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PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

## Memorandum

Subject: Completed Atrazine Actions, Summaries and Comments

From: Melba S. Morrow, D.V.M. MSN 10/1/96  
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Health Effects DivisionThru: Joycelyn E. Stewart, Ph.D. JS 10/2/96  
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The following submissions, filed in response to the PD1 for atrazine have been completed and should be logged out of Toxicology Branch I. A summary of the submission and identification by DPBarcode and MRID numbers have also been provided.

## Summaries for Submissions Provided by Ciba for Atrazine

1. Hypothesis for Mammary Tumorigenesis in Sprague Dawley Rats  
Exposed to Certain Triazine Herbicides  
MRID 435986-11 D215419

This article discussed the mammary tumor incidence for several triazine compounds. This summary will address atrazine, only. Mammary tumor incidences were compared with data from 3 studies in Sprague Dawley rats. Dose levels ranged from 0 to 1000 ppm and the study conditions were not available in the article for comparison. At 1000 ppm, a decrease in survival was reported. The incidence of mammary adenocarcinomas was significantly increased in rats receiving 70, 500 and 1000 ppm. No hormonal data were provided in these studies for atrazine. The following is a representation of the incidence rate, tumor type and dose level for Sprague Dawley rats:

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## Mammary Tumor Incidence

Dose level	Adenomas	Fibroad	Adenocarc	#w/tumors
0 ppm	1/88	29/88	15/88	35/88
10 ppm	0/69	29/69	16/69	40/69
50 ppm	-- --	10/40	5/40	13/40
70 ppm	1/69	36/69	27/69*	48/69
500 ppm	1/70	39/70	27/70*	48/70
1000 ppm	2/89	46/89	45/89*	65/89
Historical	0-7	36-48	3-19	40-51
Control range (%)				

**Comments:** The results show that while a statistically significant increase in adenocarcinomas was present from 70 to 1000 ppm, there was no additional dose response, i.e. the the tumorigenic response was flat. This provides contradiction to the belief that the mammary lesions occurred at a dose that was in excess of the MTD. Furthermore, with the exception of early mortality at 1000 ppm, no other clinical signs were reported for animals at 70 or 500 ppm that would suggest an excess in dosing. The incidence of adenocarcinoma, in addition to being significantly increased above concurrent controls, is also outside of the historical control range for this type of tumor in Sprague Dawley rats.

2. Factors Affecting Mammary Tumor Incidence in Chlorotriazine  
Treated Female Rats  
MRID 43598612 D215420

In this article, the results and conclusions from previously conducted studies were summarized. The article states that atrazine was negative when tested for uterine weight stimulation and when thymidine incorporation was measured, high doses of atrazine did not increase incorporation, indicating that there were no compound related effects on the growth of uterine tissue. In another study, atrazine was found to weakly interact with estrogen receptors and a Scatchard plot analysis suggested that atrazine possessed both competitive and noncompetitive estrogen receptor binding properties under the most optimal conditions following incubation.

The effects on estrus cycling, vaginal cytology and reproductive hormonal levels were assessed in several separate studies. In a 2 week study in which atrazine was administered at doses of 100 and 300 mg/kg estrous cycles were significantly lengthened as evidenced by the increased amount of time the animals spent in estrus. In another study in which atrazine was administered at doses ranging from 0 to 400 ppm, prolonged vaginal estrous, characterized by increased cornified epithelial cells (that increased in relationship to dose of atrazine and advancing age of the rats) was reported. Results from a study conducted in both Fisher and Sprague Dawley rats at 0, 70 and 400 ppm demonstrated a dose

related increase in estradiol at 400 ppm in Sprague Dawley rats and an increase in progesterone in Fisher 344 rats at the same dose level.

**Comments:** In the studies conducted to support this article, it was reported that with reference to hormonal levels, there were no observable changes after 3 months. Furthermore, the article concluded that the reason for the strain divergence of estrous cycling patterns in Sprague Dawley and Fisher 344 rats is unknown. Other effects, such as that on neuroendocrine control have not been fully evaluated and the effects of chlorotriazine feeding on the arcuate nucleus or center of reproductive control have not been adequately examined.

3. Rat Tumorigenesis: Relevance of Hormonal Imbalance to Dose Selection (a paper, presented by Jim Stevens)  
MRID 43598613 D215460

Jim Stevens presented a paper in which he discussed the mechanisms of hormonal imbalance and those mechanisms which may be involved in mammary tumor pathogenesis in rats. Imbalances in hormonal levels involve either blockade of hormone synthesis or secretion, interference with enzyme systems or competition with receptor sites. Hormones are under the control of the hypothalamus and the pituitary and may serve as switches that bring about a response. These hormones may also increase tissue susceptibility to compounds which act as initiators. The paper touched on the importance of binding for hormonal responsiveness and mentioned that prolactin levels influence the number of estrogen receptors in mammary tissue and estrogen levels affect the number of progesterone receptors in the liver.

The paper discusses and highlights the differences in the reproductive hormonal make-up of aging Fisher 344 rats as compared to aging Sprague Dawleys. In Sprague Dawley rats, senescence is marked by a decrease in FSH estradiol and progesterone and an increase in prolactin. An increase in the length of the estrous cycle followed by acyclicity also occurs with aging in this strain. In Fishers, reproductive aging is characterized by an increase in progesterone levels and the existence of a state of pseudopregnancy. Galactocoeles, which are considered as markers of prolactin exposure, are more prevalent in aged Sprague Dawleys than Fishers.

In his paper, Stevens asserts that the mammary endpoint observed with atrazine in Sprague Dawley rats is an over dose phenomenon and occurs only when the MTD is exceeded.

**Comments:** It should be pointed that the only clinical signs of overdosing were a reduction in body weight and an increase in the incidence of mammary tumors at the dose levels examined. Nothing else suggests an overt toxicity that would bring about other physiological changes and result in tumor formation. If hormones are, in fact switches, further examination of effects on hormonal imbalances such as a disruption in the ratios of one hormone to another should be examined. Some of Steven's proposals have already been refuted, specifically, the blockade of the synthesis of estradiol. The effects of other hormones on the carcinogenicity of atrazine have not been fully investigated nor have the effects on or the role of the hypothalamus been examined. Interference with transforming or metabolizing systems alluded to in the Stevens paper as a possible mechanism for the production of mammary tumors, has also not been investigated.

4. Short Term Effects of Chlorotriazines on Estrus in Female Sprague Dawley and Fisher 344 Rats  
MRID 43598614 D215461

This paper focused on the results of a 2 week study in which atrazine and simazine were administered daily by gavage for two weeks. The dose levels were 0, 100 or 300 mg/kg. The study was conducted to examine the effects of the compounds on ovaries, uterus, and adrenals, estrous cycling, vaginal cytology and hormonal levels. The results discussed in this entry will pertain to atrazine only.

Significant effects (reductions) in body weight were present at both dose levels (100 and 300 mg/kg) along with significant reductions in absolute and relative ovarian and uterine weights. Absolute and relative adrenal weights were increased. Plasma hormone levels were assessed in proestrus and revealed a marked decrease in estradiol at both dose levels, significant increases in progesterone at 300 mg/kg, slight and insignificant increases in prolactin at 300 mg/kg and a decrease in mean corticosterone levels at 300 mg/kg.

When atrazine was compared to the control groups, there appears to be a significant increase in cycle length. This is characterized by an increase in the length of time spent in estrus and a decrease in the amount of time spent in diestrus. This was confirmed by an increase in the number of cornified and nucleated cells at both 100 and 300 mg/kg.

**Comments:** It is noted that this study examined the effects of both atrazine and simazine. Interestingly, when the control groups are compared to each other, there is a degree of variation that suggests that some of the findings and conclusions made in this study did not take into account the variability within the species. Furthermore with a study of only 2 weeks duration, conclusions pertaining to an increase in cycle length and hormone levels based

on a single proestrus sampling, would be premature. The length of the study would be insufficient to determine whether the results represent a blip in the data or a real pattern due to the administration of the compound.

5. Chronic Effects of Atrazine on Estrus and Mammary Tumor Formation in Female Sprague Dawley and Fisher 344 Rats  
MRID 43598615 D215462

This article provided a comparison of the results obtained in long term studies in which atrazine was administered at similar dietary dose levels to two different strains of rats (0, 10, 70, 200 or 400 ppm in Fisher rats and 0, 70 or 400 ppm in Sprague Dawley rats). The authors concluded that the levels of atrazine were at or exceeded the MTD in Sprague Dawley rats, which in turn led to lengthening of the estrous cycle primarily based on the increased number of days in estrus. Earlier onset of galactocoele formation was also demonstrated at the high dose level (400 ppm) and an earlier onset of mammary tumors with no increase in the overall incidence at study termination was reported. A hormonal profile revealed that plasma estradiol levels were significantly elevated at three months, only. Other hormonal data were unremarkable and the prolactin data during the first two sampling intervals were hemolyzed.

Based on this article, Sprague Dawley rats develop mammary tumors spontaneously as a part of the aging process and the response at the dose levels administered in this study was expected. The reproductive aging process is different in Fisher rats and much of the endocrine control of ovarian function as observed in Sprague Dawley rats is not evident in this strain.

**Comments:** The MTD issue and its relevance to mammary tumor formation is mentioned in this article. It should be pointed out that in the opinion of the agency, the MTD has not been reached or exceeded, based on paucity of clinical signs of toxicity. In these studies, body weight gain appears to be the only parameter affected and only at the highest dose level in Sprague Dawley rats (15% decrease at 400 ppm). Other studies conducted with higher doses (500 ppm, See item 1, above) did not demonstrate an exacerbation or increase in the clinical signs; however tumor incidence, specifically adenocarcinomas, was significantly increased above concurrent and historical controls.

The hormonal data are inconsistent, with no patterns being established throughout the study. Additionally, at terminal sacrifice, there was an inadequate number of animals to make a meaningful assessment of the results.

6. Ultrastructural Changes in the Rat Hypothalamus Arcuate  
Nucleus following DACT Feeding  
MRID 43598616 D215463

Sprague Dawley rats, 20 weeks of age were fed DACT at dose levels of 0 or 1000 ppm. The study was conducted to test the theory that estrogens enhance hypothalamic structural gliosis. In cases of neuronal degeneration (etiology undefined), it has been proposed that the astrocytes and microglial cells enlarge and accumulate dense bodies. The degree of enlargement and granular content are used as indices of hypothalamic damage. Glial activity has been observed in the arcuate nucleus of the hypothalamus, the region that plays a role in the secretion of gonadotropins. It is also speculated in this report that the ultrastructural pathology reported in the hypothalamus may possibly be related to stimulation of endogenous peroxidase activity which has been reported to occur during periods of prolonged estrus.

In this study the granular content in astrocytes was increased by 4% at week 32 and by 36% at week 48. The microglial cells were increased in number at both weeks 32 and 48; however, the increases were not significant and the microglial cells were not classified as reactive (meaning they were not enlarged and did not contain dense bodies).

**Comment:** An evaluation of the results showed that the granular content of the astrocytes was not homogeneous and that the microglial cells, although increased in number, were not considered reactive cells analogous to those which have been reported in cases of neuronal degeneration. The results of this study as they pertain to the effects of the atrazine metabolite, DACT, are ambiguous and the contribution of the changes observed in the hypothalamus to the increased incidence of mammary tumors in Sprague Dawley rats would need to be more thoroughly probed and correlated.

7. Possible Antiestrogenic Properties of Chloro-S- triazines in  
the Rat Uterus  
MRID 435986-17 D215464

Uterine weight, thymidine incorporation and progesterone receptor assay studies were conducted with atrazine at dose levels ranging from 1 to 300 mg/kg. The atrazine was administered for short durations (2 to 3 days) and in some instances estradiol was injected to prime the sample. It was demonstrated that atrazine had no effect on uterine weights and when administered along with estradiol, uterine weights decreased. Thymidine incorporation into uterine DNA was determined and at higher doses there appeared to be less incorporation when compared to controls. In fact, thymidine incorporation was inversely affected by dose of atrazine when rats

were pretreated with estradiol.

In reference to the progesterone receptor binding, atrazine at 50 mg/kg had a minimal effect on progesterone receptor binding activity and at 300 mg/kg, binding activity was markedly reduced in the presence of estradiol.

**Comment:** A slight estrogenic response appears to be present at lower doses in the presence of estradiol priming; however, these studies provide no information on the results that would be expected from continuous low level exposure to atrazine. This study was reviewed and commented on prior to the PD1. No information has been provided which would alter comments or opinions about the significance of this study in this submission.

8. Chloro-S-Triazine Antagonism of Estrogen Action: Limited Interaction with Estrogen Receptor Binding  
MRID 43598618 D215465

In this article, the effects of atrazine on estrogen receptor binding in uterine cytosol from intact adult Sprague Dawley rats in the presence of radiolabelled estradiol were evaluated. No competitive binding was apparent under conditions of equilibrium; however, under conditions where disequilibrium was created by preincubation with excessive (10,000 fold molar excess) atrazine and optimal temperatures, a decreased estrogen binding capacity was observed. Atrazine was not considered under the conditions of this study to be a competitive inhibitor of estrogen.

**Comment:** This article suggested that there may be some other receptor response that should be investigated, and specifically mentioned the aryl hydrogen (ah) receptor. The report further stated that the mechanism for the decreased binding capacity of estrogens is still not fully understood.

9. Failure of Atrazine and Simazine to Induce Estrogenic Responses in MCF 7 Human Breast Cancer Cells  
MRID 43598619 D215480  
MRID 43933403 (Supplement)

In this article and the supplement, the results of several assays conducted to evaluate the estrogenicity of atrazine as compared to other estrogen receptor agonists were evaluated. Atrazine did not bind to the aH receptor, did not induce proliferation of MCF-7 cells, did not increase nuclear PR-PRE binding and did not induce luciferase activity. In conclusion, atrazine did not elicit the response that would have been expected of an estrogenic compound.

**Comment:** The earlier hypotheses regarding the potential estrogenic activity of atrazine proved to be incorrect in these studies by

performed by S. Safe. This indicates that other mechanisms of carcinogenicity of atrazine exist and need to be explored. (See Table I for in vitro studies that have been performed for atrazine).

10. Evaluation of a Hormonal Mechanism for Mammary Carcinogenesis:  
Consensus Panel Report  
MRID 43598620 D215485

This document provided a basis for conclusions reached by an independent group of scientists with regard to a mechanism for carcinogenesis and the additional areas where studies would be needed to account for the influence of sex, species and strain. The panel addressed the suitability of the Sprague Dawley rat as a model for evaluating mammary carcinogens and concluded that the strain was inappropriate because of the endocrine processes that take place in the strain as it ages.

With regard to an age based mechanism, the panel concluded that in the Sprague Dawley reproductive senescence is characterized by a persistent elevation in the hormones prolactin and estrogen. In affected animals, a persistent estrus exists and is due to an ovulation failure. The panel also believes that atrazine disrupts the LH secretory mechanism in Sprague Dawley rats resulting in an acceleration of age related endocrine problems and a high background incidence of mammary tumors.

Based on their proposed mechanism(s) of action, the panel suggested additional studies, primarily to determine the effect of LH on tumorigenic response in general and the effect on mammary tissue, specifically. References are made within the document to the high level of exposure, not just to atrazine, but to other chlorotriazines and to the neuroendocrine regulation of reproductive aging.

Additional references are made in response to the results from studies designed to determine the estrogenicity. The panel concluded that 7 out of 9 in vivo tests designed to establish estrogenicity were negative for atrazine. One of the remaining positive tests has not been validated and in the other test, the results were not consistent with published reports. Anti-estrogenic activity has been demonstrated in several studies.

**Comments:** Many of the assumptions made by the technical panel have not been demonstrated (LH secretory disruption, increases in prolactin, persistent estrus); others have been refuted (induction of estrogenic responses, initially believed to be the mechanism of carcinogenicity). With regard to neuroendocrine aging, no studies providing a comparison or contrast of events in normal aging animals and in those receiving atrazine have been conducted. Suggestions that a threshold mechanism exists are discredited by the fact that in at least one study in Sprague Dawley rats, an



increase in mammary tumor incidence was present at 10 ppm and in another study an increase was apparent at 70 ppm. Furthermore, the lack of other clinical signs of toxicity that would suggest that the MTD had been exceeded were conspicuously absent in studies conducted with dose levels as high as 400 ppm. The panel has focused on dose levels but has neglected to examine the different windows of susceptibility and how they may be different in humans and in rats.

As indicated in the report, atrazine does not appear to have a significant stimulatory effect on estrogen secretion; effects on prolactin remain obscure. With regard to the studies that were suggested by the panel, the results are now in and do not add clarity to the panel's proposed mechanism with regard to the effects of LH on premature aging and mammary tumorigenesis.

11. Ciba's Response to May 3, 1994 EPA Letter by Penelope Fenner Crisp, Director, Health Effects Division  
MRID 43598621 D215487

This response, contains a discussion to address the validity of the data collected in several studies that were conducted to test Ciba's hypothesis that atrazine has a hormonally mediated impact on the development of mammary tumors. The document states that changes observed in the vagina, uterus, ovary and mammary tissue are due to an acceleration of reproductive senescence.

With regard to specific issues raised by EPA in the May 3 correspondence, concerning vaginal cytology and how the samples were read, Ciba concluded that the samples were allowed to air dry but were rewet before reading. Histomorphologic staging was another area of concern identified in the May 3, 1994 letter and the response stated that estrogen levels were most often read during proestrus. Any differences that occurred in the determination of estrous stage from vaginal smears would have been consistent throughout the study. It is still the registrant's contention that based on the vaginal smears, the animals showed signs of constant estrus.

For hormone assays, Ciba states that proestrus was chosen as the time of hormonal assays because it represented the time of most active estrogen secretion and follicles are at peak development.

12. One Year Chronic Toxicity Study with Atrazine Technical in Rats  
MRID 43598623 D215495

Atrazine was administered to Crl:CDBR rats (Sprague Dawley) at dietary levels of 0, 15, 30, 50, 70 or 400 ppm equivalent to 0, .75, 1.5, 2.5, 3.5 or 20 mg/kg for 12 months. The study was conducted to determine the effects of atrazine on the mammary and pituitary glands. Other parameters measured for evaluation of toxic effects included body weights, body weight gain, food consumption along with gross and histopathological samples.

There was an increase in the number of adenocarcinomas (6/55) when the 400 ppm group was compared to controls (1/55). No trends were established for increases in tumor incidence. Galactocoeles were present but were not dose related, as no trend was apparent. A positive trend in combined adenocarcinomas and fibroadenomas was present when data were evaluated for early onset.

**Comments:** It should be noted that the events preceding the early onset of tumors were not provided. No correlation has been made in this report on the hormonal effects of the compound and the development of lesions. (Kit has this review. It is a new study).

13. Weight of the Evidence on the Oncogenic Potential of Atrazine Consensus Panel Report  
43598624 D215502

The consensus panel report concluded the following with regard to the carcinogenic potential of Atrazine:

Atrazine induces adenocarcinoma in Sprague Dawley rats and accelerates the onset at doses greater than or equal to the MTD, with the exception of one study where increases in mammary tumors were observed below the MTD. This could not be reproduced in additional studies conducted at the same dietary level.

Atrazine is negative for oncogenicity in male Sprague Dawley rats, in both sexes of Fisher rats and in both sexes of CD-1 mice.

Atrazine accelerates age related reproductive changes in Sprague Dawley rats causing a state of constant estrus (prolonged estrogen exposure) albeit atrazine has not been demonstrated to be estrogenic by in vivo and in vitro tests. The female Sprague Dawley rat is not considered to be a good model for mammary tumor induction in humans because of the differences in reproductive cyclicity between the SD rat and human females.

Atrazine was not considered to be genotoxic in 31/37 mutagenicity studies. The remaining 6/37 studies were considered either positive or equivocal.

Atrazine is completely absorbed and rapidly eliminated via the urine. Dealkylation is the major route of metabolism and the primary metabolite is DACT.

Atrazine is structurally related to simazine in that it contains a 2-chloro-4,6-bis-(alkylamino)-s-triazine ring and both have been associated with the formation of mammary tumors (increased incidence and early onset). The panel report states that atrazine at doses of 400 ppm or greater accelerates senescence in female Sprague Dawley rats and is characterized by an increase in serum estradiol levels, early onset of constant estrus and ovulatory failure. Changes in mammary histomorphology appear to be associated with an imbalance of endogenous estrogen and not caused by an exogenous source of estrogen.

**Comments:** The consensus panel suggested a mechanism for the development of mammary tumors in Sprague Dawley rats; however, the possible influences of the hypothalamus are not investigated thoroughly or followed in this report. The effect of atrazine on the ability of LHRF (GnRF) to evoke a neuronal response has not been addressed. Furthermore, it has been stated that there is an age related decline in estrogen receptors; however, this phenomenon has not been correlated with the decline in neuroendocrine control of the estrous cycle. The panel report has alluded to the possibility that a neuroendocrine component exists in the development of mammary tumors.

In the neuroendocrine scheme of aging the following series of events are believed to take place:

Under neuroendocrine control animals lose their ability to ovulate. This leads to an increase in the secretion of estradiol. New follicles are formed but, because ovulation is impaired, there is a decrease in the number of corpora lutea formed and a decrease in the progesterone level. The balance between the progesterone/estrogen activity is shifted, with estrogen becoming the most persistent hormone. This estrogenic persistence leads to overstimulation of estrogen responsive tissues such as the uterus, mammary gland and anterior pituitary. Estrogenic effects on the anterior pituitary would lead to prolactin secretion that would in turn stimulate the rate of growth of existing mammary tumors.

While portions of the different mechanisms of carcinogenic activity have been examined, they have not been proven, correlated or reproduced with a level of confidence that would rule out all other feasible mechanisms. It is possible that the carcinogenic effect of atrazine may lie in its ability to promote phenotypic expression of mammary cancer in a sensitive strain or species. It is also possible that the mechanism is not a singular, triggering incident, but a series of events.

Within the consensus panel report there was a comparison of reproductive senescence in humans and rats. The emphasis should not be on events related to aging across species, but rather the potential for this compound to cause or be associated with the development of cancer over the lifetime of a human. A factor for consideration in the risk equation, when extrapolating from rats to humans with regard to breast cancer, is the paucity of information on hormonal disruption and the impact of any such disruption during various stages of development in humans. Additionally, the etiology of human breast cancer is for the most part unknown. Mammary cancer is not a concern solely in aging humans, but a concern over the lifetime of an individual and specifically during periods when hormonal equilibrium is disturbed (i.e. menarche, pregnancy, lactation and menopause).

14. Studies Conducted to Evaluate the Effects of LH in Determining the Mechanism of Mammary Carcinogenesis in Sprague Dawley Rats  
MRID 43934404, 05 and 06 D??

These studies were conducted to evaluate the effects of LH on estrous cycle disruption and included the method validation phase of the study and the preliminary report in addition to the main study. In the main study, atrazine was administered by gavage for 28 to 31 days at dose levels of 2.5, 5.0, 40 and 200 mg/kg to ovariectomized female Sprague Dawley rats. The duration of the study from initial dosing to terminal sacrifice was approximately 45 days. According to the study report, atrazine was associated with a decrease in the LH surge and persistent diestrus and prolonged estrus at the two highest dose levels. The observed effects on LH were only significant at 200 mg/kg. At 5 mg/kg, effects on LH surge were present but inconclusive and no disruption in estrus cycle patterns was noticeable. Based on these findings, the NOEL was 2.5 mg/kg.

**Comments:** Several of the assumptions made by the registrant in support of the LH mechanism have not been fully investigated, or the results are inconclusive. Specific flaws in the LH study included the lack of comparison of the LH surge observed in atrazine treated rats to that observed in normal, untreated middle aged (10 -15 months) rats; failure to demonstrate unovulated or abnormal ovarian follicles either grossly or histologically; failure to provide data that demonstrated a stimulation of prolactin; large standard deviations that in some instances exceed the mean values and indicate a high degree of variability in the data; and the failure to demonstrate promotion of mammary tumor growth due to the short duration of the study. A critique of the studies is provided in the May 30, 1996 memo from T.M. Crisp to E. Francis, K. Hammerstrom, K. Baetcke and M. Morrow.

In addition to the flaws in the LH study, it is understood that LH is under the control of the gonadotropin releasing hormone (GnRH) that is released by the anterior pituitary. No links or assessments have been conducted to determine the role of the pituitary hypothalamic tract with regard to the hormonal perturbations that are supposedly occurring in these aging rats. Furthermore, if LH is controlled at this site in the body, the changes in this hormone could be the result of an effect at the site of hormonal control.

### **Conclusions:**

Given the information presented by Ciba Geigy in response to EPA's PD1, the Agency concludes that a mechanism for mammary tumors has not been adequately presented. Some of the statements made in the hypothesis regarding LH involvement have not been proven and other statements and conclusions are not as uncomplicated as presented, in that other factors are involved in the control of hormonal output.

As indicated in an earlier correspondence, the registrant continues to describe a phenomenon. Although the registrant has identified an association between atrazine and the suppression of a surge in leutenizing hormone (LH), there is a failure to correlate this effect with changes in the target tissue after prolonged dosing and to compare any observations to the changes occurring in a normal untreated middle-aged rat.

The registrant has stated that the differences in the reproductive aging of Sprague Dawley rats and humans makes the latter more susceptible to the development of mammary tumors. Unfortunately, there are still many unknowns in the etiology of human breast cancer. Breast cancer is not solely a disease of post-menopausal women and may require years after an initiating event to manifest. The suggestion by the company that treatment with atrazine may cause a disruption in the normal hormonal milieu in rats can not be dismissed as having no bearing on humans when the same hormones are present across species and are subject to peaks and valleys with respect to serum concentrations. Unlike the case that can be made for renal tumors in rats and the association with alpha 2 U globulins (a substance that has not been found in humans), hormonal disruption in this case is not species specific.

The registrant has also placed a great deal of significance on the aging phenomenon. The emphasis in this case appears to be misplaced, in that the stress should not be on the similarities in aging rats vs aging humans, but on the effect that a disruption in hormones may have over the a human's lifetime. Additionally, the potential to induce cancer in humans is not limited in this case to breast cancer, only, but because of the hormonal implications, other organs under endocrine control would have to be considered.

The company's statement that mammary cancer occurred at dose levels that were in excess of the MTD have been addressed by HED's Cancer Peer Review Committee. This premise of dosing excess as stated by Ciba is unfounded and is contradicted by other theories that they have proposed on the mechanism of carcinogenesis. At these purported excessive dose levels (400 ppm and above), the only consistent sign of toxicity was a decrease in body weight that ranged in several studies from between 11 to 17%. No other clinical signs of toxicity were reported at this level and effects on mortality were not reported until the dose level was increased to 1000 ppm.

If all doses and proposed mechanisms are considered, the results reported in the LH surge study indicate that the NOEL for effects on the leutenizing hormone was 2.5 mg/kg. In this study a NOEL of 2.5 was determined after only 28 days of atrazine administration. In a two year feeding study, the lowest dose at which mammary tumors were present was 70 ppm (equivalent to 3.5 mg/kg). In this study, LH levels were not measured so there is no indication on whether an attenuation of a hormonal surge may have occurred at this dose. If both the NOEL for LH attenuation and the LOEL for carcinogenicity are considered together, there does not appear to be an adequate margin of exposure from the no effect level to the level which has been associated with cancer.

The sponsor has placed an abundance of weight on the results of the LH surge studies to arrive at a mechanism for mammary tumor development in Sprague Dawley rats. As pointed out earlier, LH levels are directly influenced by the gonadotropin releasing hormone, which is controlled and released in the pituitary. No links have been made nor have adequate studies been designed to examine the role of the pituitary hypothalamic tract in the proposed hormonal disruption scenario. The effects that were seen on LH levels in the specially designed studies may be indicative of a single event in a chain of events that is required to bring about a perturbation in hormonal levels.

In conclusion, the sponsor has not provided adequate support for a hormonal mechanism of carcinogenesis nor have they adequately made a case that the response is the result of an excessive dose. Although differences between the strains in question have been demonstrated with regard to hormonal fluctuations and aging; the suitability of Sprague Dawley rats as opposed to Fisher 344 rats in determining the potential risk of atrazine to humans has not been refuted. It has been called to our attention by the registrant that since these studies have been provided in response to the PD1, additional studies have been conducted and will be submitted in the near future.

The metabolism studies provided for atrazine have been addressed in reviews by Bob Zendzian. These include studies submitted under D215354, D215358, D215359 and D215361. All of these should be removed from HED's record of pending actions. Hard copies of these reviews will be provided with the hard copy of the the memo.



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<b>Chemical:</b>	<b>Atrazine</b>
<b>PC Code:</b>	<b>080803</b>
<b>HED File Code</b>	<b>13000 Tox Reviews</b>
<b>Memo Date:</b>	<b>10/24/96</b>
<b>File ID:</b>	<b>DPD215419</b>
<b>Accession Number:</b>	<b>412-03-0019</b>

**HED Records Reference Center**  
**12/31/2002**