, including organ, age, and gender specific details. The P450s or phase II routes and metabolic profiles should be detailed with the effects of age on the expression of these enzymes and resultant profile and toxic potential. Clearance rate and effect of age, pregnancy, etc., should also be included. The PK of the metabolites should also be discussed.

e**.** End organ effect/toxicities, including which metabolites may be more or less toxic than the parent chemical. Also the effect of age on organ susceptibility or unique disease states such as functional birth defects, pregnancy losses, etc.

f. In regards to mode of action the effect of age and animal vs. human. Also unique mode of action and susceptibility in regards to pregnancy and development. The effect of age of development, pregnancy, etc. on critical mode of action pathways and how these changes might alter susceptibility or outcome.

g. Other susceptible populations might be identified. For example, with atrazine exposure, individuals with decreased pituitary hypothalamic axis function may be at risk.

h. Atrazine's potential non-cancer toxicities may be very broad considering the mechanism of action involves altering the pituitary-hypothalamic axis which results in delay of puberty and pregnancy losses. Therefore the effect of atrazine may not only be on maturation but also on growth and development. This is highlighted by the ATSDR report of the Iowa study (Burnmeister, 1990), where the offspring in the exposed population had increased incidence of intrauterine growth retardation and hypospadia.

i. Finally, the effect or possible effect of atrazine on altering amount and the success of lactation and pregnancy losses due to its neuroendrocrine effects including prolactin, etc.

Importantly, in both the Zirkin and the Cooper(EPA) laboratories, benign food restriction alone was found to produce the full spectrum of all toxic effects induced by atrazine. As noted, it is well established that food restriction reduces LH and delays puberty. No studies were done in the Cooper or Zirkin laboratories to determine if weight loss due to food restriction in the same range as the atrazine-induced weight loss produced the depressive effects on LH. In the Zirkin laboratory studies, the only atrazine groups that showed toxic effects also exhibited weight loss. In the Cooper laboratory studies, some toxic effects were seen at some lower atrazine doses that did not induce weight loss. Nevertheless, weight loss effects are variable and can be missed statistically in small groups. For example, in one Cooper laboratory study weight loss was found in SD rats at a dose of atrazine as low as 25 mg/kg. In pregnant rats, high-atrazine-dose gavage studies (for 3-12 days in rats which in proportion of lifespan might be roughly equivalent to 3 to 12 months of human life) resulted in anorexia-like effects and rapid weight loss and, consequently, could be the basis for altered fetal androgen synthesis, reduced fetal testes or liver weight, low weight fetuses, liver function changes, etc. If anorexia is a basis for the developmental effects, then such actions of atrazine should only be of human health concern under exposure conditions in which atrazine-induced unintended weight loss in humans was sufficient to alter LH actions.

Thus, it appears that a common hypothalamic-pituitary mode underlies the actions of atrazine on mammary tumors and developmental toxicity in rats, namely reduced LH secretion possibly induced by the effect of atrazine on the regulation of food consumption.

5.2 ***Does the Panel agree that the reproductive developmental findings in rats (e.g., delayed puberty, prostatitis in young animals) are a result of atrazine primary action on the hypothalamic-pituitary-gonadal axis. And if so, would the Panel also provide their view of the commonality between the cancer mode of action and the underlying basis leading to adverse reproductive/developmental outcomes and how well the EPA document addresses this commonality.***

Yes, the reproductive developmental findings in rats are a result of exposure to atrazine at relatively high doses. Data presented support the statement that the hypothalamic-pituitary-

gonadal axis is affected in the SD rat. A potential exists for reproductive and developmental disruption to exist as a consequence of HPG disturbance. There are no apparent cancer consequences.

The reproductive developmental effects in rats (delayed puberty, prostatitis) are consistent with a primary action of atrazine on the HPG axis. In the case of delayed puberty, a suppression of GnRH and LH would obviously delay increases in estradiol secretion by the ovary that characterize puberty in females. This would lead to the observed delays in vaginal opening. In the male, similar delays in increased LH secretion would lead to delays in T elevations that would result in delayed preputial separation etc. Therefore, the proposed mode of action for developmental effects shares considerable overlap with the proposed mode of action for carcinogenicity. Indeed, the neuroendocrine actions of atrazine are probably the dominant mechanisms by which atrazine exerts reproductive developmental effects.

The same endocrine perturbations that induce tumors also appear to play a role in at least some reproductive developmental effects. However, as with the cancer mode of action, the experiments and draft do not adequately address the possibility that atrazine may alter peripheral target metabolism and sensitivities to steroids.

5.3 *Do the rodent studies showing delayed puberty in both female and male rats raise a concern for children from a clinical perspective? What does it mean to have a delay in puberty in females and males caused by a compound that does not bind to an estrogen receptor?*

Alterations in puberty onset are a clear outcome of neuroendocrine and endocrine changes in the CNS mechanisms regulating reproductive functions. From a clinical point of view, puberty acceleration has occurred on a population basis over the past 50+ years in developed nations and most puberty-timing pathologies relate to precocial, not delayed puberty, especially in females. Nevertheless, an alteration in onset of puberty results from significant changes in the hypothalamaic-pituitary-gonadal axis and is an important developmental effect.

The basic hypothalamic disturbance reported was attenuation of the preovulatory spike in LH. This was presumably due to decreased pulses of GnRH leading to attenuation of the preovulatory spike of LH. This was accompanied by a corresponding decrease in hypothalamic catecholamines; but in the opinion of some neurophysiologists, hypothalamic norepinephrine levels reflect GnRH secretion rather than are causative thereof.

The neuroendocrine mechanisms regulating gonadotropin release and thus puberty are clearly influenced by the environment. For example, environmental stress, including disrupted family life, have been shown to trigger early menarche in adolescents (Belsky, Steinberg, and Draper, 1991; Wierson, Long and Forehand, 1993). Such studies reveal the exquisite sensitivity of the puberty regulating mechanism to environmental stimuli that operate via the neuroendocrine signals. Thus, alterations in neuroendocrine function by atrazine could have influence on onset of puberty. In the

cases involving social stress in humans the puberty is accelerated whereas apparently, from the rat studies, puberty is delayed as a result of atrazine consumption.

There are a number of potential mechanisms by which atrazine might control GnRH pulsatility: These include nutritional influences involving leptin and/or neuropeptide Y, sex steroids, the influence of aromatases, effects on GABA levels due to interactions with glutamic acid decarboxylase (GAD), and many others. In the absence of any data pointing to some more basic abnormality, it is impossible to evaluate any potential hazardous effects of atrazine on children other than those caused by deficient secretion of LH.

In his presentation on behalf of Novartis, Dr. John Marshall summarized in considerable detail what is known about the operation of the hypothalamic GnRH pulse generator from fetal life through the completion of adolescence. His summary was based on studies in humans and other primates. The robust pulses of LH in the fetus during the latter part of gestation are of doubtful importance to human sexual development because the fetus is supplied by ample LH in the form of chorionic gonadotropin by the placenta. There are well controlled human experiments of nature to support this conclusion: Boys born with anencephaly (and lacking any pituitary) have normally formed genitalia, although they are born with a small phallus. Boys with congenital inability to secrete GnRH (hypogonadotrophic hypogonadism or Kallman's syndrome) have a normally formed and normally sized phallus at birth. The small penis of boys with anencephaly is probably due to growth hormone deficiency since boys with congenital growth hormone deficiency are likewise born with small penises.

The LH pulsatility in the fetus continues with diminishing amplitude during the early months of postnatal life, and then LH secretion becomes barely detectable during much of the first decade of postnatal life (the juvenile pause). The restraint of GnRH pulses during this juvenile pause is probably due to impulses transmitted by g-amino-isobutyric acid (GABA) through its type A receptor. This was demonstrated by E.I. Terasawa in juvenile primates. When she blocked the type A receptor by blocking agents or blocked the formation of GABA by injecting an antisense oligonucleotide to GAD, (the enzyme that converts glutamic acid to GABA), there was prompt conversion of the nearly flat GnRH pulses to the spikes characteristic of adolescence.(PNAS 1994, 91, 17-20; Endocrinology 1999, 140, 705-712)

In the human, the first harbinger of puberty is the reemergence of LH pulses while sleeping. The diagnosis of emerging puberty is made clinically by injecting GnRH (or one of its analogues) and comparing the subsequent rise of FSH and LH. This stimulation test is often used in the diagnosis of both boys and girls with delayed onset of adolescence.

Boys and girls differ greatly in their timing of the adolescent growth spurt and onset of adolescence. Girls take their adolescent growth spurt about 2 years earlier than boys. A plot of the frequency distribution of the time at which each sex reaches its peak growth velocity (Tanner showed the close correlation of this benchmark with the emergence of secondary sexual characteristics) reveals that the age at which this occurs is not normally distributed: girls are more prone to sexual precocity and their age-frequency curve is skewed to the left, whereas the

frequency curve is skewed to the right in boys, who frequently have a physiologic delay in the onset of adolescence.

The proposed mode of action of atrazine suggests that sustained exposure to very high levels of this substance might be responsible for delaying the onset of puberty in either sex. Epidemiologists would be hard pressed to detect such an occurrence in boys because no clear cut markers of puberty exist in the male. In females the onset of menses is a clear event signaling puberty. The long-term effects of decreased LH secretion in childhood are difficult to assess. Experience with both boys and girls with hypogonadotropic hypogonadism suggests that such individuals function well when they are given appropriate sex steroids and do not suffer from behavioral or cognitive problems or from other unusual health problems.

5.4 ***Given that atrazine treatment of dams during lactation results in a decrease in prolactin, which may lead to altered TIDA neuron development and eventually prostatitis in young animals, what kind of concern does this finding mean to humans, especially as an early life stage susceptibility?***

The finding that inhibition of prolactin transmission by atrazine exposure in milk early in life leads to increased incidence of prostatitis in young adult rats is clear from the Agency report. Previous studies have shown that early post- natal availability of prolactin from milk affect the TIDA neurons in the hypothalamus to reduce dopamine (DA) production. DA is a known neuromodulator of several functions, including pituitary release of hormones and behavior. This finding may signal an important toxic effect of atrazine and should be explored in greater detail.

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