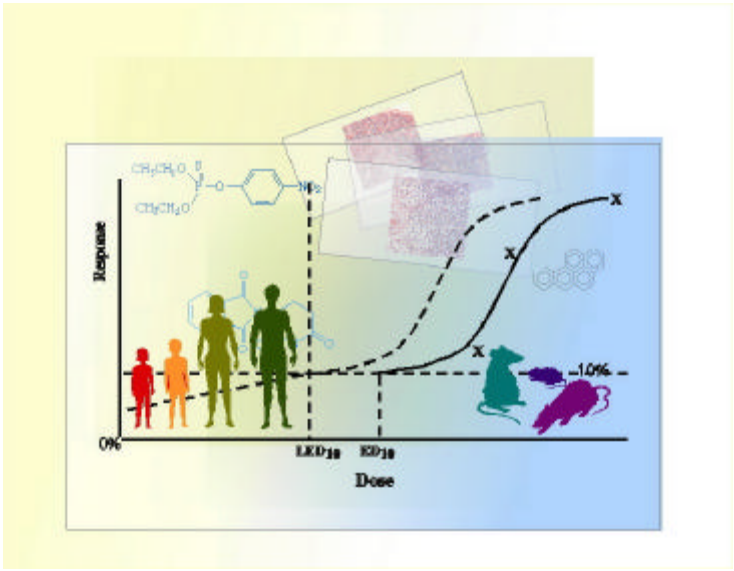
PRELIMINARY DRAFT

Hazard And Dose-Response Assessment And Characterization

Atrazine



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U.S. Environmental Protection Agency
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Health Effects Division (7509C)

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Preface

Over the last several years there has been increasing concern about the possible human health effects posed by chemicals that may alter the normal function of the endocrine system. Within the scientific community there is much debate and discussion about the extrapolation of animal findings on endocrine disruptors to predict and quantify such potential effects in humans, including children.

Agency guidance regarding endocrine perturbations in health risk assessment is limited to thyroid follicular cell carcinogens (US EPA, 1998a). Laboratory animal studies available on atrazine indicate that its mode of action in rats involves a perturbation of the neuroendocrine system that results in prolonged exposure to endogenous estrogen and prolactin. This endogenous exposure to estrogen leads to carcinogenic effects on the mammary and pituitary gland. There are also animal data available showing that there is an association between the adverse effects of atrazine on neuroendocrine control of reproductive developmental function. Given the complexity and multiplicity of effects that result from exposure to atrazine, the Office of Pesticide Programs (OPP) is at a point in its assessment of atrazine where external peer review by the FIFRA Scientific Advisory Panel (SAP) would facilitate further development and refinement of the draft health assessment document. Furthermore, very little is understood about the long term consequences that may result from prenatal and early postnatal exposures to neuroendocrine-perturbing chemicals. Thus, presenting the atrazine health assessment to the SAP at this time also allows the OPP an opportunity to obtain comments on the adequacy of the approach taken by OPP to address potential hazard to children.

The aim of the SAP review is to obtain advice and comment on the draft document on specific science issues, such as: what factors should be considered in evaluating this particular neuroendocrine mode of action?; what are the relevance and implications of this type of perturbation in humans?; what are the key biological events driving the hazard concern; and what are the potential cumulative effects and hazards on the developing brain that could result from the effects of atrazine on the function of the endocrine system? This external scientific peer review is a significant and critical step as the OPP proceeds to develop a sound and scientifically credible health risk assessment on atrazine as part of the mandate under the 1996 Food Quality Protection Act to protect public health and the environment. OPP intends to use the SAP's comments, as well as public comments that are received to further refine this draft document. Thus, the conclusions and analyses presented here within are considered preliminary.

Introduction

Over 10 years ago, atrazine was found to induce mammary gland tumors in Sprague Dawley female rats (Mayhew, 1986). Shortly afterwards, the Office of Pesticide Programs (OPP) classified atrazine as a possible human carcinogen (*Group C)* based on "limited evidence for the oncogenicity of the chemical in rats" (Hauswirth, 1988a,b). In 1988, OPP asked the FIFRA Scientific Advisory Panel (SAP) to comment on the cancer classification. The SAP agreed with OPP's classification of atrazine as a *Group C* carcinogen. The 1988 SAP also raised the possibility of a hormonal mode of action underlying atrazine's carcinogenicity (Copley, 1988). Accordingly, OPP 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 potential mode of carcinogenic action to explain the mammary gland tumor response found in female SD rats. The Agency's National Health and Environmental Effects Laboratory has also generated information on atrazine's neuroendocrine effects, as well as its effects on reproductive development in young rats.

The purpose of this draft document is to update and revise OPP's previous cancer assessment of atrazine by considering new information bearing on it's postulated mode of action. The draft document presents an integrative approach that uses a common neuroendocrine mode of action to evaluate the potential for both cancer and noncancer health effects (especially reproductive and developmental outcomes). This preliminary assessment also addresses how the available mode of action information influences decisions about the human hazard potential including sensitive subpopulations (*e.g.,* children). This draft document is organized into three parts, A, B, and C. Each has its own List of Contents.

- **Part A** summarizes the key conclusions on the cancer and reproductive developmental hazard potential and mode of action, and provides an integrated synthesis and characterization of the main findings:

  - *Chapter 1* provides a summary of tumor and other key data supporting the carcinogenicity of atrazine, as well as data on the reproductive developmental effects of atrazine.

- *Chapter 2* provides a technical hazard characterization and presents the mode of action analysis. The mode of action analysis is based on a framework described in the Agency's 1999 draft revisions to its guidelines for carcinogen risk assessment (US EPA, 1999a). This framework is used for judging whether the available evidence supports the mode of carcinogenic action in rats postulated for atrazine. This Chapter also discusses the common events in this mode of action which may lead to consequences on reproductive development.

- *Chapter 3* addresses what inferences can be made about the human relevance of the rat based findings on the mode of action conclusions presented in Chapter 2, and discusses whether there is special concern for children. The proposed dose-response extrapolation approach for cancer is also presented.

❑ **Part B** of the document (Chapters 4-9) presents a detailed carcinogenicity assessment and evaluation of the available epidemiology, toxicology, metabolism, mutagenicity, and mode of action studies on atrazine that are summarized in Chapter 1 of Part A.

❑ **Part C** of the document (Chapters 10-13) presents an evaluation of special reproductive/developmental studies performed on atrazine, as well as a review of available reproductive epidemiology studies.

List of Acronyms

CI	Confidence Ratio
CL	Corpea Lutea
DA	Dopamine
DACT	Diaminochlorotriazine
DMBA	Dimethylbenzanthracene
ED_{10}	Effective Dose - Central estimate on a dose associated with a 10% response adjusted for background
F-344	Fischer-344
FSH	Follicle Stimulating Hormone
GD	Gestational Day
GnRH	Gonadotrophin Releasing Hormone
HDT	Highest Dose Tested
LE	Long Evans
LED_{10}	Lower Limit on a Effective Dose - 95% Lower confidence limit on a dose associated with 10% response adjusted for background
LH	Lutenizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
MTD	Maximum Tolerated Dose
NHL	non-Hodgkins Lymphoma
NOAEL	No Observed Adverse Effect Levels
OR	Odds Ratio
OVX	Ovariectomized/Ovariectomy
PCOS	Polycystic Ovarian Syndrome
PND	Postnatal Day
PoD	Point of Departure
PPS	Preputial Separation
SD	Sprague-Dawley
PRL	Prolactin
PIF	Prolactin Inhibiting Factor

Organizations

CARC	Cancer Assessment Review Committee
HED	The Office of Pesticide Program's Health Effects Division
IARC	The International Agency for Research on Cancer
MARC	Metabolism Assessment Review Committee
NTP	National Toxicology Program
SAP	Scientific Advisory Panel

PART A

Preliminary Hazard and Mode of Action Characterization

Chapter 1

1. Summary of Effects

This Chapter summarizes the data discussed in the hazard assessment portion of this document (Parts B and C). The summary forms the basis for the analysis of the mode of carcinogenic action information presented in Chapter 2 and draft OPP science policy positions on human relevance of the animal tumor findings and the classification of atrazine for human carcinogenic potential developed in Chapter 3. This Chapter also presents a summary of data on the reproductive and developmental toxicity of atrazine.

1.1 Effects Attributable to Treatment of Rats and Mice with Atrazine

Treatment of female 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 carcinomas and adenomas, mammary fibroadenomas, and pituitary adenomas. Atrazine treatment of female SD rats also leads to certain non-neoplastic responses which precede and some of which may be antecedents to the neoplastic responses. A prominent effect is an attenuation of the luteinizing hormone (LH) surge that is necessary for normal reproductive cycling and a disruption of the estrous cycle. Effects on mammary tissue, namely markers of estrogen and prolactin (PRL) exposure, include increased incidences or increased severity of alveolar development, acinar development, dilated ducts, increased secretory activity, and galactoceles. Prolactin exposure is more strongly associated with the development of mammary fibroadenomas while estrogen exposure is more supportive of the development of adenomas/carcinomas. Estrogen also stimulates prolactin secreting cells and predisposes them to neoplasia. Data from short-term, high-dose studies suggest that a primary site of action of atrazine is the hypothalamus.

Results of mutagenicity assays mostly are negative. Assays designed to evaluate direct estrogenic activity of atrazine have failed to attribute exogenous estrogenic activity to atrazine treatment. Treatment with the close structural analogues, simazine and propazine, also lead to the formation of mammary tumors in female SD rats. Treatment of male SD rats or CD-1 mice of either sex with these chemicals does not result in an increased incidence of tumors at any site.

1.2 Carcinogenic Effects

Evidence from epidemiologic studies is not sufficient to establish whether atrazine may be carcinogenic to humans. Therefore, any inferences as to human carcinogenic potential must be determined from animal studies (see Part B, Chapter 4).

Table 1-1 summarizes the data on the incidence and onset of mammary adenomas/carcinomas found in carcinogenicity bioassays following administration of atrazine to female SD rats. The data generated on the formation of mammary fibroadenomas in female SD rats treated with atrazine is summarized in Table 1-2. These benign tumors are considered separate from mammary carcinomas because they are of a different cell origin than the tubular and glandular adenomas and carcinomas. Carcinomas arise from undifferentiated terminal end buds and terminal ducts of the mammary gland; fibroadenomas arise from more differentiated structures such as alveolar buds and lobules (Russo and Russo, 1996). In addition to increased incidence/early onset of mammary gland tumors, an early onset is found for pituitary adenomas. Table 1-3 summarizes data regarding the associations between atrazine treatment and the formation of pituitary adenomas.

1.2.1 Mammary Carcinomas

Treatment of female SD rats with atrazine leads to an increased incidence of mammary carcinomas and adenomas in one and two year bioassays (Mayhew, 1986; Morseth, 1998; Pettersen and Turnier, 1995). Serial sacrifice data show that atrazine treatment of female SD rats results in an early onset of mammary carcinomas (Thakur, 1991a; Pettersen and Turnier, 1995). Data on time of onset of mammary carcinomas as determined by palpation also show an early onset of mammary carcinomas (Thakur, 1992a; Morseth, 1998). The lowest dose of atrazine associated with an increased incidence in mammary carcinomas is 3.5 mg/kg/day (Mayhew *et al*., 1986). The NOAEL in the same study was 0.5 mg/kg/day.

Table 1-1. Carcinogenicity Bioassays with Atrazine: Incidence and Onset of Mammary Adenomas/Carcinomas in Female SD Rats

Study	Duration	Tumor Incidence
Mayhew *et al.*, 1986	2-year	Dose (mg/kg/day) 0 0.5 3.5 25 50 15/88**16/67 **27/69* 27/68* 45/60****
Thakur, 1991a	2-year serial sacrifice month 9 12 15 18 24	Dose (mg/kg/day) 0 4.23 26.63 0*** 0 4 0 1 2 2 0 1 5 2 4 2 1 0
Thakur, 1992a	2-year terminal sacrifice week of onset# ≤ 52 53-78 79-104 0-104 mean wk. onset	Dose (mg/kg/day) 0 3.79 23.01 **0/14*** 3/11 **6/18*** 8/14 3/11 5/18 6/14 5/11 7/18 17/60 13/59 22/60 78.9 72.5 65.4
Morseth, 1998	2-year week of onset# ≤52 53-78 79-104 0-104 mean wk. onset	Dose (mg/kg/day) 0 1.5 3.1 4.2 24.4 1/11 2/15 0/14 2/10 6/23 5/11 6/15 7/14 6/10 7/23 5/11 6/15 7/14 2/10 10/23 12/80 18/80 20/79 14/80 **27/80**** 72.6 77.2 78.6 64.4 64.8
Pettersen and Turnier, 1995	1-year serial sacrifice month 9 12 (no tumors at 3 & 6 mo.)	Dose (mg/kg/day) 0 0.8 1.7 2.8 4.1 23.9 1/10## 1/11 0/10 0/10 0/10 1/10 1/25 1/24 1/25 2/25 2/24 6/25

* = p=≤0.05; **=p=≤0.01; at control=trend, at dose group=pairwise versus control; ***per 10 animals; #=onset as determined by first palpation of a tumor; ## incidences for adenomas and adenocarcinomas combined.

Table 1-2. Carcinogenicity Bioassays with Atrazine: Incidence and Onset of Mammary Fibroadenomas in Female SD Rats

Study	Duration	Tumor Incidence
Mayhew *et al.*, 1986	2-year	Dose (mg/kg/day) 0 0.5 3.5 25 50 20/88 24/65 21/69 21/68 20/89
Thakur, 1992a	2-year terminal sacrifice week of onset# ≤52 53-78 79-104 0-104 mean wk. onset	Dose (mg/kg/day) 0 3.79 23.01 2/35 1/27 3/39 16/35 15/27 18/39 17/35 11/27 18/39 39/60 30/59 41/60 76.4 76.1 72.7
Thakur, 1991a	2-year serial sacrifice month 9 12 15 18 24	Dose (mg/kg/day) 0 4.23 26.63 0 ## 0 2 1 0 2 2 5 1 2 4 4 3 3 4
Morseth, 1998	2-year week of onset# ≤52 53-78 79-104 0-104 mean wk. onset	Dose (mg/kg/day) 0 1.5 3.1 4.2 24.4 0/15 1/18 3/26 1/26 1/22 9/15 11/18 13/26 14/26 9/22 6/15 6/18 10/26 11/26 12/22 16/78 **25/79* 34/77** 29/78* 25/77*** 76.1 72.4 73.7 73.3 76.3
Pettersen and Turnier, 1995	1-year month 9 12 (no tumors at 3 & 6 mo.)	Dose (mg/kg/day) 0 0.8 1.7 2.8 4.1 23.9 1/10 0/10 0/10 0/10 1/10 1/10 1/25 2/24 2/25 0/25 3/24 3/25

*p=<0.05;**p=<0.01; at control=trend, at dose group=pairwise versus control; #=Time of onset as determined by first palpation of tumor; ## = Incidence per 10 animals.

In one study, treatment of male and female F344 rats with a high dose of about 38 mg/kg/day of atrazine was reported to lead to an increased incidence of benign mammary tumors in males (Pinter *et al.,* 1990). The finding is difficult to evaluate because, among other shortcomings, no control animals survived to study termination, the study covered a lifetime and at approximately 30 months of age when the study was terminated, background mammary tumor incidence in untreated male rats would be expected to be similar to the incidence reported in the high dose group. Further, a separate study with F344 male and female rats did not show atrazine treatment induced the formation of tumors of any kind (Thakur, 1992b).

1.2.2 Mammary Fibroadenomas

With one exception (Morseth, 1998), atrazine treatment has not been shown to lead to a statistically-significant (pairwise comparisons, treatment group versus control) increased incidence of mammary fibroadenomas. The apparent increased incidence in fibroadenomas in the single study may not be treatment related because there is no dose-response trend among treatment groups over a 16-fold increase in doses; the control group incidence is low compared to historical control rates; and the incidences in atrazine treatment groups are within historical control ranges. As illustrated in Figure 1-1, data from one serial sacrifice study (Thakur, 1991a) support an association between atrazine treatment and an early onset of mammary fibroadenomas. However, an early onset of mammary fibroadenomas was not evident in the other serial sacrifice study (Pettersen and Turnier,1995). The Thakur data suggest an early onset of mammary fibroadenomas at the lowest atrazine dose administered, 4.23 mg/kg/day.

1.2.3 Pituitary Tumors

There are no increases in the incidences of pituitary tumors at the terminal sacrifice (24 month) in any of the carcinogenicity studies performed with atrazine. Because the background incidence of pituitary tumors is in the range of 80-90% at 24 months of age in SD rats, the lack of an increased incidence in pituitary tumors at terminal sacrifice may not be surprising. However, there is evidence for an earlier onset of pituitary tumors at nine and 12 months in female SD rats treated with atrazine in one serial sacrifice study (Thakur 1991a) but not in a second 12-month

study which included a nine month interim sacrifice (Pettersen and Turnier, 1995). Figure 1-1 depicts the dose-response data for the cumulative incidence of pituitary tumors over time and shows that there is an apparent early onset of pituitary tumors in the Thakur (1991a) serial sacrifice study. The information in Figure 1-1 shows that an early onset of pituitary tumors can be attributed to atrazine treatment at a dose level of 26.23 mg/kg/day. Neither an early onset nor an increased incidence of pituitary tumors is evident at an atrazine dose level of 4.23 mg/kg/day. Table 1-3 provides the incidences of pituitary tumors found at each sacrifice interval.

Figure 1-1. Cumulative Incidence of Pituitary â-Adenomas (Thakur, 1991a)

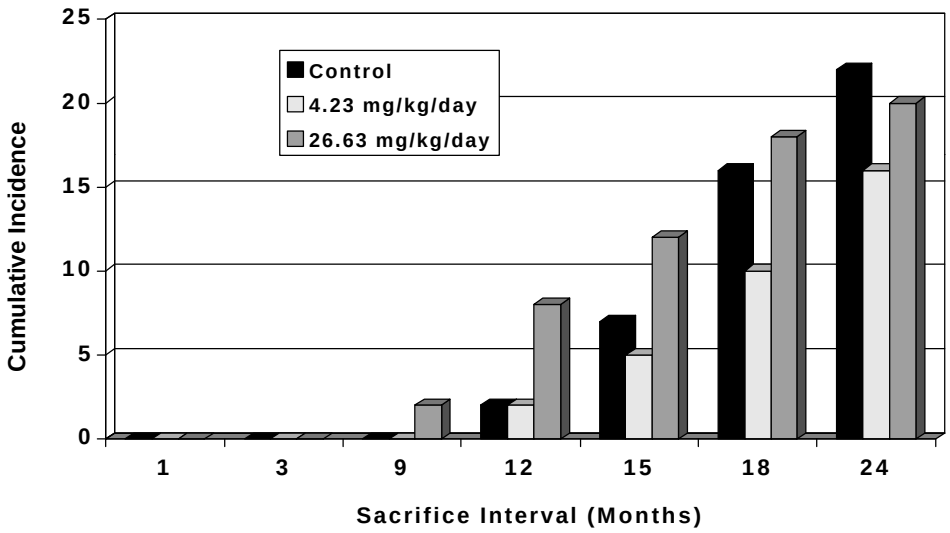


Table 1-3. Carcinogenicity Bioassay: Incidence of Pituitary Adenomas (Thakur, 1991a)

Sacrifice Time (Months)	Dose (mg/kg/day)		
	Control	4.23	26.63
9*	0**	0	2
12	2	2	6
15	5	3	4
18	9	5	6
24	6	6	2

* = No tumors at one and three months; ** = Incidence/10 Animals

1.3 Potential Antecedents to Carcinogenicity

Chronic atrazine treatment of female SD rats leads to the expression of a number of non-neoplastic neuroendocrine disruptions and of histomorphologic effects on mammary and pituitary glands. Neuroendocrine effects include attenuation of LH surges, disruption of the estrous cycles, and an increase in pituitary weights. Endocrine associated histomorphologic effects on mammary tissue include increases in the incidences of acinar/lobular development and secretory activity and severity of galactoceles, in atrazine treated animals.

As discussed in Part B, Chapter 9.3, preliminary data implicate the hypothalamic-pituitary axis as a primary site of atrazine toxicity (Cooper *et al*., 1996, 1998; Cooper *et al.,* 2000). Atrazine appears to affect the catecholamine neurotransmitters in the hypothalamus by decreasing norepinephrine (NE) and increasing dopamine (DA) (Cooper *et al.*, 1998). The decrease in NE results in a decrease in gonadotropin releasing hormone (GnRH), with a corresponding diminution of surges of luteinizing hormone (LH). If serum LH levels do not display a proestrus afternoon surge above a critical level then ovulation does not occur, and the ovarian cycle is disrupted. The inhibition of ovulation following continued atrazine exposure leads to maintenance of a state (prolonged or constant estrus) where ovarian follicles continue to secrete estrogen. Removal of the estrogen stimulus by ovariectomy abolishes the induction of mammary tumors by atrazine treatment.

1.3.1 Attenuation of the LH Surge

Table 1-4 is a summary from a one month study on the effects of atrazine treatment on the preovulatory surge of LH in female SD rats while Table 1-5 provides a summary of the LH surge effects following six months treatment. Although LH data were collected at several time periods in addition to those shown, table entries are limited to periods when LH blood levels should be near or at baseline values (1100 hours) and the period when LH blood levels should be near or at the peak surge value (1800 hours). Thus, these time periods are appropriate points for evaluating the fold increase in serum LH compared to baseline values and for ascertaining the effects of atrazine on the preovulatory surge.

Table 1-4. LH Data (mean ± sd) from Animals Repeatedly Bled in the One-Month Study (Morseth, 1996a) (LH values given are in picograms/mL)

Dose mg/kg/day	1100 Hours	1800 Hours	Fold Increase*
0	732 ± 461	2650 ± 2389	3.6
2.5	1101 ±652	3015 ±3220	2.7
5.0	810 ±519	2717 ±2542	3.3
40	755 ±389	1450 ±857	1.9
200	514 ±503	812 ±470	1.6

*Increase = 1800 hour values (peak values) divided by the 1100 hour values (baseline values)

Table 1-5. LH Data (mean ± sd) from Animals Repeatedly Bled in the Six-Month Study (Morseth, 1996b) (doses are in LH values given are in picograms/mL)

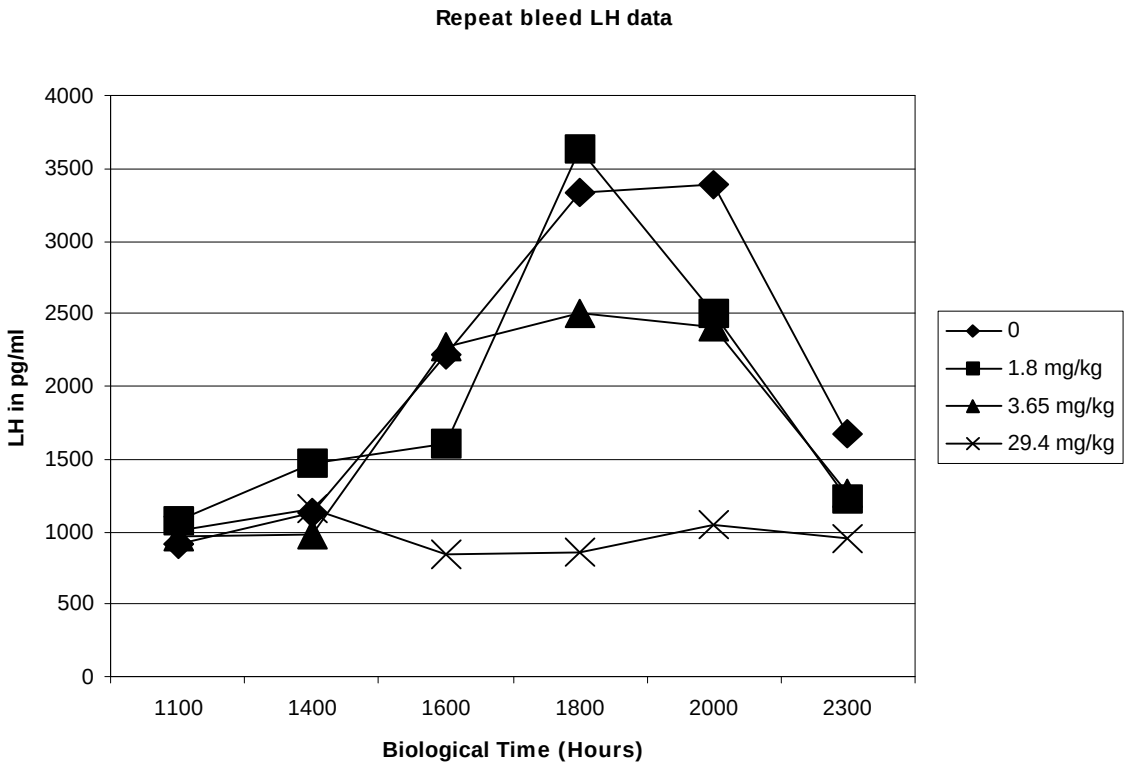
Dose mg/kg/day	1100 Hours	1800 Hours	Fold Increase*
0	909±410	3336±3138	3.7
1.8	1075±621	3631±2732	3.4
3.65	972±353	2500±1897	2.6
29.4	1005±482	858±416	<1.0

*Increase = 1800 hour values (peak values) divided by the 1100 hour values (baseline values)

As shown in Table 1-4, treatment of female SD rats with 200 mg/kg/day of atrazine for one month leads to a pronounced attenuation of the LH surge while treatment with 40 mg/kg/day suppresses the preovulatory surge to a lesser degree. Treatment with atrazine over a six month period (Table 1-5) results in effects at lower doses: an abolishment of the preovulatory surge at 29.4 mg/kg/day and an attenuation of the LH surge at 3.65 mg/kg/day. Figure 1-2 presents graphically the LH levels over the entire sampling period (1100 to 2300 hours) in the six month study. Atrazine treatment suppresses the LH surge in a time and dose dependent fashion. In other words, lower doses of atrazine require longer periods of time to produce an attenuation of the LH surge.

1
2

Figure 1-2. Effects of Atrazine Treatment on the LH Surge in Female SD Rats After Six Months of Dosing



1.3.2 Estrous Cycle Disruptions

In the normal female SD rat, approximately 20-25% of the days of the estrous cycle are spent in estrus. Atrazine treatment leads to a disruption of the normal reproductive cycle as evaluated by vaginal smears (Table 1-6) (Morseth, 1996b). As early as 13 weeks following initiation of treatment and continuing throughout the remainder of the six month study, there is a statistically-significant increase in the percentage of days spent in estrus (control - 31%; 29.4 mg/kg/day - 40%). By 21 to 22 weeks of treatment, the effect on the days in estrus is also statistically-significant in animals treated with 3.65 mg/kg/day atrazine (control - 32%, 3.65 mg/kg/day - 45%).

Table 1-6. Percentage of Days (± sd) in Estrus for SD Females Following Six-Month Exposure to Atrazine through the Diet (Morseth, 1996b)

Dose (mg/kg/day)	9-10 weeks	13-14 weeks	17-18 weeks	21-22 weeks	21- 26 weeks
0	25 ± 9.4	31 ± 22.4	34 ± 24.2	32 ± 25.4	47 ± 32.2
1.8	25 ± 4.8	28 ± 18.0	33 ± 24.7	41± 31.9	48± 35.5
3.65	26 ± 10.2	31 ± 21.1	36 ± 25.1	**45± 32.2***	54 ± 35.1
29.4	26 ± 9.3	**40 ± 27.6***	**45 ± 32.1***	**51 ± 34.8****	**63 ± 37.0***

* $p \leq 0.05$; ** $p \leq 0.01$

1.3.3 Effects on Pituitary Weights

Atrazine treatment of female SD rats leads to an early increase in pituitary weights by nine months (Table 1-7). Pituitary weights were increased by 54% over control weights at a dose level of 26.23 mg/kg/day. A less pronounced effect was observed at 4.23 mg/kg/day (25% increase over control pituitary weights) at nine months but not at other times).

Table 1-7. Effects of Atrazine Treatment on Group Mean Absolute Pituitary Weights (mg ± sd) in Female SD Rats (Thakur, 1991a)

Dose (mg/kg/day)	3 months	9 months	12 months
Control	23 ± 4	24 ± 6	37 ±20
4.23	21.2 ± 30.0 (-8%)*	30 ± 6 (+25%)	35 ± 26 (-4%)
26.23	21 ± 8 (-11%)	37 ± 8 (+54%)	42 ± 15 (+13%)

*Values in parenthesis represent percent change relative to control

1.3.4 Histomorphology of Mammary Tissue

Endocrine associated histomorphologic effects on mammary tissue found following treatment of female SD with atrazine include increases in the incidence and severity of acinar development, acinar/lobular development, secretory activity, dilated ducts with secretion, and galactoceles. Each of these effects are considered to be associated with exposure of mammary tissue to estrogen and/or prolactin (Part B, Chapter 9).

The incidences and severity of acinar development, which is primarily associated with estrogen secretion, seemed to be increased at three and nine months in both the low and high dose groups.

Secretory activity is primarily associated with prolactin exposure. At nine months, incidences of animals determined to have increased incidence and severity of secretory activity increased as a function of increasing atrazine dose-levels.

The development of dilated ducts is primarily influenced by prolactin secretion. The incidences and severity of dilated ducts (with secretion) increased markedly at the low and high dose at nine months and at the high dose at 12 months. There is also a suggestion that the incidence of lesions of ducts was increased at the high dose at three months.

The incidence and severity of galactoceles, primarily a marker of prolactin secretion, were reported to increase at both nine and 12 months in a serial sacrifice study (Thakur, 1991a). This increase is pronounced in the 26.23 mg/kg/day atrazine treatment group. The response at the 4.23 mg/kg/day does not indicate a treatment-related effect.

An examination of the individual animal data from Thakur (1991a) is quite useful in demonstrating the relationships between mammary and pituitary tumors, pituitary weights, and histomorphological indications of hormone exposure in the mammary gland. Also evident, when individual animal data from the nine month time point in this study is examined, is the early onset of these parameters. Appendix Tables 27, 28, and 29 display these parameters for each individual animal at the nine month time point in this study.

Early onset of tumors is clear from comparing the control to atrazine-treated animal data displayed in Appendix Tables 27, 28 and 29. None of the ten control animals at this time point had a mammary or pituitary tumor while five of ten and two of ten 400 ppm animals had a mammary tumor or pituitary tumor respectively. Early onset of histomorphologic markers of hormone exposure of the mammary gland is also evident. Only one of the ten control animals had a galactocele or had index weighted scores of 3 or greater for secretory activity or dilated ducts with secretion at nine months. At 400 ppm, eight of the ten animals had galactoceles and eight of the ten had weighted index scores of either three or four for secretory activity or dilated ducts with secretion.

The relationship of these parameters to each other is clear when the data from each animal at these time points is examined. For example, the one and only animal which had a galactocele in the control group, also was the only animal with a three or four weighted index score for secretory activity and dilated ducts and also had the heaviest pituitary in the group. A pituitary would be expected to be enlarged due to lactotroph hyperplasia. Lactotroph hyperplasia is associated with increased prolactin secretion; thus, the animal with the heaviest pituitary is secreting the most prolactin and this is why it is the only animal in the group with a galactocele and high scores for markers of prolactin exposure in the mammary gland. Similar examples can be found in the 70 ppm group where the two animals with the heaviest pituitaries both had galactoceles. Two other animals in this group also had a galctocele, but had pituitaries that were close to the average pituitary weight of the group. Though the pituitaries in these two animals did not weigh an exceptional amount, they were the only two animals in this group in which histopathology detected increased focal hyperplasia of the pituitary. Thus, all four animals with galactoceles (a marker of prolactin exposure) had either heavy pituitaries or focal hyperplasia of the pituitary as detected by histopathology.

1.4 Mutagenic and Estrogenic Activity

The totality of the evidence from a variety of *in vitro* and *in vivo* studies does not support a role for mutagenicity or DNA damaging potential for atrazine. A detailed evaluation of the genotoxicity studies available on atrazine, its metabolites, and structural analogues is provided in Part B, Chapter 6. Additionally, as discussed in Part B, Chapter 7, numerous studies indicate that atrazine does not have exogenous estrogenic activity.

The mutagenic compound *N*-Nitrosoatrazine (NNAT) can be formed *in vitro* when atrazine and nitrite are mixed at an acid pH. Because nitrites and atrazine can be found together in drinking water, concern has been raised about this mutagenic chemical. Although the hypothesis has been advanced that NNAT can be formed in the acid pH found in the stomach, the formation of NNAT in the stomach *in vivo* has yet to be demonstrated. Further, cancer bioassays in female Swiss mice and female Wistar rats failed to show a carcinogenic response following NNAT exposure.

1.5 Structure Activity Relationships

Like atrazine, treatment of female SD rats with simazine and propazine leads to an increased incidence and/or early onset of mammary tumors. Also like atrazine, treatment of male SD rats or CD-1 mice of either sex with simazine or propazine does not lead to an increase in tumor incidences at any site (see Part B, Chapter 8).

1.6 Doses Associated with Effects

Tables 1-8 and 1-9 list NOAELs and LOAELs for the neoplastic and non-neoplastic effects reported to be associated with treatment of female SD rats with atrazine.

1.7 Chronic, Developmental, and Reproductive Toxicity

The data summarized in sections 1.1 through 1.6 indicate that primary underlying events that lead to decreases in LH and prolactin release by the pituitary, irregular estrous cycles, and mammary and pituitary tumor formation following treatment of female SD rats with atrazine involve disruption of the hypothalamic mechanisms involved in the regulation (release) of pituitary hormone secretion. The proximal effects of atrazine that lead to these outcomes have been identified as increased dopamine levels and decreases in norepinephrine, and diminished ability to release GnRH from the hypothalamus (Cooper *et al.*, 1998). Because reproduction and development are controlled by the neuroendocrine system, there are concerns that atrazine treatment could lead to reproductive or developmental toxicity.

Table 1-8. NOAELs and LOAELs (mg/kg/day) Associated with Neoplastic Responses of Female SD Rats Treated with Atrazine

Response #	Duration of Exposure (Months)	Dose in mg/kg/day (Incidence)			Reference
		Control	NOAEL	LOAEL	
Carcinomas	24	0 (15/88)	0.5 (16/67)	3.5 (27/69)	Mayhew *et al*., 1986
Carcinomas	24	0 (12/80)	4.2 (14/80)	24.4 (27/80**)	Morseth, 1998
Carcinomas	24	0 (17/60)	3.79 (13/59)	23.01 (22/60)	Thakur, 1992a
Carcinomas	12	0 (1/25)	4.12 (2/24)	3.9 6/25)	Pettersen & Turnier, 1995
Carcinomas	12	0 (0/14)	3.79 (3/11)	23.01 (6/18*)	Thakur, 1991a
Carcinomas	12	0 (1/11)	4.22 (2/10)	4.4 (6/23)	Morseth, 1998
Fibro-adenomas	9-15	0 (3/30)	<4.2 (5/30)	4.2 (5/30)	Thakur, 1991a
Pituitary adenomas	9-12	0 (2/20)	4.23 (2/20)	26.63 (8/20)	Thakur, 1991a

#= mammary unless otherwise specified; *p=<0.05; **p=<0.01; *** when adjusted for survival

Table 1-9. NOAELs and LOAELs (mg/kg/day) Associated with Non-Neoplastic Responses in Female SD Rats Treated with Atrazine

Response	Duration of Exposure (Months)	Dose in mg/kg/day (Response) 0	NOAEL	LOAEL	Reference
Percent days in estrus	~5	0 (32% days)	1.8 (41% days)	3.65 (45% days*)	Morseth, 1996b
LH-repeat bleed; fold increase above baseline	1	0 (3.6X)	5.0 (3.3X)	40 (1.9X)	Morseth, 1996a
LH-repeat bleed fold increase above baseline	6	0 (3.7X)	1.8 (3.3X)	3.65 (2.6X)	Morseth, 1996b
Mammary galactoceles	9	0 (10%)	4.23 (40%)	26.23	Thakur, 1991a
Mammary secretory activity[1]	9	0(24)	<4.23 (28)	4.23 (28)	McConnell, 1995
Mammary dilated ducts[1]	9	0(17)	<4.23 (24)	4.23 (24)	McConnell, 1995
Mammary acinar development[1]	3	0 (23)	<4.23 (28)	4.23 (28)	McConnell, 1995
Pituitary weights relative to control[2]	9	0	<4.23 (+25%)	4.23 (+25%)	Thakur, 1991a

*p=<0.05; **p=<0.01; when adjusted for survival; 1 - Index Score shown in parenthesis. Each grade was assigned the following values: absent=0; minimal=1; mild=2; moderately severe=3; marked=4. The sum of these values is the index score; 2 - Increase in pituitary weight relative to control shown in parenthesis.

Standard (EPA Guideline) chronic and subchronic studies conducted with atrazine do not provide insight regarding the potential of atrazine to produce lesions of reproductive organs or tissues that might lead to adverse reproductive or developmental outcomes in male or female animals. Similarly, results of developmental or reproductive toxicity guideline studies with atrazine do not show that the dam or her offspring express effects of atrazine treatment that can be associated with disruption of the hypothalamic-pituitary-ovarian axis. However, results of mode and mechanism of action studies conducted with atrazine in the adult cycling or adult, ovariectomized, estrogen-primed female rats suggest that treatment with atrazine, its structural analogues or metabolites, during other periods of the life cycle would also alter reproductive or developmental function in the dam or offspring. Special studies have been conducted that show that atrazine has reproductive and developmental effects that can be attributed to alterations in endocrine function. Summaries of the guideline and special studies are presented below. Implications of the data summaries presented are discussed in Chapter 2.

1.7.1 Chronic and Subchronic Toxicity of Atrazine

There is no clear evidence that chronic or subchronic treatment of rats or dogs with atrazine, its metabolites or structural analogues leads to effects on reproductive organs and tissue with the exception of the carcinogenicity and histomorphologic effects involving mammary tissue discussed previously. The principal effects reported in female SD rats following chronic dietary treatment with high doses of atrazine (50 mg/kg/day) include altered hematology and clinical chemistry parameters, retinal degeneration, centrolobular necrosis in the liver, rectus-femoris muscle degeneration, myeloid hyperplasia, transitional epithelial hyperplasia in the bladder and kidney, and extramedullary hematopoiesis (Mayhew *et al.,* 1986). Other effects observed in this combined chronic/carcinogenicity rat study at the high dose were histopathology findings in male rats consisting of statistically-significant increases in incidences of prostate epithelial hyperplasia and acinar hyperplasia of the mammary gland at the high dose. These effects were observed at the end of the study at which time there was increased survival in the high dose male rats compared with control male rats. Thus, the significance of the effects observed is unclear because the apparent increases may reflect the increased number of animals that survived for 24 months at the

high dose compared with the controls.

When atrazine was fed to dogs for one year, the prominent effect observed was cardiac dysfunction (O'Conner *et al.,* 1987). Chronic effects observed in a 91-week dietary study in mice were limited to hematologic alterations and decreased mean group absolute brain and kidney weights (Hazelette and Green, 1987). The only effect occasionally seen and potentially associated with endocrine alterations following subchronic or chronic treatment with atrazine, its metabolites, or structural analogues is an effect on the weight of the testes in rats and dogs. However, this effect is variable in different studies. Atrazine treatment produced no effects on the testes in a two-year rat bioassay or in a 18-month mouse bioassay. Simazine treatment resulted in a decrease in gonadal weights in males and females in a 90-day rat study. DACT did not produce effects on the gonads when administered to dogs in 90-day orone-year studies or when administered to rats in a 90-day study. G-28279, when administered to dogs for 90-days produced decreased testes weights. On the other hand, treatment with this metabolite led to increased testes weights when administered to rats for 90-days. G-30033 treatment led to increased relative testes weights when fed to rats for 90-days but produced no effects on testes weights in a 90-day dog study. The overall conclusion regarding effects on gonadal tissue is that there is no clear pattern of increased or decreased weights.

1.7.2 Developmental Toxicity of Atrazine

Results of standard (guideline) rat developmental toxicity studies with atrazine show that effects in maternal animals are confined to increased mortality and decreases in body weight gains and food consumption (Infurana, 1984; Ginkis, 1989). Fetal effects observed in the Infurana study (1984) included incomplete or delayed ossification of skull bones or other sites (NOAEL, 10 mg/kg/day and LOAEL, 70 mg/kg/day). The developmental NOAEL and LOAEL for delayed ossification in the Ginkis study (1989) were 25 and 100 mg/kg/day, respectively. Developmental effects observed in a rabbit developmental toxicity study were reduced litter sizes, increased resorptions, and delayed ossification at maternally toxic doses (appearance of blood in the cage or on the vulva, reduced body weight gain, and reduced food consumption) (Arthur, 1984a). The NOAEL and LOAEL (developmental) in this study were 5

mg/kg/day and 75 mg/kg/day, respectively. There are no data that would suggest that the delays in ossification in fetal animals are due to disruption of the hypothalamic-pituitary-ovarian axis by atrazine and the dose-levels for producing the delays in ossification (NOAELs 5-25; LOAELs 70 - 100 mg/kg/day). Because of the limited histopathology and the lack of measurements of developmental delays (*e.g.,* vaginal opening and preputial separation) in traditional developmental studies, it is not expected that developmental effects of atrazine treatment that are associated with endocrine perturbations would be seen in the guideline rat and rabbit developmental studies.

1.7.3 Reproductive Toxicity of Atrazine

The effects on gonadal weights (both increases and decreases) occasionally observed in subchronic and chronic studies with atrazine or its metabolites were seen in multi-generation reproduction studies. In the rat multi-generation studies with atrazine, simazine, and propazine, increases were observed in relative but not absolute testes weights of adult P_0 and F_1 rats following treatment with atrazine, simazine or propazine at doses ranging from 29 to 50 mg/kg/day (Mainiero *et al.,* 1987; Epstein *et al.*, 1991; Jessup, 1979). No effect on testes weights were observed in juvenile pups. The increases in relative testes weights may be due to decreased body weights of the adult animals. As noted from the data on testes weights from subchronic and chronic studies, the significance of this finding is unclear. The multi-generation study results provided no evidence of reproductive or developmental toxicity. However, as in the case of the developmental studies performed with atrazine, the traditional, EPA Guideline studies for reproductive effects do not include observations or measurements that were selected to determine effects related to endocrine imbalances.

1.7.4 Special Studies

Several special studies have been performed with atrazine with the objective of evaluating the effects of atrazine, or its metabolites, on pregnancy maintenance and postnatal development. Table 1-10 provides a listing of key findings reported in the special studies along with NOAELs and LOAELs for the effects.

- **Pregnancy Maintenance**

When 0, 50, 100, or 200 mg/kg/day of atrazine was administered by gavage to SD, F344, Holtzman, or LE rats during GD 1-8 just prior to the diurnal prolactin surge or just prior to the nocturnal surge of prolactin, a small but significant decline in mean number of implantation sites was seen only in Fischer-344 rats at the two highest doses. Holtzman rats alone showed an increase in postimplantation loss at the two top doses (Cummings *et al.*, submitted). Serum LH levels were significantly decreased in Holtzman, or LE-hooded rats treated with 100 mg/kg, at 200 mg/kg/day in F344 rats, but at no dose in SD rats. A decrease in serum progesterone levels was seen only in Holtzman rats treated with 200 mg/kg.

In a series of experiments assessing the effect of atrazine on pregnancy maintenance in the female rat by Narotsky *et al.*, (submitted, 1999), atrazine was administered by gavage to F344, SD, and LE rats during GD 6-10. The F344 strain was the most sensitive to atrazine's effects on pregnancy maintenance (full-litter resorption); the LE strain was the least sensitive. In F344 rats, surviving litters appeared normal; however, parturition was delayed. In SD rats, full-litter resorptions were also observed, but at higher dose levels: parturition was delayed at the same dose levels as for F344 rats. In contrast, the LE hooded strain showed full-litter resorption at the same dose level as SD rats, but there were no effects on parturition.

❑ **Reproductive and Postnatal Effects**

In a study examining the effect of atrazine on pubertal development, young Wistar rats were treated by gavage with atrazine (12.5, 25, 50, 100 or 200 mg/kg/day) during PND 22-41 (Laws *et al*.,submitted; Laws *et al.,* 2000). Vaginal opening was significantly delayed (three or four days) by 50 and 100 mg/kg respectively. The 200 mg/kg per day treatment with atrazine for the same period delayed vaginal opening by more than seven days in 18 of 32 females. When vaginal opening did occur, irregular cycles were observed in the 50 and 100 mg/kg dose groups during the ensuing two weeks. Vaginal opening occurred shortly after dosing was stopped in the 200 mg/kg dose group, and these females also demonstrated irregular estrous cycles for the next two weeks. All animals returned to regular estrous cycles by PND 70.

In a study evaluating the pubertal development in the male, weanling Wistar rats were dosed with atrazine during PND 23-53 (Stoker *et al.*,2000a; Stoker *et al.,* submitted). The significant finding from this study was that atrazine delayed preputial separation. The LOAEL for delay in preputial separation was <12.5 mg/kg/day. No consistent effect on serum prolactin and testosterone concentrations was observed, but the serum levels of these two hormones in animals of this age fluctuate widely making significant difference difficult to identify. However, there was a significant dose-related decrease in serum LH on PND 53 ($r = -0.92$. $P < 0.0024$).

Other studies have shown that reproductive tissues in the offspring can also be affected if the dam is treated during lactation. Suckling-induced PRL release was measured in Wistar dams treated with atrazine by gavage, twice daily with 0, 6.25, 12.5, 25, or 50 mg/kg (the total daily dose was 13, 25, 50 or 100 mg/kg/day) during PND 1-4 (Cooper *et al.*, 2000). Serum PRL in dams was measured on PND 3. A significant rise in serum prolactin release was noted in all control dams within 10 minutes of the initiation of suckling. The 25 and 50 mg/kg/day treatment with atrazine inhibited prolactin release in 40% or 60% of the dams, respectively; the daily dose of 100 mg/kg inhibited this measure in all dams. In this same study, the effect of postnatal atrazine on the incidence and severity of inflammation of the lateral prostate of the offspring was examined in adult males at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was shown to increase in those offspring of atrazine-treated dams (50 or 100 mg/kg/day). Combined treatment of dams with ovine prolactin (oPRL) and atrazine on PND 1 - 4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in inflammation seen after atrazine alone resulted from the suppression of prolactin in the dam. These data demonstrate that atrazine suppresses suckling-induced prolactin release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND1-9. It should be noted that vaginal opening was delayed in the offspring of these dams (Stoker *et al.*, submitted). Whether this effect is also related to changes in prolactin secretion in the dam remains to be determined.

Table 1-10. NOAELs/LOAELs (mg/kg/day) for Reproductive and Developmental Effects Following Treatment of Dams or Offspring of Several Rat Strains with Atrazine or its Metabolites[1]

Response and exposure period	F344	SD	Wistar	LE	Holtz-man	Ref.
Decrease in mean number of implantation sites- GD 1-8	50/100	>200	NA*	>200	> 200	Cummings *et al.,* submitted
Delayed parturition-GD 6-10	50/100	50/100	N.A.	>200	N.A.	Narotsky *et al.,* submitted; 1999
Full litter resorptions-GD 1-8 or 6-10						
atrazine	25/50	100/200	N.A.	100/200	50/100	Cummings *et al.,* submitted; Narotsky *et al.,* submitted; 1999
DACT	<67/67	N.A.	N.A.	N.A.	N.A.	
DEAT	<87/87	N.A.	N.A.	N.A.	N.A.	
DIAT	> 80	N.A.	N.A.	N.A.	N.A.	
OHA	<275/275	N.A.	N.A.	N.A.	N.A.	
Reduction in serum LH-GD 1-8	100/200	> 200	N.A.	50/100	50/100	Cummings *et al.,* submitted;
Decreased prolactin release- PND 1-4 (dams)	N.A.	N.A.	13/25	N.A.	N.A.	Stoker *et al.,* 1999
Increased incidence of prostatitis-PND 1-4	N.A.	N.A.	13/25	N.A.	N.A.	Stoker *et al.,* 1999
Increased incidence and severity of prostatitis-PND 1-4	N.A.	N.A.	25/50	N.A.	N.A.	Stoker *et al.,* 1999
Delayed vaginal opening-PND 22-41	N.A.	N.A.	25/50	N.A.	N.A.	Laws *et al.,* submitted; 2000
Delayed preputial separation-PND 23-53	N.A.	N.A.	<13/13	N.A.	N.A.	Stoker *et al.,* submitted; 2000a

[1]Data are for atrazine unless otherwise noted; * not available

Chapter 2

2. Hazard Characterization And Mode of Action Analysis

This Chapter presents information characterizing the neoplastic and non-neoplastic effects reported from studies conducted with atrazine and considers them in the context of an analytical framework for evaluating a postulated mode of action as described in the proposed revisions to the guidelines for carcinogen risk assessment (EPA, 1999). The framework is used to judge how well the available data support a mode of action postulated for a carcinogenic agent. This Chapter draws on the information summarized in the preceding Chapter. Complete details on the carcinogenicity and chronic toxicity of atrazine are presented in Part B of this document. This Chapter also evaluates the neuroendocrine effects of atrazine on the development and function of the reproductive system. The details of these studies can be found in Part C.

2.1 Human Cancer Studies

Several epidemiologic studies have examined cancers among populations with exposures relevant to the assessment of atrazine, especially among farmers or farm residents (see Part B, Chapter 4 for details). Most are case control studies, although there are ecologic investigations and also a worker mortality study of workers directly employed in the manufacture of triazines. Studies examining the association of triazine exposure with colon cancer, leukemia, multiple myeloma, soft tissue sarcomas, and Hodgkins disease failed to find firm associations. The pooled results of three separate case-referent studies investigating atrazine exposure in the development of non-Hodgkins lymphoma (NHL) concluded that there was essentially no risk of NHL attributable to farm use of atrazine. A mortality study of workers in two triazine manufacturing plants did not find any significant excesses of deaths for any disease category. There were, however, two cases of NHL in plant workers - one of whom was relatively young (31 years). These two cases do not provide evidence of an association between atrazine exposure and NHL, but do indicate that further follow-up of workers in these triazine manufacturing plants is desirable.

Associations between triazine exposure and cancer for three hormone-responsive cancers--ovary, breast and prostate cancer has been reported. Although suggestive, these associations should not be considered as conclusive evidence of a correlation between triazine exposure and these tumor types. The studies that showed possible relationships between these tumor types and triazine exposure should be interpreted with caution because of limitations, such as misclassification of subjects, use of surrogate data for exposure, or concurrent exposure to other potentially carcinogenic compounds.

To summarize, there is suggestive evidence of a possible association of triazine exposure and NHL, prostate, breast and ovarian cancers. This evidence does not show a direct cause and effect relationship between atrazine or triazine exposures and carcinogenicity because of confounding factors and limitations in the available studies. The available evidence emphasize the need for further epidemiologic research into the association of these tumor types with atrazine exposure.

2.2 Carcinogenicity in Female SD Rats

There were dose-related increases in the incidence of mammary tumors (adenomas, adenocarcinomas, and carcinosarcomas combined) in female Sprague-Dawley (SD) rats in the seminal carcinogenicity test performed with atrazine (Mayhew *et al*., 1986). No dose-related increases in tumor responses were observed in male SD rats. Results of subsequent bioassays, some of which included serial and/or one year sacrifices, confirmed that the predominant response observed following testing of atrazine in female SD rats is an increase in the incidence and/or early onset of mammary adenomas/carcinomas. Although less compelling, there is evidence that there is decreased latency for the formation of mammary fibroadenomas and pituitary adenomas (Thakur, 1991a and 1992a; Petersen and Turnier, 1995) and an increased incidence of mammary fibroadenomas (Morseth, 1998). An increased tumor incidence is not found at any other site in female SD rats, or at any site in male SD rats, or in either sex of Fischer 344 rats and CD-1 mice (Mayhew *et al*., 1986; Hazelette and Green, 1987; Thakur, 1992a,b). Mammary tumors were reported in one study in male Fischer 344 rats that involved lifetime treatment with atrazine (Pinter *et al*., 1990), but the finding is difficult to evaluate in light of the experimental design and shortcomings of the study. Furthermore, this finding is in conflict with the results of a conventional 24-month carcinogenicity study with

F344 male rats that showed no increases in mammary tumors (Thakur, 1992b). The closely related structural analogues to atrazine, simazine and propazine, also produce mammary tumors in the female SD rat but no other tumors of any type in the female SD rat and no tumors of any kind in the male SD rat or in CD-1 mice of either sex.

2.3 Postulated Mode of Carcinogenic Action

Before presenting the postulated mode of action for atrazine, it is instructive to consider aspects of the normal reproductive biology of the female SD rat and its relevance to tumor formation.

2.3.1 Reproductive Aging in Rats

With advancing age, the female Sprague-Dawley, as most strains of rats, normally undergoes a transition from regular ovarian cycles to an acylic pattern of "persistent" or "constant" estrus (Cooper and Walker, 1979; Also, see Part B, Chapter 9.1). Typically, this transition occurs prior to one year of age and is related to a disruption in both the timing and amplitude of the preovulatory surge of lutenizing hormone (Cooper *et al.,* 1980). As a result of this inability to achieve ovulation, the ovaries of the constant estrous female may contain many large follicles *(i.e.,* polyfollicular ovaries) but no corpora lutea (Huang and Meites, 1975). These follicles continue to secrete estradiol, while progesterone secretion is minimal (Huang *et al.,* 1978). This pattern of hormone secretion has been shown to facilitate the development of mammary gland tumors in aging rats and in young females in which a constant estrus has been induced (Nandi *et al.,* 1995; Russo *et al.,* 1990; Cutts and Noble, 1964; Meites, 1972). The inability to achieve an ovulatory surge of LH is the result of changes in the ability of the hypothalamus to achieve the proper release of GnRH. Changes in norepinephrine concentration occur prior to the onset of the loss of regular ovarian cyclicity (Wise *et al.,* 1997; Wise *et al.,* 1999). Conversely, treatment with CNS acting compounds such as the catecholaminergic precursor, L-dopa, will result in a reinitiation of regular cycles (Quadri *et al.,* 1973). Similarly, the age at which regular estrous cycles are disrupted can be extended if the female is placed on a diet containing L-tyrosine (*i.e.,* the amino acid precursor of L-dopa). Persistent or constant vaginal estrus, the accompanying pattern of persistent estradiol secretion and no progesterone, also leads to an

increase in pituitary weights, development of pituitary hyperplasia, and formation of pituitary adenomas in the aged female rat (Blankenstein *et al*., 1984; McConnell, 1989a; Nelson *et al*., 1980; Meites, 1980; McConnell, 1989b). The majority of the pituitary adenomas seen in the aged female SD have been found to originate from lacotrophs (*i.e*., prolactin-secreting cells of the anterior pituitary) (Sandusky *et al*., 1988). The increased number of prolactin-secreting cells results in an increased serum level of prolactin and extended or prolonged exposure of mammary tissue to higher than normal levels of prolactin. As indicated above, dietary supplementation with L-dopa and L-tyrosine (precursors to catecholamine synthesis in the central nervous system) delays reproductive aging as evidenced by maintained LH surges, normal reproductive cycling, and delayed onset of mammary gland tumor formation in treated animals compared to controls of the same age. No female Long-Evans (LE) rat developed mammary tumors by 21 months of age when fed a diet supplemented with L-tyrosine compared with a mammary tumor incidence of 67% in control (no supplement) animals (Cooper and Walker, 1979). Restored vaginal cycling is also found when aged female rats are administered L-dopa and L-tyrosine. Ovariectomy also reduces exposure of mammary tissue to estrogen and reduces or eliminates mammary tumor formation.

In summary, reproductive aging in the female rat appears to result from a disruption of hypothalamic neurotransmitter and neuropeptide (primarily noradrenergic) regulation of GnRH, and subsequently LH secretion. Importantly, the normal age-related disruption of regular cycling can be modified by pharmaceutical treatment or dietary supplementation. Finally, the resultant endorine milieu of enhanced or unopposed estrogen and prolactin secretion, provides an environment that is conducive to the development of mammary gland and pituitary tumors.

2.3.2 Atrazine Effects Relevant to Carcinogenicity

It is postulated that the carcinogenicity of atrazine is a consequence of the disruption of the normal secretory activity of the hypothalamic-pituitary-ovarian axis. Atrazine exposure adds to the formation of mammary tumors by inducing a sequence of events which intersects, at some point, with the normal reproductive aging pathway. The point of intersection appears to be the attenuation of the proestrous afternoon LH surge. Both *in vivo* and *in vitro* experiments demonstrate that atrazine exposure does not directly affect the pituitary (Cooper *et al.,* 2000) and that a decreased ability of the hypothalamus to release GnRH is likely the cause of the attenuated LH surge in the atrazine exposed SD female. Finally, pituitary weight and histomorphologic data in the mammary gland demonstrate that continued estrogen secretion also stimulates prolactin secretion by the pituitary. Again, ongoing secretion of estrogen and prolactin create an endocrinological milieu conducive to mammary gland and pituitary gland cell proliferation and eventual tumor development.

Females of the F-344 rat strain have a rather low background incidence of mammary tumors. In contrast to SD, LE, and Wistar females, this strain goes through a different pathway for reproductive senescence. F-344 females age through a process termed repeated pseudopregnancy, a condition where there are normal LH surges and ovulation occurs but continued secretion of progesterone by corpus lutea leads to a vaginal cytology indicative of diestrous. Mammary tumors are not induced by atrazine in F344 female rats. It would seem that the differences in reproductive aging between the F-344 and SD strains influence their sensitivity and response to atrazine administration.

Figure 2-1 illustrates the postulated mode of action of atrazine in female SD rats on the activity of the hypothalmic-pituitary-ovarian axis and the development of mammary and to some extent pituitary neoplasms. Effects associated with atrazine treatment on the activity of this axis are:

1. Atrazine exposure affects - either directly or indirectly - the hypothalamus, leading to a decreased secretion of hypothalamic norepinephrine (NE) (Cooper 1998)[1].

2. Hypothalamic NE normally modulates the release of gonadotropin releasing hormone (GnRH) from the hypothalamus. Decreased NE levels result in decreased release of GnRH from the hypothalamus (Cooper, 1998).

3. GnRH is the hormone responsible for inducing the pituitary gland to release luteinizing hormone (LH). A decreased GnRH level leads to an attenuated LH release (Cooper *et al*., 2000, Morseth, 1996a, b).

4. LH normally provides a signal to the ovaries promoting ovulation. Below some critical level, the decreased serum levels of LH are insufficient to stimulate ovulation.

5. Estrogen from ovarian follicles normally provides a feed back to the hypothalamus to stimulate a pituitary LH surge which promotes ovulation. Following atrazine exposure, there is insufficient GnRH to stimulate ovulation. Under the tonic secretion of LH and FSH, the ovarian follicles persist and continue to secrete estradiol. In turn, under the continued stimulation of estradiol, the pituitary lactotrophs become hypertrophied and secrete increasing amounts of prolactin.

[1]Cooper (1998) has also shown that acute atrazine treatment results in an increase in hypothalamic dopamine which in turn results in a decrease in pituitary prolactin. This acute effect is not expected to be associated with neoplasia but has potential reproductive consequences under certain circumstances.

6. Estrogen acts on the mammary gland increasing the risk of mammary tumors, especially carcinomas and adenomas.

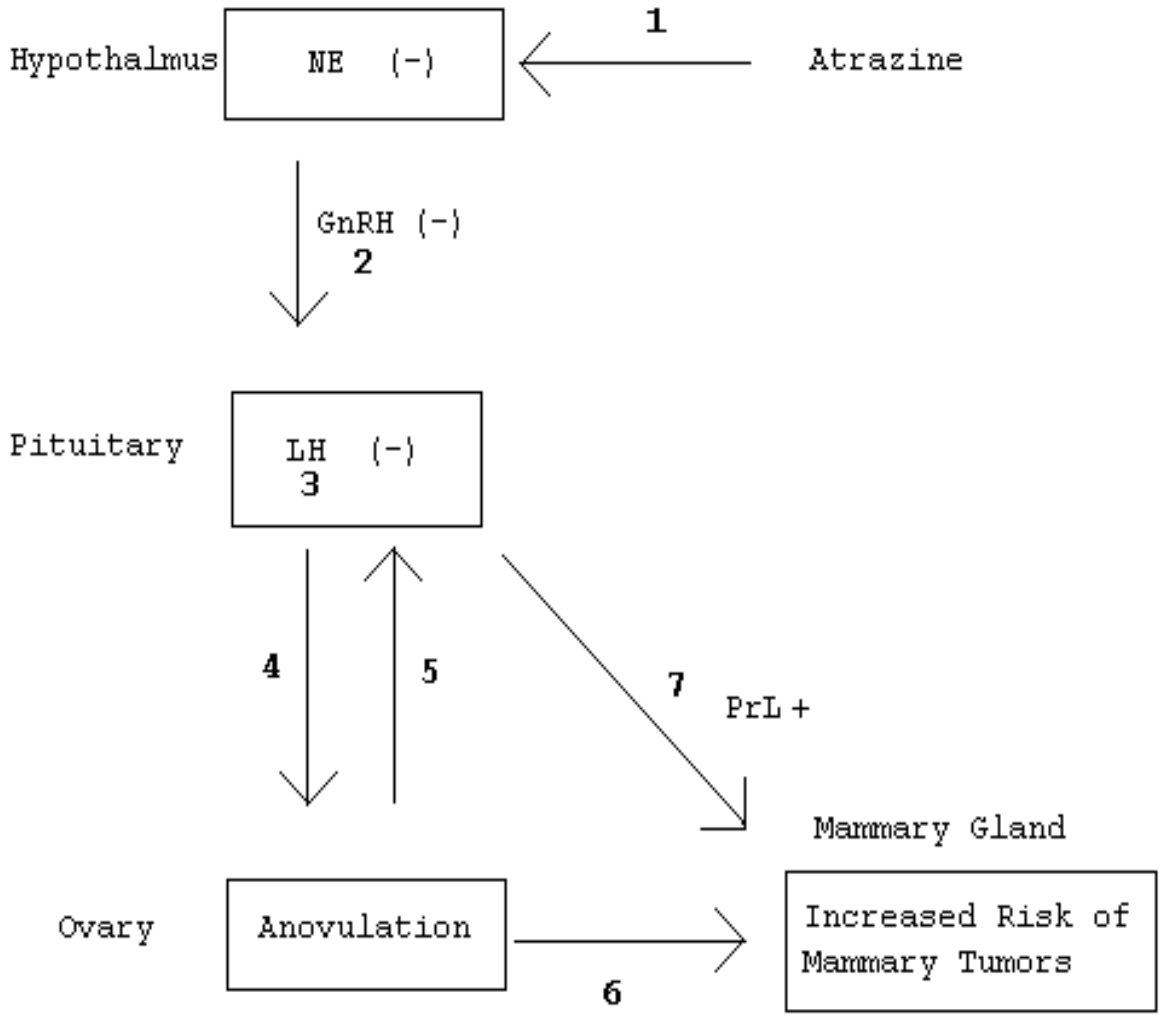
7. Prolactin derived from the hyperplastic lactotrophs (prolactin secreting cells) described in step 5 also acts on the mammary gland (in concert with estrogen) to increase the risk of mammary tumors, particularly fibroadenomas.

8. Tumor formation by atrazine does not appear to involve direct mutagenic effects nor does atrazine act as a direct estrogen agonist.

2.4 Evaluation of the Postulated Mode of Carcinogenic Action

In this section, the evidence linking the formation of mammary and pituitary tumors in female SD rats with disruption of biochemical activities in the hypothalamic-pituitary-ovarian axis is examined. These sections also examine the evidence supporting or refuting the postulated mode of action described in Figure 2-1 as the causal mode of action associated with the carcinogenicity of atrazine in female SD rats.

Figure 2-1. Postulated Effects of Atrazine Treatment on the Hypothalamic-Pituitary-Ovarian Axis



2.4.1 Key Events

Data showing that the hypothalamus appears to be a primary initial site of action for atrazine primarily come from short-term, high dose studies conducted in Long Evans (LE) females[2] by the EPA's National Health and Effects Research Laboratory (Cooper *et al.*, 1998; Cooper, 2000). These studies provide evidence that atrazine affects hypothalamic catecholamine levels. A decrease in NE results in a decrease in gonadotropin releasing hormone (GnRH), with a corresponding diminution of pituitary surges of luteinizing hormone (LH). These *in vivo* observations are further supported by *in vitro* studies using pheochromocytoma cells. In this cell line, both dopamine and norepinephrine are synthesized constitutively. Das *et al*. (2000, in press) have shown that catecholamine synthesis is suppressed, in a dose dependent manner, following exposure to atrazine. Evidence for a hypothalamic site of action for the neuroendocrine disrupting effects of atrazine include the following observations:

- ❑ the pulsatile release of GnRH from the hypothalamus is impaired in the female rat following atrazine exposure (Cooper *et al.*, 1998)

- ❑ the atrazine-induced suppression of LH secretion can be reversed following treatment with synthetic GnRH (Cooper *et al.*, 2000)

- ❑ there is a dramatic increase in the hypothalamic concentration of GnRH following exposure to atrazine demonstrating that release (and not synthesis) of GnRH is impaired (Ford *et al.*, 2000)

[2]Some rat strains (LE, Wistar and SD included) undergo a similar reproductive aging process which is characterized by the appearance of persistent (or constant) estrus by approximately one year of age and under similar neuroendocrine events. Thus, the LE female rat is considered to be a valid model for evaluating atrazine's mode of action resulting in mammary tumors in SD females.

- related to these effects on GnRH release, a hypothalamic site of action also appears to be responsible for the inhibition of prolactin release as the atrazine-induced suppression of prolactin secretion is not observed if the pituitary is removed from its normal location (within the sella turcica, beneath the hypothalamus) and placed beneath the kidney capsule (Cooper *et al*., 2000).

Suppression of the LH surge in female SD rats is considered to be a necessary precursor for the development of atrazine-induced mammary gland tumors. This is because LH blood levels must reach a sufficient magnitude to induce ovulation and to maintain normal reproductive cycles. When atrazine reduces LH output to the critical point where there is not enough to trigger ovulation, a physiological state results which is characterized by prolonged or persistent estrous. This state leads to continued stimulation of mammary tissue by estrogen. Evidence for an attenuation of the LH surge and an early onset of prolonged and/or persistent estrus is provided in several studies (Morseth 1996a,b; Thakur 1991a; Eldridge *et al*., 1993a). Removal of the estrogen stimulus by ovariectomy completely abolishes the formation of mammary tumors following chronic administration of atrazine (Morseth, 1998). Estrogen has been strongly implicated in mammary gland cell proliferation and the enhancement of neoplastic transformation in rodents and humans (for review see Russo and Russo, 1996; Nandi, 1996).

The attenuation of LH surges and disruption of the normal reproductive cycles in female SD and Long-Evans hooded rats treated with atrazine mirrors prominent features of the normal reproductive aging process in these strains. This process features a diminution of LH blood levels, a failure to ovulate, and a state of persistent estrus.

Prolonged estrogen secretion resulting from atrazine treatment appears to lead to other consequences. There is evidence that sustained exposure of the pituitary gland to estrogen leads to an increase in pituitary weights, pituitary hyperplasia, development of lesions characteristic of prolactin secretion, and the formation of pituitary adenomas (Thakur, 1991a; McConnell, 1995). The sustained secretion of prolactin is believed to play a role in the development of mammary tumors, in general, but a more prominent role in the development of mammary fibroadenomas (Welsch, 1985).

2.4.2 Correlation of Effects and Dose

There is a strong association between dose levels of atrazine that lead to an early onset and increased incidences of mammary tumors and doses that produce biochemical perturbations that have been linked to reproductive aging *(i.e*., suppression of LH surges and prolonged or persistent estrus). Table 2-1, lists the lowest dose (LOAEL) which elicited each of the effects associated with atrazine treatment. Tables 1-8 and 1-9, Chapter 1, may be referred to by the reader for NOAELs and LOAELs of all data on tumor and non-neoplastic effects.

A dose of 3.5 mg/kg/day and above that leads to an early onset and/or increased incidence of mammary carcinomas in female SD rats also leads to attenuation of LH secretion. Examination of Figure 1-2, Chapter 1, indicates that administration of atrazine at a dose level of 3.65 mg/kg/day results in a diminution of the LH surge. This is the same dose that results in estrous cycle perturbations. At a dose level of 29.4 mg/kg/day, the LH surge is completely suppressed. If attenuation of the LH surge were indeed a key event in mammary and pituitary tumor formation, then doses that result in an attenuation of the LH surge would be expected to result in an increased incidence or early onset of these tumors. Doses of 4.2 and 24.4 mg/kg/day resulted in an early onset of mammary carcinomas (Morseth, 1998). Doses of 3.79 and 23.01 mg/kg/day resulted in an early onset of mammary carcinomas in another study (Thakur, 1992a). The evidence for an early onset of mammary fibroadenomas and pituitary tumors is less strong as these effects were only seen in one study (Thakur, 1991a).

Table 2-1. LOAELs for Tumor Formation and Non-Neoplastic Effects in Female SD Rats

Effect/Time of Observation	LOAEL (mg/kg/day)	Reference
LH-repeat bleed; increase above baseline (6 months)	3.65	Morseth, 1996b
Prolonged days in estrus (6 months)	3.65	Morseth, 1996b
Mammary carcinomas - decreased latency (12 months)	3.79	Thakur, 1992a
Mammary carcinomas - increased incidence (24 months)	3.5	Mayhew *et al*., 1986
Mammary galactoceles (9 months)	4.23	Thakur, 1991a
Increased pituitary weights (9 months)	4.23	Thakur, 1991a
Pituitary adenomas - decreased latency (9 months)	26.23	Thakur, 1991a
Mammary fibroadenomas - decreased latency (15 months)	4.23	Thakur, 1991a

There is also a correlation between time spent in estrus and tumor formation. The data from the 1998 Morseth study, as described in Thakur (1999), shows that there is a statistically-significant correlation between percent days spent in estrus during both the one to 46 week and 17 to 26 week time intervals, and an increased mammary carcinomas incidence. Moreover, examination of the animals in this study, where there was an especially early tumor onset (prior to 52 weeks), showed that there was an unusually long period of time spent in estrus. Five of six female SD rats that developed mammary carcinomas by 52 weeks spent >70 % of the days in estrus between weeks 17 to 26.

As discussed previously, estrogen stimulation of the pituitary gland is believed to cause an increase in the secretion of prolactin, a hormone closely associated with the development of mammary tumors, especially fibroadenomas. There is histomorphologic evidence (*e.g.,* acinar development, dilated ducts, and increases in the incidence and severity of galactoceles) of an early onset of increased prolactin secretion at 4.23 mg/kg/day (McConnell, 1995). It is biologically plausible that this early exposure to prolactin may contribute to the early onset of mammary fibroadenomas as seen in Thakur (1991a). There is also an early onset of increased pituitary weights in this study. Absolute pituitary weights at 4.23 mg/kg/day are increased by 25% at nine months. The increase is likely due to the mitogenic effect on pituitary lactotrophs of estrogen derived from unovulated follicles. The larger pituitaries would be expected to secrete increased amounts of prolactin. This is indicated by the early onset of prolactin-dependent histomorphologic parameters and the early onset of mammary fibroadenomas.

The lowest atrazine dose showing effects on LH, the pituitary gland, and the estrous cycle is somewhere between 3 and 4 mg/kg/day. The LH surge attenuation occurred at 3.65 mg/kg/day, but did not occur at 1.8 mg/kg/day. The estrous cycle alterations occurred at 3.1 and 3.65 mg/kg/day in two separate studies. In these studies, the estrous cycle alteration did not occur at 1.5 and 1.8 mg/kg/day. There is only one study on serum estradiol levels. Although this study shows an early onset of increased estradiol levels at 4.23 mg/kg/day, a clear dose effect level is uncertain due to variability in the data and the lack of confirmatory data at other timepoints in the same study (*e.g.,* six months). The main factor is that estrogen secretion is prolonged during persistent estrus which results in continuous stimulation of the mammary gland.

2.4.3 Temporal Association of Effects

Data from chronic studies in female SD rats administered atrazine consistently show that there is an early onset of mammary tumors. This is what would be expected if atrazine accelerated the reproductive aging process. Therefore, it is anticipated that precursor events to mammary tumors would have their onset in atrazine treated females before that of untreated SD females undergoing normal reproductive aging. The temporal pattern of effects found following atrazine treatment are summarized in Figure 2-2.

In untreated aging female SD rats, prolonged days in estrus begin as early as nine months and shortly thereafter they enter into persistent estrus. Extended days in estrus, a key event associated with the formation of mammary tumors begins earlier in rats treated with atrazine than in controls. An increased number of days in estrus begins as early as 3.5 or 5.5 months in females administered 29.4 mg/kg/day or 3.65 mg/kg/day of atrazine, respectively (Morseth, 1996b). These data were confirmed in a separate study which showed that by 3.3 months SD females exposed to 24.4 mg/kg/day were spending approximately 26% more days in estrus than control animals (Thakur, 1999). Dietary administration of 3.65 mg/kg/day of atrazine leads to attenuation of the proestrus afternoon LH surge after as little as six months of atrazine exposure. Thus, exposure to atrazine decreases the onset time of attenuated LH surge and persistent estrus. These effects have been identified as the precursor events in the pathway towards mammary tumors in rats. In keeping with these findings, animals receiving 4.23 mg/kg/day (lowest dose tested) manifest an early onset of histomorphologic changes in mammary tissue (*e.g.,* increased incidences and severity of acinar formation, secretory activity, and galactoceles) following six to nine months of treatment of female SD rats with atrazine (McConnell, 1995). These changes are primarily indications of exposure of mammary tissue to prolactin and estrogen. This broad time line illustrates the sequence of events that occur prior to tumor development (as well as the associated effective dose levels for the response).

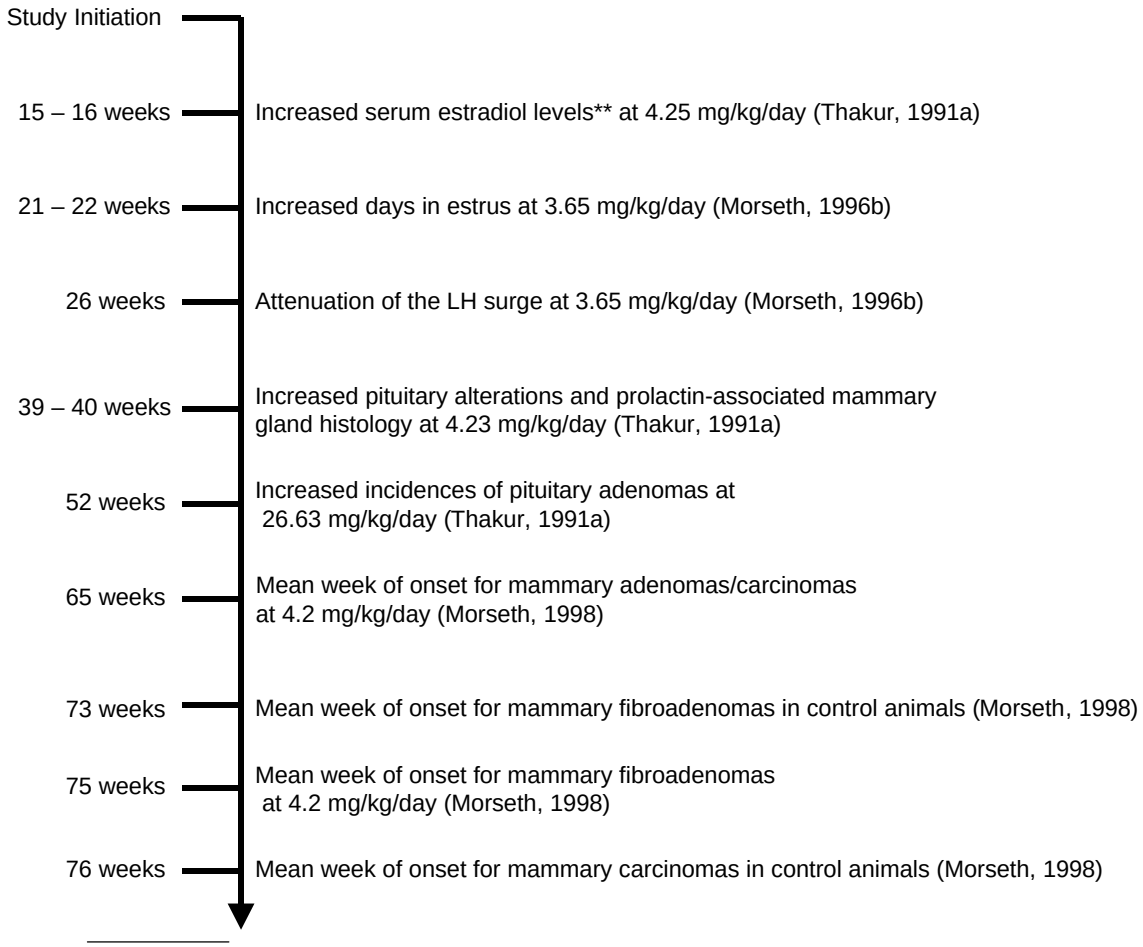
2.4.4 Biological Plausibility and Coherence of the Database

The process of normal reproductive senescence in the female SD rat has been implicated in creating a hormonal milieu conducive to mammary tumor and pituitary tumor formation, including:

- ❑ Attenuation of the pre-ovulatory LH surge;
- ❑ Increased days in estrus; and
- ❑ Prolonged exposure to endogenous estrogens and prolactin.

The events listed above have been well described in the open literature as normal and expected events in the reproductive aging of the female SD rat (Cooper and Walker, 1979; Lu, 1994; Mobbs, 1996; Smith and Conn, 1983; Zuo, 1996). Prolonged exposure to endogenous estrogens has been generally accepted as a major contributor to the high spontaneous mammary and pituitary tumor rates seen in the SD female (Welsch, 1987; Cooper, 1983; Cutts and Noble, 1964). Prolonged exposure of mammary tissue to prolactin, which results from the estrogen-induced pituitary tumors, also has been well established as a contributor to mammary carcinogenesis in the normally aging female SD (Welsch, 1970a; Welsch, 1970b; Meites, 1971; Goya *et al.,*1990).

Figure 2-2. Temporal Pattern of Atrazine Effects*



*Time when effects are first noted was dictated by study design; **Only one study available.

The biologic plausibility for the mode of action proposed for atrazine-induced mammary and pituitary carcinogenicity lies in the observation that atrazine exposure has been shown to induce an earlier onset of all three of the events outlined above: attenuation of the pre-ovulatory LH surge; increased days in estrus; and prolonged exposure to endogenous estrogen. Because this sequence of events has been generally accepted as leading to mammary and pituitary carcinogenesis in the normally aging SD female, one can reasonably expect that atrazine administration would lead to the same events, including tumors, only at earlier times than in normally aging females.

Atrazine dose levels that lead to attenuation of the LH surge also are associated with disruption of the estrous cycle and an early development or increased incidence of mammary and pituitary gland tumors. One study provides histomorphologic evidence that an early onset of pituitary tumors and mammary fibroadenomas may be explained by prolonged secretion of estrogen by the anovulatory female rat, stimulation of the pituitary to undergo cell proliferation, and increased prolactin secretion by the estrogen-stimulated pituitary gland. The formation of both mammary carcinomas and mammary fibroadenomas are influenced by prolonged exposure of the mammary gland to follicle - derived estrogen and pituitary-derived prolactin. Thus, in several respects, the effects of atrazine treatment mirror biochemical alterations that have been attributed to the onset of reproductive aging and spontaneous tumor formation in the female SD rat. The mode of action proposed to account for the tumor responses in female SD rats treated with atrazine is biologically plausible because the major key biological and biochemical events shown to be altered by atrazine treatment are the same ones that have been identified as contributors to the formation of mammary and pituitary tumors in aging female SD rats.

2.4.5 Other Modes of Action

❑ **Mutagenicity**

Because cancer is the result of a series of genetic defects in genes controlling cell growth, division, and differentiation, an initial and prominent question to be examined is whether atrazine (or an atrazine metabolite) interacts directly with, and mutates DNA. The totality of evidence for atrazine, including data on several metabolites of atrazine and close structural analogues, does not support a mutagenic potential for atrazine, and indicates that a direct DNA reactive, mutagenic mode of action is unlikely to be an influence on atrazine tumor development. The genetic toxicology database for atrazine shows consistent negative responses in bacterial tests and inconsistent positive responses across other phylogenetic lines (which are typically weak, found at high doses, or cannot be reproduced). No subset of data points clearly establishes a direct DNA reactive mode of action for atrazine associated with the carcinogenicity.

❑ **Estrogen Agonistic Action**

The available evidence from *in vivo* and *in vitro* studies indicates that atrazine does not bind to the estrogen receptor or possess direct estrogenic activity. Under equilibrium conditions, atrazine does not compete with estradiol for binding to SD rat estrogen receptors (Tennant *et al*., 1994b). Atrazine treatment does not induce changes in estrogen-responsive tissues (*e.g.,* increased uterine weight, increased uterine cell proliferation, uterine peroxidase activity and uterine progesterone receptors) in ovariectomized SD rats. Atrazine does not affect basal or estradiol induced cell proliferation in a human breast cancer cell line (MCF-7) (Safe *et al*., 1995). Atrazine does not have agonist or antagonist action against estradiol induced luciferase activity in MCF-7 cells transfected with a Gal4-regulated human estrogen receptor chimera, thus showing failure to bind to the estrogen receptor (Conner *et al*., 1996). Although estrogen binding was found for atrazine (Tennant *et al*., 1994b), it was demonstrated only under conditions that favored binding and at very high doses of atrazine

relative to those that induced mammary tumors in SD females. DACT (a metabolite of atrazine) and simazine (analogue of atrazine) also do not appear to bind to the estrogen receptor (Tennant *et al*., 1994b; Connor *et al*., 1996).

❑ **Other Endocrine Imbalances**

Data presented in abstract form indicates that atrazine depresses hypothalamic norepinephrine (NE) levels and increases hypothalamic dopamine (DA) levels (Cooper, 1998). NE levels correlate directly with hypothalamic GnRH release (*i.e*., increased NE leads to increased GnRH release) while DA levels are inversely related to pituitary prolactin release *(i.e*., increased DA levels leads to decreased prolactin secretion). Thus, a decrease in hypothalamic GnRH secretion and a decrease in pituitary prolactin secretion might be expected from alterations of these neurotransmitters.

Chronic exposure to doses of atrazine as low as ~4 mg/kg/day leads to elevated prolactin secretion, as indicated by histomorphologic markers, presumably because of estrogen-induced pituitary lactroph hyperplasia in the anovluatory female. Prolonged exposure to serum prolactin contributes to mammary gland carcinogenesis in the rat (Welsch, 1985) because of its proliferative effect on the mammary gland tissue.

The pituitary does not appear to be a direct target of atrazine toxicity. When pituitary hormone secretion is removed from the influence of CNS hypothalamic factors (*i.e.*, by placing pituitary grafts under the kidney capsule) in LE females, there is no effect on prolactin release when animals are exposed to a dose of atrazine that suppresses the prolactin surge (Cooper, 2000). The atrazine-induced attenuation of the LH surge can be reversed by intravenous exposure to exogenous GnRH (Cooper, 2000). This implies that the pituitary is functional and the deficit responsible for LH surge attenuation is a hypothalamic insufficiency of GnRH release. *In vitro* studies provide additional support that effects on the LH and prolactin surges are not due to a direct pituitary response to atrazine exposure (Cooper, 2000). No differences in

either LH or prolactin release were found from the pituitaries of untreated females exposed to atrazine *in vitro*. These three lines of evidence indicate that the effect of atrazine on the LH surge (and the high-dose effect on the prolactin surge) involves a disruption of the GnRH pulse from the hypothalamus, rather than a direct effect on the pituitary.

There is some evidence that atrazine may enhance estrogenic activity by stimulating aromatase activity. Aromatase is an enzyme that converts androgens to estrogens. Treatment of human adrenocortical cells *in vitro* with atrazine has been shown to stimulate aromatase activity (Sanderson *et al.,* 2000). Crain *et al.* (1997) have also shown that atrazine treatment of male hatchling alligators leads to an increase in aromatase activity. Although an increase in aromatase activity would be consistent with dose-response increases in estradiol and estrone and decreases in testicular testosterone noted in a study that examined the effects of atrazine on pubertal development, the doses that resulted in effects on these hormones were well above doses that led to reproductive developmental effects (Stoker *et al.,* submitted; 2000a). It is plausible that enhanced aromatase activity may have some influence on the development of mammary tumors in SD female rats but whether or not enhanced aromatase activity is a significant contribution to the carcinogenicity, or other effects, of atrazine remains to be determined.

No other modes of action, apart from disruption of the hypothalamus-pituitary-ovarian axis, are plausible biochemical processes that could account for the early onset and increased incidence of mammary and pituitary gland tumors in female SD rats.

2.4.6 Uncertainties and Limitations

Despite numerous studies directed at understanding the mode of action for the carcinogenicity of atrazine, several uncertainties remain. In themselves they do not discount the postulated mode of action. Although the available data show that attenuation of the LH surge and disruption of the estrous cycle occur before mammary tissue and pituitary gland tumor formation, precise dose and time correlations are not available for each of the key events due to differences in study design and dose selection. Serum LH values are highly variable within dose groups, which makes it very difficult to determine accurately biologically relevant doses that are associated with effects.

There is some concern that the lack of direct effects on the pituitary was established using ectopic pituitaries and using prolactin secretion as a marker of LH secretion. There also is a lack of robust data on blood prolactin measures and serum estradiol measurements. Because prolactin measurements are not available from chronic studies, confirmation of the role of the hormone in the formation of the histomorphologic changes in mammary tissue is not possible. Histomorphologic markers are, however, generally viewed as valid indicators of prolactin secretion.

Stop-dose studies to demonstrate that induced toxicological processes leading to cancer are reversible are limited but this deficiency is offset, once again, by the lack of effects in ovariectomized female SD rats.

Finally, the initial interaction between atrazine and the rat brain has not been established, albeit effects on hypothalamic catecholamine neurotransmitter levels have been shown.

2.4.7 Preliminary Conclusions on the Postulated Mode of Carcinogenic Action

Support for atrazine's mode of action comes from several lines of evidence.

- ❏ Atrazine's induced LH and cyclicity effects have been shown in two different laboratories and in two different strains of rats (LE and SD);
- ❏ A strong correlation has been shown for atrazine induced persistent estrus and induction of mammary tumors;
- ❏ Generally, there is a strong temporal and dose-response correlation between tumor formation and precursor effects; precise correlations are not possible due to differences in study designs and dose selection;
- ❏ Although robust data on estrogen and prolactin levels are not available, ovariectomized SD rats treated with atrazine do not develop tumors, thus demonstrating the role of ovarian estrogen in atrazine's mode of action;
- ❏ A strong correlation was demonstrated between increased pituitary weights and histomorphological markers of prolactin exposure in the mammary gland, thus supporting the role of prolonged estrogen and prolactin exposure in tumor development; and
- ❏ Despite the lack of precise effective dose levels (LOAELs), data from multiple hormonal and carcinogenicity studies show that no effects of atrazine treatment are observed at a dose level between 0.5 and 1.8 mg/kg/day.

The absence of data on the detailed steps in the hypothalamus, would provide insights regarding the mechanism of action of atrazine. However, as stated in the proposed Guidelines for Carcinogen Risk Assessment (USEPA 1999), mode of action is contrasted with mechanism of action which implies a more detailed molecular description of events than does mode of action. The focus of a mode of action analysis is on a sequence of key events which lead to cancer formation and whether data are sufficient to establish a cause and effect relationship between key events and cancer. This guidance was followed in reaching the conclusion stated below.

Given the overall strengths, consistency, and specificity of the evidence, it is concluded that it is biologically plausible that treatment of female SD rats with atrazine leads to an increased incidence and/or decreased latency in the formation of mammary adenomas, carcinomas, fibroadenomas, and pituitary adenomas through a mode of action involving disruption of the hypothalamic-pituitary-ovarian axis. Disruption of the axis leads to suppression of LH surges, prolonged days spent in estrus, and exposure of mammary tissue and the pituitary gland to estrogen for a extended period. Exposure of the pituitary gland to estrogen stimulates the secretion of prolactin. Exposure of the mammary tissue to estrogen and prolactin and the pituitary gland to estrogen creates an endogenous endocrine milieu conducive to cell proliferation and tumor formation. The available data do not support a role for direct mutagenic or direct estrogenic activity for effects attributed to atrazine treatment.

2.5 Reproductive and Developmental Toxicity

The natural progression from prepubertal to postpubertal status is dependent upon the normal function of the hypothalamic-pituitary-gonadal axis. Likewise, many of the same hypothalamic mechanisms controlling pituitary function and the pituitary hormones themselves (especially LH and prolactin) play a key role in pubertal development. For example, it has been shown that an increased turnover rate in hypothalamic GnRH, NE and DA precedes the gonadal development (Matsumoto *et al.,* 1986; Ojeda, 1986).

At the time of puberty (*e.g.,* vaginal opening and first ovulation) the CNS and pituitary respond to increased concentrations of estradiol in a positive feedback fashion culminating in the first LH surge (Ojeda and Urbanski, 1994).

These processes are not specific to the rat. Inhibition of GnRH release in neonatal rhesus monkeys suppresses gonadatrophin secretion and testosterone production; this effect is associated at the time of puberty with compromised testicular growth and testosterone secretion (Plant, 1994). This same author postulated that there is a coupling between a rise in circulating LH and FSH concentrations and the transition into puberty that is a general characteristic of sexual maturation in higher primates. Thus, given that atrazine treatment of rats suppresses GnRH, LH, and prolactin release, there is a concern for potential adverse reproductive and developmental effects of atrazine in maternal animals and their offspring.

Adverse reproductive or developmental consequences have been identified following treatment of different strains of pregnant rats or neonates with atrazine or its metabolites. As noted in Chapter 1, Section 1.7, this evidence does not come from results of EPA guideline studies but from results of special studies conducted with atrazine or its metabolites. The results of these studies show that atrazine or its metabolites produce effects in pregnant, neonatal, or young adult SD, F344, Wistar, Holtzman, or LE rats that may be associated with disruption of the hypothalamic-pituitary axis. The developmental/reproductive effects observed in these studies include reductions in implantation sites, failure to maintain pregnancy, attenuation of suckling-induced prolactin release and the development of prostatitis, delayed vaginal opening, and delayed preputial separation. Table 2-2 provides a listing of the lowest NOAELs and LOAELs reported for these effects. NOAELs and LOAELS for effects on prolactin and LH release following acute or short-term repeat

dosing treatment of rats with atrazine are also provided in order to allow comparisons of developmental/reproductive NOAELs/LOAELs with the NOAEL/LOAELs that result in disruption of neuroendocrine parameters.

Treatment of young Wistar rats with atrazine during PND 22-41 delays vaginal opening three or four days and produces irregular cycles (Laws *et al.,* submitted; 2000). Treatment of weanling male Wistar rats with atrazine during PND 23-53 leads to delays in preputial separation (Stoker *et al.*, submitted; 2000a). No consistent effects on serum progesterone or LH were observed in this study but variability in hormonal levels in animals of the age studied makes comparisons with control animals difficult.

In addition to affecting the onset of puberty, the offspring of dams exposed to atrazine have also been found to be affected adversely. Administration of atrazine to dams during PND 1-4 inhibits suckling-induced prolactin release in the dams and leads to lateral prostate inflammation in the offspring. The effect on the prostate is reversible if the offspring are treated with ovine prolactin, which provides evidence that prolactin has a role in the development of prostatitis. Also, the incidence and severity of lateral prostate inflammation correlates with decreases in serum levels of prolactin. The effects on the prostate of offspring of dams treated with atrazine and the delays in pubertal developmental observed when young rats are treated with atrazine are associated with the endocrine imbalances that have been identified as critical events in the neuroendocrine mode of action attributed to the carcinogenic activity of atrazine.

As stated earlier, atrazine may also affect pregnancy maintenance in the rat. The full-litter resorptions reported following atrazine exposure on GD 6-10 (roughly coinciding with the LH-dependent period of pregnancy) are consistent with a neuroendocrine mode of action (Narotsky *et al.*, submitted). Although this effect was observed at maternally toxic doses (as defined by a decrease in body weight), treatment after the LH-dependent period caused a similar degree of maternal toxicity, but had no effect on pregnancy maintenance. Hormone measurements on GD 9 (following treatment on GD 1-8) did not show a consistent pattern across strains for prolactin, estradiol, or progesterone. However, for the Holtzman strain, the only strain of four tested to show full-litter resorptions following treatment on GD 1-8, there were reductions in serum progesterone and LH; although not proof, these data are consistent with an LH-mediated mechanism of pregnancy loss. In contrast to treatment on GD 6-10,

exposure on GD 1-8 did not cause full-litter resorption in F344 or SD rats. An explanation for the lack of effect following exposure on GD 1-8, remains unclear, but may be related to the truncated dosing regimen within the LH-dependent period, or examination of the litter on GD 9, possibly prior to the actual time of pregnancy loss.

Because pubertal development is under neuroendocrine control, it may be expected that administration of atrazine to young rats leads to delays in vaginal opening or preputial separation. The dose levels that led to delays in vaginal opening also produced irregular ovarian cycles in offspring, which supports a role for disruption of neuroendocrine control in young animals treated with atrazine or its metabolites. The reductions in implantation sites and the full-litter absorptions reported following treatment of dams with atrazine during the LH-dependent phase of pregnancy are also consistent with an effect on neuroendocrine control but other modes of action can not be discounted (*e.g.*, general toxicity at high-dose levels).

There are uncertainties, in particular, regarding the dose-response data on preputial separation (PPS). A statistically-significant effect was reported at a dose-level of 13 mg/kg/day (PPS ~42 days in controls and PPS ~ 44 days at 13 mg/kg/day, the lowest dose tested). It should be noted that this dose has a significance of $p \leq 0.05$. The next higher dose of 25 mg/kg/day approached statistical significance but did not achieve significance (*i.e.,* $p = 0.07$). Statistical-significance ($p \leq 0.05$) was achieved at the next three dose levels (50, 100, or 150 mg/kg/day). At 200 mg/kg/day there was a statistically-significant effect of delayed preputial separation (~42 days in controls and ~45 days in the high-dose rats). There was a significant dose-related decrease in LH; however, no statistically-significant effects were observed for testosterone or prolactin concentrations. The variability in levels of these hormones in young rats should be considered before much weight is placed on these data.

In summary, reproductive and developmental effects in various strains of rats that are associated with atrazine treatment include preimplantation and postimplantation losses, prostatitis in adult male offspring of treated lactating females, delays in vaginal opening and preputial separation, and disruption of the estrous cycle in young females. A reduction in prolactin release in nursing dams is strongly associated with the development of prostatitis in male adult offspring. Decreases in serum LH or prolactin were not observed to occur at dose-levels that led to delays in vaginal opening (50 mg/kg/day) and preputial separation (13 mg/kg/day) in the same study but it is presumed that the variability in levels of these hormones in juvenile animals preclude obtaining definitive data. On the other hand, a separate study using dams showed that a daily dose of ~13 mg/kg/day was sufficient to depress serum levels of prolactin in the lactating dam. To the extent that decreased prolactin levels can serve as a marker for effects on neuroendocrine control, there is a linkage between pubertal development and an effect on the hypothalamic-pituitary axis.

Table 2-2. Lowest NOAELs/ LOAELs (mg/kg/day) for Reproductive and Developmental Effects Following Short-term (1-30 Days) Treatment of Rats During Various Stages of the Reproductive Cycle with Atrazine or its Metabolites

Response	Exposure Period	Rat Strain	NOAEL/LOAEL	Reference
Preimplantation loss-nocturnal dosing only	GD 1-8	F344	50/100	Cummings *et al*., submitted
Postimplantation loss-diurnal and nocturnal dosing	GD 6-10	Holtzman	50/100	Cummings *et al.,* submitted
Dams prolactin release decreased	PND 1-4	Wistar	13/25	Stoker *et al.,* 1999
Increased incidence of prostatitis in offspring	PND 1-4	Wistar	13/25	Stoker *et al.,* 1999
Increased incidence and severity of prostatitis in offspring	PND 1-4	Wistar	25/50	Stoker *et al.,* 1999
Delayed vaginal opening	PND 22-41	Wistar	25/50	Laws *et al.,* submitted; 2000
Delayed preputial separation	PND 23-53	Wistar	<13/13	Stoker *et al.,* submitted; 2000a
Attenuation of LH surge	Adult females - single dose 3 daily doses 21 daily doses 21 daily doses 30 daily doses Dams- GD 1-8	 LE LE LE SD SD LE & Holtzman	 200/300 <50/50 <75/75 <75/75 5/40 50/100	Cooper *et al.,* 2000; Morseth, 1996a; Cummings *et al.*, submitted
Attenuation of prolactin release	Adult females - single dose 3 daily doses 21 daily doses 21 daily doses	 LE LE LE SD	 200/300 serum <50/50 pituitary <75/75 pituitary <75/75 pituitary	Cooper *et al.,* 2000
Disruption of estrous cycle	PND 22-41	Wistar	25/50	Laws *et al.,* submitted; 2000

Chapter 3

3. Science Policy Considerations: Human Relevance, Children's Health Concerns, and Dose-Response Analysis

This Chapter evaluates and characterizes the human relevance of the rat toxicological findings of atrazine and postulated mode of action. This analysis focuses on the question of whether the mode of action found to be operative in rats is also operative in humans and whether any human subpoulations or life stage are apt to qualitatively respond to the mode of action differently than the general population. The key questions and rationales are presented in addressing the issue of human relevance. Also, based on the mode of action understanding, a dose-response extrapolation approach is proposed for atrazine.

3.1 Human Relevance

3.1.1 Potential Neuroendocrine Disruption

As discussed in Chapter 2, there are data supporting an understanding of how atrazine induces tumor development in the rat. Briefly, the mode of carcinogenic action underlying mammary and pituitary gland tumor formation in female SD rats involves a lack of adequate secretion of pituitary LH to stimulate ovulation, the development of persistent estrus, and prolonged stimulation of the mammary and pituitary glands by estrogen and prolactin. These hormones promote cell proliferation and predispose cells to become neoplastic. Other endocrinopathies found in the rat (*e.g.,* delayed puberty, prostatitis) are also associated with the neuroendocrine effects of atrazine on pituitary function (*i.e.,* secretion of LH and/or prolactin).

There is clear evidence (discussed in Chapter 2) that atrazine alters hypothalamic GnRH release in rats. There are some data that show that atrazine diminishes NE in the rat hypothalamus as a initial or early site of action which in turn leads to diminished GnRH release. Atrazine also increases dopamine levels which can result in a diminished pituitary prolactin secretion. Therefore, a key question to address is whether this neuroendocrine mode of action at the level of the hypothalamus may be operative in humans. In both humans and rats, hypothalamic GnRH controls pituitary hormone secretion (*e.g.,* LH, prolactin). The hypothalamic-pituitary axis is involved in the development of the reproductive system, and its maintenance and functioning in adulthood. Additionally, reproductive hormones modulate the function of numerous other metabolic processes (*i.e.,* bone formation, and immune, CNS and cardiovascular functions) (Cooper *et al.*, 1986, Plant, 1994). Given that the primary site of atrazine's effect on GnRH secretion in the rat is at the level of the hypothalamus, it is important to address the questions below:

Question. Is there evidence in primates including humans of central neural modulation of GnRH secretion by the hypothalamus? Is this central mechanism conserved across species?

Although GnRH secretion is influenced by a number of factors in primates and humans (such as circulating steroids), and the precise control mechanisms remain to be fully understood, the prevailing view is a central neural control system is involved in governing GnRH release (as reviewed by Marshall and Eagleson, 1999; Plant, 1994). For example, there have been studies in both rats and primates showing that CNS-altering drugs (*e.g.,* opiates) can alter the menstrual cycle or pubertal development (see review by Plant, 1994; Ojeda, 1986). Further, there is evidence that endogenous opioids are involved in GnRH/LH secretion in primates (Ferin and Van de Wiele, 1984), indicating that GnRH neurons are modulated by other hypothalamic neural inputs like in the rat. Therefore, if atrazine affected the hypothalamic GnRH in humans like in the rat, it is plausible to assume that this neuroendocrine mode of action would apply to humans.

3.1.2 Potential Human Health Consequences Associated with Altered GnRH/Pituitary Function

Given that the rat neuroendocrine mode of action may be operative in humans, it is important to address:

Question: What neuroendocrinopathies may result in humans if exposed to atrazine?

Atrazine interferes with the CNS control of pituitary-ovarian function and leads to irregular cycles and inhibition of ovulation in SD and LE rats. In humans and primates reproductive function/ovarian cycling is also influenced by the hypothalamic GnRH (Goldfien and Monroe, 1997; Plant 1994; Nishihara *et al.,* 1992; Terasawa and Nyberg, 1997). Therefore, a potential consequence in humans is disrupted or irregular menstrual cycles which can lead to gynecological problems such as diminished fertility, prolonged menses or excessive bleeding.

It is important to evaluate what is understood about the role of altered GnRH secretion in human ovulatory disorders. Also, a further evaluation of human anovulatory conditions may give some clues as to potential downstream endocrine effects and other health consequences. Hypothalamic amenorrhea (HA) is one model of disrupted cyclicity. HA is a manifestation of a variety of disorders associated with emotional stress, heavy exercise, self-imposed weight loss and oral contraceptive use and occurs in the absence of pathology in the pituitary and ovaries (Reame *et al.,* 1985). HA has been found to represent a spectrum of disordered GnRH secretion (presumably low frequency and variable or low amplitude pulses) that can vary over time (Perkins *et al.,* 1999). Clinically, persons fail to ovulate, as in atrazine treated SD rats. HA is characterized by normal to moderately low serum estrogen and normal to low serum LH. When serum LH is lowered, the cause appears to be a reduction in hypothalamic GnRH secretion (Perkins *et al.,* 1999). These manifestations of HA are similar to those seen with atrazine treated SD and LE rats: decreased hypothalamic GnRH, decreased pituitary LH, and failure to ovulate. These observations suggest that certain of the manifestations may be the same in humans and rats if atrazine affects the hypothalamic neurons in similar ways. In addition to the gynecological

problems associated with disrupted ovarian cycling, HA patients can suffer other health consequences. For example, they can be at an increase risk of osteoporosis later in life given that these women are estrogen deficient, and thus can experience significant losses in bone density. Women who are hypoestrogenic may also suffer from vasomotor symptoms, urogenital atrophy, cardiovascular disease, and possibly diminished cognitive and memory functions (Wren, 1997).

Polycystic ovary syndrome (PCOS) is another model of anovulation, which occurs in 6% to 8% of premenopausal women (Marshall and Eagleson, 1999). PCOS is often characterized by irregular menstrual cycles or amenorrhea, infertility, obesity, and ovaries that are polycystic with many unovulated follicles in various stages of development and atresia. Hirsutism is associated with PCOS (Schildkraut *et al.*, 1996; Hershlag and Peterson, 1996). Some of the other manifestations of PCOS are very different from that seen in atrazine treated rats. There commonly is an increase in LH secretion from the pituitary and increased synthesis of androgens (hyperandrogenism) and their conversion to estrogens. This can result in unopposed exposure to estrogen. The mechanism underlying the excess ovarian androgen secretion is unknown but may be multifactorial, and include abnormalities of steroidogenesis, effects of hyperinsulinemia, and abnormal gonadotropin secretion in stimulating ovarian steroidogenesis (Ehrmann *et al.,* 1995; Utiger, 1996; Marshall and Eagleson, 1999). PCOS is not an exact model for evaluating the consequences of atrazine exposure in humans, other than in some cases it is associated with abnormal GnRH secretion (with presumably high frequency-low amplitude pulses), anovulation, and unopposed exposure to estrogen.

As discussed in Chapter 2, atrazine has also been shown to increase the hypothalamic neurotransmitter, dopamine, which in turn results in a decrease of pituitary prolactin secretion in female rats. In both rats and humans, prolactin is one of the hormones involved in lactogenesis. It is the suckling action of the neonate that stimulates prolactin secretion, and thus the maintenance of milk production. Therefore, in humans, diminished production and secretion of milk could result if atrazine were to affect hypothalamic dopamine and suppress prolactin as in the rat. Given that the initial sucking induced prolactin response is relatively robust, atrazine exposure would not be anticipated to impact the initiation of lactation, but could potentially impact the ability to sustain milk production with continuous exposure.

Therefore, there is support from the primate literature that atrazine's neuroendocrine mode of action (CNS perturbation of GnRH secretion) may apply to humans. Human ovulatory disorders can be associated with aberrant hypothalamic GnRH pulses. These conditions indicate that altered hypothalamic GnRH secretion can broadly affect an individual's functional status, and thus lead to a variety of clinically important health consequences. These human conditions, HA and PCOS, do not prove but raise the possibility that if atrazine produced effects on hypothalamic GnRH in the human, like that seen in atrazine-treated rats, adverse health effects may ensue. The potential ability of atrazine to affect dopamine and prolactin in humans must also be considered. Below, the potential human cancer risk associated with this neuroendocrine mode of action is discussed.

3.1.3 Potential Cancer Risk Associated with Altered GnRH/Pituitary Function

It is standard Agency practice to assume that chemically induced tumors in animals have human relevance unless there are data to the contrary (US EPA 1999a). Target organ concordance is not necessarily a prerequisite for evaluation of the implications or relevance of animal tumor findings for humans. Even if there is a mode of carcinogenic action understanding for the rodent tumor findings, site concordance may or may not be expected. In the case of atrazine, there is an increased incidence and early onset of mammary gland tumors in female SD rats. As discussed below, it does not seem plausible that humans would be at an increase risk for breast cancer given that atrazine would potentially reduce the cumulative number of normal ovarian cycles (*i.e.,* one of the risk factors for humans). In fact, the neuroendocrine mode of action for atrazine raises the possibility of tumor development at other hormone-responsive site.

In assessing potential human risk, human data are generally preferable over animal data when of good quality, and should be given greater weight in the hazard characterization of an agent. Therefore, an obvious question to address is:

> **Question:** Are there data in humans to determine the human cancer potential and neuroendocrine mode of action for atrazine?

As summarized in Chapter 1 (and discussed in detail in Part B-Chapter 4), there is suggestive evidence of a possible association of triazine exposure and cancer occurrence for three hormone-responsive cancers--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. There are no human or primate studies that directly examine the potential for atrazine to induce endocrine effects as have been described in the SD or LE rat special studies.

One important aspect of atrazine's postulated mode of carcinogenic action involves components in common with the reproductive aging process in SD female rats. It is well recognized that in SD female rats, as well as in other strains of rats such as LE and Wistar, reproductive aging is due to failure of hypothalamic-pituitary-gonadal function resulting in the normally aging female spending an increased percentage of days of their ovarian cycle in estrus *(i.e.*, constant estrus) (discussed in detail in Part B-Chapter 9.1). Therefore, an aging female SD rat experiences a dampening of the preovulatory pituitary LH surge which results in prolonged exposure to estrogen. In contrast, the prevailing view for humans is that reproductive aging results from a depletion of follicles from the ovary (*i.e.,* atresia). However, the potential that an age-associated loss of the hypothalamic control of GnRH secretion may contribute to significant changes in menstrual function during the perimenopausal period in women can not be discounted (*e.g.,* Wise *et al.,* 1996; 1999).

Nevertheless, to the extent that the carcinogenic effects of atrazine in SD rats are intimately tied to an interaction between effects of the chemical and the normal aging process in rats, then there may be questions as to the applicability of the carcinogenic effects to humans.

Question: Can atrazine lead to cancer through a process not involving reproductive aging; and can the neuroendocrine effects of atrazine alone set up a milieu favorable to the development of cancer in humans?

In addressing the above questions, it is important to note that of the key events identified in Figure 2-1 based on laboratory *in vitro* and *in vivo* data, atrazine's initial site of action appears to be at the level of the hypothalamus (*i.e.*, effects on hypothalamic catecholamine and GnRH levels). As discussed above, CNS control of hypothalamic GnRH is similar in primates and humans, and human conditions of anovulation, which can be associated with aberrant GnRH release, lead to a variety of health consequences. It is important to look at the cancer risk associated with the human ovulatory conditions discussed above.

HA has not been found to be associated with a cancer risk based on epidemiologic studies, although it is clearly associated with other health consequences as discussed above. Because of the prevalence of PCOS in the population, several epidemiologic studies have assessed its role in breast cancer. One showed a significantly increased risk, but only in the postmenopausal period (Coulam *et al.*, 1983); the remaining three failed to show breast cancer increases (Gammon and Thompson, 1990; Anderson *et al.*, 1997; Pierpoint *et al.*, 1998). Such findings have been interpreted as lending little support for PCOS being a risk factor for breast cancer (Solomon, 1999). A small number of patients, however, have enough estrogen to maintain the endometrium, which has the potential to become hyperplastic over time (Mansfield and Emans, 1989; Schachter and Shoham, 1994); endometrial hyperplasia is a risk factor for endometrial cancer (Rose, 1996). Case reports suggest that PCOS may predispose women to endometrial cancer at an early age, in contrast to this cancer's usual occurrence with advancing age (Jafari *et al.*, 1978; Dahlgren *et al.*, 1991). A statistically-significant increase in relative risk for endometrial cancer was noted among documented PCOS patients (Coulam *et al.*, 1983). Information linking PCOS to ovarian cancer is less well developed. One study of epithelial ovarian cancer showed a statistically-significant increase of persons with PCOS (Schildkraut *et al.*, 1996), while another did not (Coulam *et al.*, 1983).

The human conditions of anovulation only supply inferential information concerning potential cancer risks. These human disease models do not prove a potential cancer risk associated with atrazine's neuroendocrine mode of action. But on the other hand, they do not allow one to discount the possibility that if atrazine produced effects on hypothalamic GnRH like is seen in atrazine treated SD rats, disrupted cyclicity may result in an endocrine environment that may be conducive to tumor development at hormone-responsive sites. Mammary gland site concordance with SD rats should not be expected (as discussed further below), but the mode of action responsible for the rat tumors and information on PCOS raise the possibility of other endocrine sites (*i.e.,* endometrial and ovarian). Also, conditions of human anovulation disorders suggest that atrazine exposure alone may produce an endocrine imbalance that may be conducive to tumor development. Given that hypothalamic GnRH control of the preovulatory pituitary LH surge is similar in rats and primates, it seems possible that this process could be independent of the reproductive aging pattern as seen in rats.

3.1.4 Breast Cancer

Given that atrazine induced mammary gland tumors in SD rats, it is important to evaluate what is understood about endocrine influences for breast cancer.

- ❑ Estrogen seems to be an important influence in breast cancer development, as indirect indicators of estrogen stimulation are known risk factors for the disease: early age of menarche, late onset of menopause and nulliparity. However, it seems that the cumulative number of regular and not irregular ovarian cycles is the important input into breast cancer development (Henderson *et al.*, 1988; den Tonkelaar and de Waard, 1996). Consistent with this, regular exercise is associated with reduction in breast cancer risk, possibly by reducing the number of normal ovulatory cycles as is seen in hypothalamic amenorrhea (Bernstein *et al.*, 1994).

- ❑ Prolactin plays a role in mammary gland carcinogenesis in rodents, but its importance in human breast cancer development is not at all established. Prolactin together with estrogen, stimulates the human breast tissue during lactation. Unlike rats where there are significant changes in prolactin levels throughout the ovarian cycle, there is little modification during the human menstrual cycle (Goldfien and Monroe, 1997). Rats and humans do show circadian variations in prolactin. Two prospective studies among postmenopausal women have found increases in breast cancer with elevated prolactin levels, although only one was statistically-significant; retrospective studies of premenopausal women have been variable in their outcomes (Wang *et al.*, 1992; Hankinson *et al.*, 1999).

Many different pharmaceuticals induce hyperprolactinemia, including the raulwolfia drugs used to treat hypertension, tricyclic antidepressants, antipsychotic phenothiazines and methyldopa, the drug used to treat Parkinson's disease. A number of epidemiologic studies have been conducted with the raulwolfia derivatives. Some have noted no increase in breast cancer risk while others have indicated rather limited increases in postmenopausal women (Shapiro *et al.*, 1984; Williams *et al.*, 1978). It has been argued that agents which produce about a 50% increase in prolactin levels may account for the small increase in cancer risk in some of the studies (Ross *et al.*, 1984). One investigation showed that with dosing for at least 10 years or with initiation of dosing at least 10 years prior to diagnosis, significant risk ratios of about four were found (Stanford *et al.*, 1986). Antidepressants also lead to increases in prolactin levels. The relationship between their use and breast cancer have led to differing outcomes (CoHerchio *et al.,* 2000; Kelly *et al.,* 1998; Wallace *et al.,* 1982). There has been less investigation of other psychiatric drugs that produce hyperprolactinemia and its association with increases in breast cancer risk. An investigation of all 9156 schizophrenic patients in Denmark that had there first hospital admission between 1970-1987 showed no indication of increase in breast cancer risk (Mortensen, 1994), in keeping with other studies. More work is needed to probe these relationships.

Interestingly, mammary gland and breast cancers have receptors for prolactin, and studies show that prolactin mRNA and the hormone itself are synthesized by tumor cells (Clevenger *et al*., 1995; Mershon *et al*., 1995). It has been hypothesized that the local formation of prolactin may serve autocrine or paracrine functions within the mammary gland (Ben-Jonathan *et al.*, 1996; Vondehaar, 1999). These observations reopen the question of the role of prolactin in human breast cancer development. As of yet, the regulation and effects of locally synthesized prolactin on the breast have not been determined.

3.1.5 Cancer Classification

In the past, OPP had classified atrazine as a Group C, possible human carcinogen based on an increased incidence of combined mammary carcinomas/adenomas and fibroadenomas in female SD rats, in accordance with the 1986 cancer risk assessment guidelines. Recently, the OPP Cancer Assessment Review Committee (CARC) proposed that atrazine should be classified as a **likely human carcinogen** in accordance with the draft 1999 revisions to the cancer risk assessment guidelines (*i.e.,* US EPA, 1999a). The basis of this current proposal is as follows:

- ❏ Consistent findings in female SD rats of an increased incidence and early onset of mammary gland carcinomas/adenomas in several studies, and suggestive evidence of an early onset of pituitary adenomas and mammary fibroadenomas;

- ❏ Mode of action evidence that indicates hypothalamic disruption of GnRH control of pituitary function by atrazine, and critical reductions in LH and resultant anovulation; and

- ❏ Similarity in humans and rats for CNS control of pituitary function.

Therefore, if atrazine affected hypothalamic GnRH as in the rat, this opens the possibility that an endocrine imbalance may result which could lead to several different health consequences including cancer at hormone responsive tissues.

3.2 Potential Health Effects of Atrazine in Children

3.2.1 Reproductive/Developmental Hazard

The data summarized in Chapter 2 indicates that the primary underlying process that leads to mammary and atrazine involves disruption of the hypothalamic pituitary-gonadal-axis. pituitary gland tumor development in female SD rats following treatment with This axis is also involved in reproductive development. Therefore, as summarized in Chapter 1.7, it is not surprising that atrazine treatment also results in adverse reproductive and developmental outcomes in special studies using several different strains of rats *(i.e.,* F344, SD, Wistar, LE, Holtzman). These outcomes include interruption of regular ovarian cycling, decreased suckling induced prolactin release and increased incidence and severity of prostatitis, and delays in vaginal opening and preputial separation.

Rat and human reproductive development and puberty are under similar hypothalamic-pituitary control, especially LH and prolactin (Matsumoto *et al.*, 1986, Ojeda, 1986). After the first trimester in humans, fetal LH and FSH are used to complete genital maturation (Hsing, 1997). There is an appreciable release of LH commencing at parturition that extends until four to six months of postnatal life. Thereafter, LH is suppressed until puberty begins. There is a re-awakening of the hypothalamic-pituitary-gonadal axis at puberty. The exact mechanism underlying this pubertal LH release is unknown. For male sexual development, LH is required to stimulate the Leydig cells for testosterone production, and androgens are responsible for the outward signs of pubertal development. LH and FSH are required to begin ovarian activation, follicle growth, and steroid production in female sexual development. Estrogen secreted from the ovary triggers breast growth and other body changes. Some adolescent patients with delayed puberty display low levels of LH and/or FSH (Styne 1997; Kulin 1996). Therefore, there is concern that if children were exposed to atrazine and if it affected the hypothalamic-pituitary-gonadal axis and the pituitary LH and PRL releases as in rats, there is the potential for delayed puberty or altered pubertal growth in both female and male adolescents. Delayed puberty is not without health consequences. For example, girls with delayed

menarche show a higher incidence of scoliosis, stress fractures, and osteopenia than do girls with normal time of menarche (Goldfien and Monroe, 1997). Additionally, abnormal puberty may result in problems manifested later in life (*e.g.,* osteoporosis (Styne, 1997).

Exposure to atrazine in lactating dams (Wistar rats) suppresses suckling-induced prolactin release which eventually results in hyperprolactinemia and prostatitis in the lateral prostate in young adult offspring. It is reasonable to assume that this suppression of pituitary prolactin secretion in the dam is due to atrazine's effect on hypothalamic catecholamine levels (*i.e.,* dopamine). Prolactin does play a role in the development and maintenance of the human prostate. Critical periods for developmental exposures and the hormonal involvement in the induction of prostatitis remain unknown in humans. In humans, nonbacterial prostatitis of undefined etiology is an important clinical problem that has been associated with infertility (Meares, 1998; Huaijin *et al*., 1998). There is a suggestion in the literature that chronic proliferative inflammation in the prostate may be a precursor event to prostatic carcinogenesis (De Marzo *et al.,* 2000; Leav *et al.,* 1999). It should be acknowledged that the relevance of effects in the rat prostate as a human model has been debated. However, Because the dorsal and lateral prostate of the rat are considered to be the most homologous to the human prostate (Price, 1963), the increase in inflammation observed in young male rat offspring should not be discounted.

In summary, because of the similarity between rats and humans of the influence of hypothalamic GnRH on the growth and morphogenesis of the reproductive system, the concern is raised about the potential health effects due to early life exposure to atrazine, some of which may not be manifested until later in life.

3.2.2 Cancer Hazard

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 needs to be made as to whether this mode of action is relevant to children. When there is no cancer information on children *per se*, a "cogent biological rationale needs to be developed regarding whether the mode of action is applicable to children." In the case of atrazine, although there are no animal data directly evaluating its neoplastic potential from pre- and postnatal exposures *per se*, there is information indicating that atrazine can affect the hypothalamic-pituitary axis and cyclicity in young animals. So reliance is placed upon both data concerning the neuroendocrine effects in young animals as well as using biological arguments to evaluate children's cancer concern.

If atrazine were to produce neuroendocrine effects in humans like it does in SD rats, projections can be made as to potential consequences in children, using what is understood about the key events described for its postulated mode of action. Components of the neuroendocrine system develop during fetal life, with varying manifestations at different times. As discussed above, the preovulatory LH surge controlling ovulation does not happen until puberty. Considering the purported mode of atrazine action involving attenuation of the preovulatory LH surge and disruption of ovarian cycling as a critical event, it is reasonable to assume that this mode of action may also be operative in children from puberty onward. Furthermore, the rodent cancer bioassays on atrazine as well as the accompanying LH/cyclicity mode of action studies used young pubertal rats (six to eight weeks of age). Thus, there is a potential cancer concern for children as a result of exposure during puberty and continued over a lifetime. The rat studies on decreased suckling induced prolactin release and increased incidence and severity of prostatitis in male offspring, however, raise the question of whether prepubertal exposure may lead to a potential prostate cancer risk later in adult life. At this time there is no indication of such an outcome, however, conventional cancer testing may not screen for such potential. Further study would be needed to determine whether there is or is not any hazard capability.

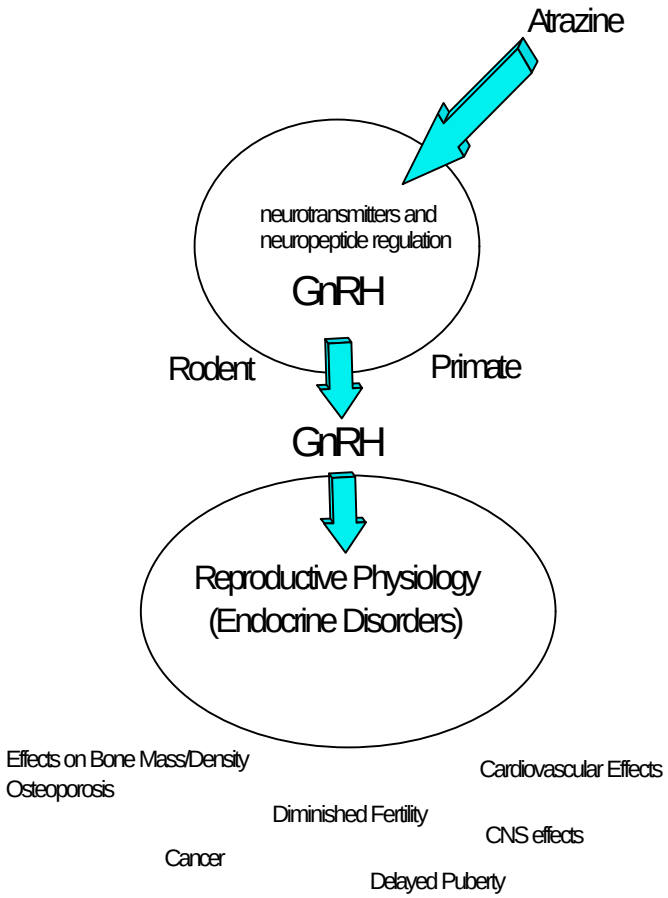
3.2.3 Summary of Children's Health Concern

Rat studies using atrazine treatment *in utero* or during early life demonstrate a wide spectrum of endocrinopathies (*e.g.,* delayed puberty, disrupted cycling, prostatitis, reproductive organ weight changes, hyperprolactemia) associated with the disruption of the neuroendocrine control of pituitary function. There are numerous studies in the literature indicating that altered neuroendocrine status in children lead to a variety of health outcomes. Furthermore, as discussed previously, CNS-GnRH control of reproductive development is similar in primates and rats. Thus, the rat studies on atrazine raise concern for the susceptibility of the fetus and young child if exposed to atrazine. The consequence in children due to this neuroendocrine mode of action would depend on the developmental stage of exposure and the duration of exposure. For example, prepubertal exposures would most likely result in developmental effects**,** and postpubertal exposure may result in a variety of health consequences including cancer. There is no direct information on cancer responses following pre- or postnatal exposure.

3.3 Summary of Atrazine Human Hazard Potential

As shown in Figure 3-1, atrazine operates via a neuroendocrine mode of action that alters hypothalamic GnRH and pituitary LH and PRL secretions. It is recognized that across species and even among different strains of a species endocrinological interactions can differ significantly (Neumann *et al*., 1996). However, atrazine's central neuroendocrine mode of action is likely to be operative in humans given that in both rats and primates a central neural control influences GnRH and pituitary function. The variety of endocrinopathies found in the atrazine treated rats (*e.g.*, mammary and pituitary gland tumors, delayed puberty, disrupted cyclicity, prostatitis in young rats) raise concern about the potential human health consequences that may ensue from this neuroendocrine perturbation, including adverse reproductive and developmental outcomes or delayed acquisition of normal reproductive potentialities. This neuroendocrine mechanism also raises concern for potential cancer risk in humans.

Figure 3-1. Atrazine's Neuroendocrine Mode of



3.4 Dose-Response Analysis

In 1988, the U.S. EPA presented a dose-response assessment of atrazine (Hauswirth 1988a; US EPA 1988). That assessment used the female SD mammary tumor incidence from the study by Mayhew *et al.* (1986) and the linearized multistage (LMS) model to estimate an oral slope factor and a unit risk of 2.22×10^{-1} $[mg/kg/day]^{-1}$. The current dose-response analysis considers the mode of action data as discussed in Chapter 2. Additionally, the two-step approach to dose response assessment as described in the proposed revisions to U.S. EPA Guidelines for Carcinogen Risk Assessment (US EPA, 1999a) are utilized in this dose-response analysis. This two-step process distinguishes between the observed range of empirical data and the range of extrapolation.

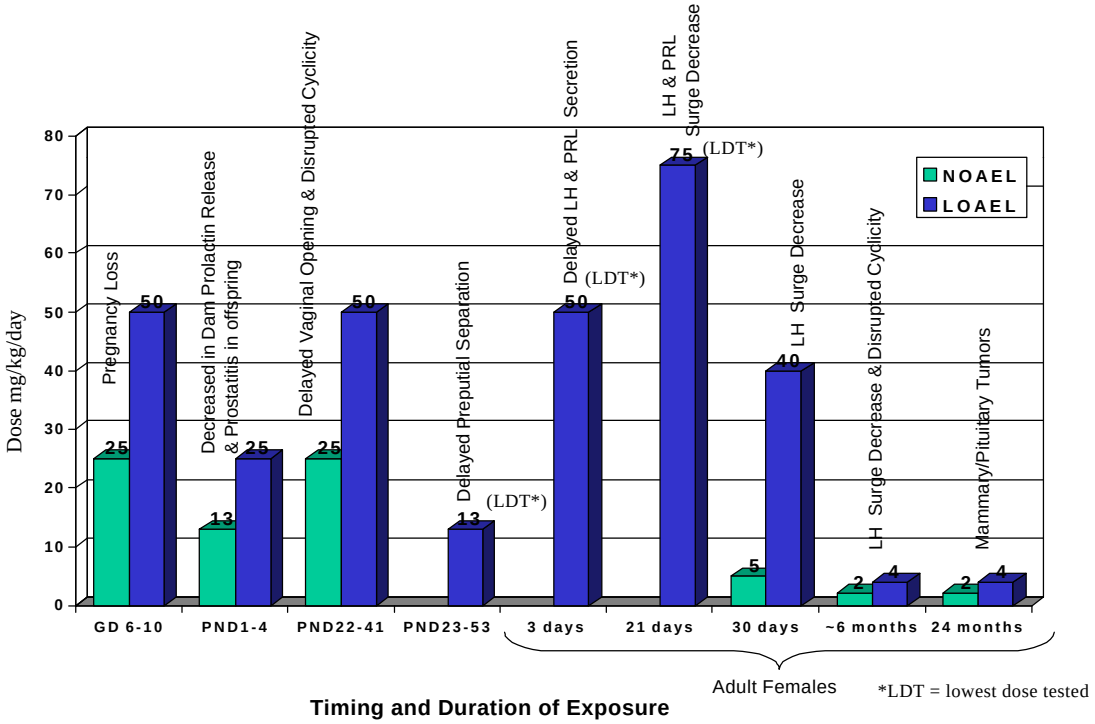
The weight of evidence does not support mutagenicity nor direct estrogenicity as components of atrazine's mode of carcinogenic action. As discussed in Chapter 2, the weight of evidence supports a conclusion that atrazine acts to cause mammary and pituitary gland tumors in female Sprague-Dawley rats by causing a attenuation of the preovulatory surge of LH which results in anovulation and an endocrine milieu that is conducive to tumor development. The critical event, the attenuation of the LH surge, is consistent with a nonlinear phenomenon in that there is a dose of atrazine that does not affect the LH surge or disrupt cyclicity. Therefore, it is proposed that dose-response assessment should proceed by a margin of exposure analysis.

An increased incidence and/or early onset of mammary and pituitary gland tumors in the rat is only one endocrinopathy found after atrazine treatment. The reproductive and developmental consequences (*e.g.,* disrupted cyclicity, delayed puberty, prostatitis in male offspring) that are found after atrazine treatment are of equal concern. These reproductive/developmental effects also originate from the effects of atrazine on the hypothalamic control of pituitary function through its interference with hypothalamic catecholamines and GnRH neurotransmitters. Thus, given the commonality in the mode of action, it is recommended that a point of departure for dose-response extrapolation be based on the most sensitive effects associated with atrazine's neuroendocrine mode of action.

3.4.1 Selecting a Point of Departure

Margin of exposure analysis begins with selection of a point of departure (POD) considered to represent the lowest reliable endpoint in the range of observation, being either tumor incidence, a key endocrine related effect or data on a proximal event that is an integral part of the mode of action process. Figure 3-2, provides an overview of the NOAELs/LOAELs for key endocrine related effects of atrazine from various studies in rats at different life stages and for different treatment durations. As discussed in Chapter 1 and Part C, the NOAELs for the effects of atrazine on pregnancy, pubertal onset and prostatitis are, for the most part, at or above 25 mg/kg/day. The exceptions are:

Figure 3-2. Key Endocrine-Related Effects Following Atrazine Treatment of Rats



- The NOAEL for delay of preputial separation in Wistar rats is not clear as a significant delay was seen at ~13 mg/kg/day (albeit near statistical significance; p = 0.07), but not at the next highest dose of 25 mg/kg/day; and

- The NOAEL for dam decreased prolactin release and resultant prostatitis in male offspring is ~13 mg/kg/day.

Dose-response data from long term repeat dosing studies are lacking for the effects on hypothalamic catecholamines and GnRH, *i.e.,* atrazine's initial site of action. However, it is the pulsatile GnRH secretion from the hypothalamus that determines the pituitary LH secretion (a critical event in atrazine's mode of action). Therefore, it is assumed that effects on LH secretion are a mirror of effects on GnRH secretion and that data on the serum LH are reasonable surrogate measures of the GNRH secretion. There are more data over different doses and time points for the attenuation of the LH surge. Thus, this is an appropriate POD. As illustrated in Figure 3-2, the NOAELs/LOAELs for the LH data are compared to the other key end points (mammary gland tumors, increased days in estrus, delayed puberty, suppression of suckling-induced prolactin and resultant prostatitis) that result from this neuroendocrine mode of action (also see Tables 1.9, 1.10, 2.1, and 2.2).

Selecting the NOAELs for the attenuation of the LH surge to determine a point of departure rather than from curve-fitting in the observable range of LH data is done here because it is not known over just what level of attenuation of the LH surge is necessary in order to produce clinically relevant effects. As discussed in the draft 1999 guidelines for carcinogen risk assessment, "the observed range of data may be represented by a NOAEL/LOAEL procedure when a margin of exposure analysis is chosen as the default procedure for nonlinear dose-response extrapolation" (US EPA,1999a).

As the treatment duration is increased, the dose that is needed to attenuate the preovulatory LH surge decreases. For example, it takes an extremely high dose of a single day of dosing of atrazine to attenuate the LH surge *(i.e*., NOAEL = 200; LOAEL = 300mg/kg; Cooper *et al*., 1999). However, with longer durations of dosing, much lower doses of atrazine can attenuate the LH surge (*i.e.,* NOAEL = ~2 mg/kg; LOAEL = ~4 mg/kg after six months of dosing; Morseth, 1996b). As shown in Figure 3-1, several types of reproductive/developmental effects can arise in postnatal rats following a few days of dosing up to several weeks of dosing with atrazine (*e.g.,* delayed puberty, prostatitis, increased days in estrus). As depicted in Table 1-10, NOAELs for these reproductive effects range from13 mg/kg/day up to 100 mg/kg/day.

With respect to effects that result from longer durations, LOAELs for precursor events associated with carcinogenesis (increased days in estrus, attenuation of the LH surge) and tumors consistently ranged between ~3 to 4 mg/kg/day. Likewise, NOAELs for various parameters were ~2 mg/kg/day or higher in all cases except one. In the Mayhew (1986) study, a significant tumor increase was noted at ~4 mg/kg/day but not at the lowest dose tested, 0.5 mg/kg/day. Based on consideration of the all the bioassay studies in SD rats and the repeat dose LH studies, as well as consideration of the dose spread in the Mayhew (1986) bioassay, LOAELs for carcinogenic, LH, and cyclicity effects tended to be approximately 4 mg/kg/day and NOAELs tended to be ~2 mg/kg/day. Clearly, there is a correspondence of doses that lead to tumor formation and doses that produce effects on LH levels and cyclicity. Thus, the point of departure for chronic effects is the dose of 1.8 mg/kg/day which is the NOAEL for attenuation of the proestrus afternoon LH surge in Morseth (1996b).

3.4.2 Point of Departure Using LED_{10} From The Tumor Data

Although data based on the attenuation of the LH surge is the preferred POD, for comparative purposes PODs based on the modeling of tumor data to derived $LED_{10's}$ are also presented. The most appropriate study to use in selecting a point of departure for tumors is Morseth, 1998. Five bioassays using the SD rat are available which examine tumor incidence and early onset. One of these studies (Petterson and Turnier, 1995) is a one year study and is not deemed appropriate for that reason. Another study (Thakur, 1991a) is not considered because only two dose groups were used and the study employed many serial sacrifices which resulted in a very small "n" value by the later timepoints in the study. A third bioassay (Thakur, 1992a) used only two dose groups. The two remaining studies, Mayhew (1986) and Morseth (1998), which employed four dose groups, both may be considered for use in selecting a point of departure. LED_{10s} for both of these studies are presented in Table 3-3. And ranged from ~2 to 3 mg/kg/day for mammary gland carcinomas and adenomas combined. These values represent equivalent human doses.[3] The NOAELs/LOAELs for mammary gland tumors are 0.5 and 3.5 mg/kg/day; and 4.2 and 24.4 from the Mayhew and Morseth studies, respectively. It should be noted that Morseth (1998) provides time to tumor information and used contemporary criteria for pathological evaluations. Also, Morseth (1998) had accompanying estrus cycling data.

The NOAEL for the LH surge attenuation and the LED_{10} for carcinomas and adenomas from Morseth (1998) are 1.8 mg/kg/day *(i.e.*, 0.48 mg/kg/day in human equivalents. Therefore, a POD based on the NOAEL for attenuation of the LH surge is comparable to a POD based on tumor response.

[3]Conversion to human equivalents performed by multiplying the rat dose in mg/kg/day by 0.266.

Table 3-2. LED_{10}s in Human Equivalents (And Revised Q_1*)

Study	Mammary Gland Tumors	LED_{10} (mg/kg/day)
Mayhew, 1986	Combined adenomas, carcinomas, and adenosarcomas	2.1
Mayhew, 1986	Fibroadenomas	3.0
Morseth, 1998	Combined adenomas and carcinomas	1.8
Morseth, 1998	Fibroadenomas	3.5
Morseth, 1998	Incidence of combined carcinomas and adenomas	(Q_1* = 1.12 x 10^{-1} mg/kg/day)

Data in this table from US EPA, 1999b and 1999d.

Table 3-2, also provides a revised Q* estimate for comparison purposes only. Given the mode of action understanding for atrazine, the nonlinear extrapolation approach is preferred over the linear default approach. The linear extrapolation is not supported by the mode of action data.

3.5 Summary and Conclusions on the Proposed OPP Science Policy Positions: Mode of action, Human Relevance, Children's Health Concerns, and Dose-Response Extrapolation

Listed below are the proposed science policy conclusions regarding the postulated mode of carcinogenic action in SD female rats. The relevance of the rat reproductive/developmental studies and the female SD rat tumor findings their mode of action to humans, including concerns for children. Recommendations are also made for the dose-response approach that should be considered in the cancer risk assessment.

3.5.1 Postulated Rat Tumor Mode of Action

Members of the pesticide program Cancer Assessment Review Committee (CARC) reviewed information on atrazine bearing on the formation of mammary and pituitary tumors in female SD rats. The CARC concluded that the increased incidence and early onset of mammary gland carcinomas and adenomas were well supported by several rat bioassay studies. The evidence for an early onset of mammary fibroadenomas and pituitary adenomas was considered to be suggestive.

Based on the *Mode of Action Framework Analysis* presented in Chapter 2, judgments were made on three considerations underpinning the mode of action of these tumors. The Committee agreed that:

- ❑ Atrazine does not have a significant mutagenic component to its mode of action;

- ❑ Direct atrazine binding to the estrogen receptor is not an influence on tumor development; and

- ❑ The neuroendocrine mode of action for the mammary and pituitary tumors is “biologically plausible” and is supported overall by the weight of the evidence.

As discussed in Chapter 2, there are several strengths of the mode of action proposal. For example, atrazine’ s induced LH and cyclicity effects have been shown in two different laboratories and in two different strains of rats (LE and SD). Furthermore, there is a strong correlation has been shown for atrazine induced persistent estrus and induction of mammary tumors. Generally, there is a strong temporal and dose-response correlation between tumor formation and precursor effects. Ovariectomized SD rats treated with atrazine do not develop tumors, thus demonstrating the role of ovarian estrogen in atrazine’s mode of action. Finally, a strong correlation was demonstrated between increased pituitary weights and histomorphological markers of prolactin exposure in the mammary gland, thus supporting the role of prolonged estrogen and prolactin exposure in tumor development. Although significant amounts of data have been developed to demonstrate how atrazine may produce

mammary and pituitary tumors in SD rats, there are uncertainties or limitations in the available data base (as discussed in Chapter 2.4.5). It should be emphasized that the uncertainties or limitations in the data in themselves do not discount the postulated mode of action, and that the strengths of the data provides compelling evidence in support of the postulated mode of action. However, the uncertainties/weaknesses in the data should be should be considered in the final risk characterization.

3.5.2 Relevance of Rat Mode of Action to Humans and Carcinogenicity Classification

It is proposed that **the postulated mode of action is assumed as being relevant to human cancer potential given that a primary initial site of action in rat involves the CNS control of pituitary function**. 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. This default is intended to be public health protective and departure from this default must have a strong accompanying scientific basis. OPP views the differences between reproductive aging in humans and rats as an insufficient scientific basis to depart from the default. Therefore, if atrazine were to act on the hypothalamus of humans as in the rat and caused CNS alterations which influence endocrine function on physiological processes including ovarian cycling, there is the potential for various adverse health outcomes, including cancer.

The OPP Cancer Assessment Review Committee proposed that atrazine should be classified as a **likely human carcinogen** (US EPA, 1999a).

3.5.3 Children's Hazard

Data are available from animal studies on atrazine to assess potential effects in children that may be associated with its neuroendocrine mode of action. **Based on the endocrinopathies found in postnatal rats, it is reasonable to assume that children would potentially be susceptible to atrazine's neuroendocrine mode of action which may lead to a variety of health consequences** (See section 3.2). How atrazine's neuroendocrine mode of action is manifested depends on the life stage exposed as well as the duration and level of exposure. Data following prepubertal exposures in rats demonstrate adverse developmental effects including delay in puberty and prostatitis. In reference to the mammary tumors in rats and their mode of action, a cogent biological rationale informs that situation. LH secretion is quiescent until puberty. Therefore, it is not expected that atrazine would pose a cancer hazard following prepubertal exposure. However, starting with exposures at puberty, cancer hazard may be evident. As with adult exposures, certain endocrine responsive sites in the female may be at risk for cancer development.

3.5.4 Dose-Response

Based on atrazine's mode of carcinogenic action, a nonlinear dose-response extrapolation approach is the preferred approach for quantifying the cancer risk. A cancer hazard in adults resulting from infant and children exposure to atrazine cannot be ruled out. Infants and children, however, would not be expected to demonstrate a unique susceptibility to tumors induced by this mode of action, with the possible exception of an increased postpubertal risk of tumors. In order to assure adequate protection of all susceptible subpopulations (*i.e.*, women and children) for both cancer and noncancer effects for potential exposures throughout their lifetime, **it is recommended that the health risk assessment be performed utilizing the most sensitive endpoint associated with atrazine's neuroendocrine mode of action**. A NOAEL of ~2 mg/kg bw/day, based on attenuation of the LH surge following six months of atrazine treatment, is recommended as the point of departure for the health risk assessment using the MOE approach. For continuous exposures, this NOAEL is viewed as appropriate given atrazine's neuroendocrine mode of action which potentially leads to a variety of health consequences including cancer, and is viewed protective of all populations (including women and children).

3.6 Other Reviews

There have been a number of reviews on the carcinogenicity of atrazine by other organizations:

- ❏ Draft report of the Cornell University Program on Breast Cancer and Environmental Risk Factors in New York State (Snedeker and Clark, 1999);.
- ❏ The International Agency for Research on Cancer (IARC, 1999);
- ❏ The National Registration Authority for Agricultural and Veterinary Chemicals of Australia (NRA, 1997);
- ❏ The United Kingdom - in a report to the European Commission (United Kingdom Pesticide Directorate, 1996);

- ❑ A report published by a U.S. consulting group under contract to the Triazine Network (a national coalition of grower organizations and individuals) (Cantox, 2000); and

- ❑ A consensus report of a scientific panel commissioned by Novartis Crop Protection (Consensus Panel, 2000).

It should be noted that the current EPA draft atrazine assessment has generally reached similar conclusions with the above reviews on several issues concerning the carcinogenicity of atrazine (see Table 3-3). There appears to be consensus that mutagenicity and direct binding to the estrogen receptor do not play a significant role in atrazine's carcinogenic action in SD rats (IARC, 1999; Snedeker and Clark, 1999; Cantox 2000; Consensus Panel 2000; NRA, 1997; United Kingdom Pesticide Directorate, 1996). Further, these reviews have also concluded that an endocrine mode of carcinogenic action in SD rats is biologically plausible and is supported by the evidence. Although there is general agreement about support for a mode of action, there are different views on the role of accelerated reproductive senescence in the SD rat tumor response. For example, the United Kingdom Pesticide Directorate (1996) states that the reproductive aging hypothesis is not adequately proven, but that the tumors do appear to be caused by a "disturbance of endogenous hormone levels." Also, Snedeker and Clark (1999), concluded that there were inconsistencies or lack of data on certain hormonal measures (such prolactin and estradiol) which did not lead support to the premature reproductive aging hypothesis, but "there is evidence that it can affect hormones along the hypothalamic pituitary gonadal axis."

Although there is general agreement among different organizations, there are differences in the conclusions regarding human relevance and cancer classification. Snedeker and Clark (1999) concluded that atrazine is a "possible breast carcinogen." This document concludes that site concordance should not be assumed and that the potential exists for cancer at other hormone-responsive sites (*e.g.*, endometrium). Several other organizations including IARC (1999) concluded that the mode of carcinogenic action in SD rats in not relevant to humans. EPA/OPP may have had more data on the mode of action than these reviews, particularly on the hypothalamus as a primary site of action (Cooper *et al.,* 2000; Das *et al.,* 2000). But more importantly, these analyses considered the disruption of hypothalamic control by atrazine in a broader sense leading to several neuroendocrinopathies (*e.g.,* delayed puberty, prostatitis, mammary

gland tumors) in the rat, rather than focusing on the reproductive aging process and induction of mammary gland tumors in rats. Unlike these reviews, this analyses evaluated the neuroendocrine controls of pituitary function in rodents and primates, including humans, and concluded that there is a potential for carcinogenic effects independent of reproductive aging, and that primates may have some aging components in common with rat. Also, the LH response were not limited to SD rats also found in LE rats. The reproductive effects were also found in other strains such as Wistar rats.

Table 3-3. Other Reviews on the Carcinogenicity of Atrazine*

	Mutagenic	**Direct Estrogenicity**	**Mode of Carcinogenic Action**	**Human Cancer Concern**
EPA/OPP (This Draft)	No	No	Support	"Likely human carcinogen"
Snedeker and Clark (1999)	"	"	Some Support	"Possible breast carcinogen"
IARC (1999)	"	"	Support	"Not relevant" (Group 3)
Cantox (2000)	"	"	Support	'Not likely to be carcinogenic"
Consensus Panel (2000)	"	"	Support	"Not relevant"
NRA (1997)	"	"	Support	"Not considered to be relevant"
United Kingdom Pesticide Directorate (1996)	"	"	Support	"A strong case for non-classification"