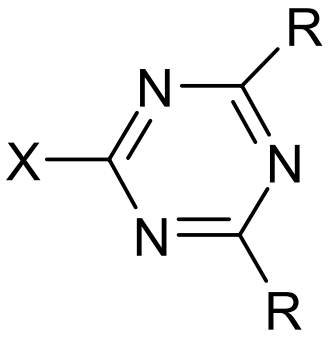
The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity



U.S. EPA Office of Pesticide Programs
Health Effects Division
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ACRONYMS

CNS	Central Nervous System
DEA	Desethyl-s-atrazine
DIA	Desisopropyl-s-atrazine
DACT	Diaminochlorotriazine
F344	Fischer 344
FQPA	Food Quality Protection Act
FSH	Follicle-stimulating Hormone
GD	Gestation Days
GnRH	Gonadotropin Releasing Hormone
HA	2-Hydroxyatrazine
HLZ	Holtzman
HPG	Hypothalamic-pituitary-gonadal
LE	Long Evans
LH	Luteinizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
NE	Norepinephrine
NOAEL	No Observed Adverse Effect Level
OPP	Office of Pesticide Programs
PND	Postnatal Days
SAP	Scientific Advisory Panel
SD	Sprague Dawley
US EPA	United States Environmental Protection Agency
WOE	Weight-of-Evidence

Executive Summary

This document discusses the available scientific evidence for determining whether a common mechanism of toxicity exists among certain triazine-containing pesticides. The weight-of-evidence (WOE) analysis used is similar to the general approach outlined in the January 29, 1999 ***Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity*** (http://www.epa.gov/oppfead1/trac/science/ #common and http://www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf). The group of triazine-containing chemicals considered as candidates for grouping in this document consists of the following pesticides: **atrazine, simazine, propazine, tribenuron-methyl (Express)**, and the degradants **2-hydroxyatrazine, desethyl-s-atrazine (DEA), desisopropyl-s-atrazine (DIA)**, and **diaminochlorotriazine (DACT)**.

Treatment of laboratory animals with these chemicals results in toxic effects such as mammary gland tumors in only female rats, attenuation of the lutenizing hormone (LH) surge, alteration of the estrous cycle, altered pregnancy maintenance, and delayed pubertal development. The development of mammary gland tumors in female rats is postulated to be associated with disruption of the hypothalamic-pituitary-gonadal (HPG) axis. In summary, the proposed mode of action for induction of mammary gland tumors in female rats by atrazine involves altered secretory activity of the HPG axis, beginning with a decrease in the release of gonadotropin releasing hormone (GnRH) by the hypothalamus followed by a consequent attenuation of the afternoon LH surge during the estrous cycle. As a result, ovulation does not occur and the estrous cycle is prolonged, thereby increasing the exposure to estrogen. Increased estrogen also stimulates prolactin secretion from the pituitary. The resultant endocrine milieu of enhanced or unopposed estrogen and prolactin secretion provides an environment that is conducive to the development of mammary gland tumors. Likewise, attenuation of the surge in LH, alteration of the estrous cycle, altered pregnancy maintenance, and delayed pubertal development are considered to be either manifestations or direct consequence of disruption of the HPG axis. This proposed mode of action of atrazine for reproductive developmental effects in female SD rats (considered in this document for grouping by a common mechanism of action) was presented by the Agency to the FIFRA Scientific Advisory Panel (SAP) in June 27-29, 2000 and found to be plausible.

Based on the available WOE, only **atrazine, simazine, propazine,** and the degradants **DEA, DIA** and **DACT** can be grouped by a common mechanism of toxicity for disruption of the hypothalamic-pituitary-gonadal (HPG) axis. Although some of the evidence may support including **Express** and/or **2-hydroxyatrazine**, the overall weight-of-evidence does not support their inclusion in the common mechanism group. If additional data become available to directly support their inclusion in the common mechanism group, these data would be considered.

Thus, in the absence of additional evidence that may support an alternative grouping, **atrazine, simazine, propazine,** and the degradants **DEA, DIA** and **DACT** will be considered as a common mechanism group for purposes of a cumulative risk assessment and as part of the tolerance reassessment process for triazine pesticides.

The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity

I. Introduction

A. Background

The Food Quality Protection Act (FQPA) amended the laws under which EPA evaluates the safety of pesticide residues in food. Among other types of information EPA is to weigh when making safety decisions, the amendments direct EPA to consider "available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity." Sec. 408(b)(2)(D)(v) of the Federal Food Drug and Cosmetic Act. FQPA also directs EPA to apply the new safety standard to tolerances established prior to the passage of FQPA. Further, in carrying out the tolerance reassessment provisions of FQPA, EPA "shall give priority to review of the tolerances or exemptions that appear to pose the greatest risk to public health." Sec. 408(q)(2).

B. Purpose

The purpose of this document is to evaluate whether there is a common mechanism for the triazine pesticides or between the triazine pesticides and other pesticides or metabolites containing a s-triazine ring. OPP used a weight-of-evidence (WOE) approach that considered all pertinent information to determine whether triazine pesticides act via a common mechanism of toxicity. A stepwise process is outlined in the 1999 Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity (http://www.epa.gov/oppfead1/trac/science/#common). The process starts with an initial grouping of chemicals based on having shared structural, toxicological and/or pesticidal properties (US EPA, 1999a). In a second phase, the steps that define the mechanism of toxicity for one or more chemicals in the group are identified. Finally, structural, toxicological and pharmacokinetic/pharmacodynamic data for the remaining chemicals in the group are examined to determine by WOE which of these possess the same mechanism of toxicity as the other compound(s) in the group. All those chemicals found to share the same mechanism of toxicity for a common toxic effect are considered to have been grouped by a common mechanism of toxicity.

It should be noted that since the passage of the FQPA, the term "mechanism of toxicity" has taken on a specific meaning in Agency-wide guidance documents. In the draft EPA guidelines for carcinogen risk assessment, the term "mode of action" is contrasted with "mechanism" which implies a more detailed molecular description of events than is meant by mode of action (US EPA, 1999b). The definition of "mechanism of toxicity", as implemented under FQPA, and thus used in OPP's common mechanism guidance (US EPA, 1999a), is equivalent to the definition of the term "mode of

action." Thus, "mechanism of toxicity" in this document is defined as "*the major steps leading to an adverse health effect following interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction (with biological targets) that are required in order to describe a mechanism of toxicity."*

II. The Candidate Group of Pesticides

A. The Triazines

The term “the triazines” has traditionally been used by EPA to refer to a group of 3 pesticides, atrazine, simazine, and cyanazine. See "Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review," 59 FR 60412 (November 23, 1994). OPP labeled a slightly larger group of pesticides as 1,3,5-triazines in the schedule for tolerance reassessment. In addition to atrazine, simazine, and cyanazine, pesticides so labeled included propazine, ametryn, cyromazine and prometryn. Additionally, several other pesticides or pesticide metabolites contain a s-triazine ring.

The triazines, the 1,3,5- triazines, and other pesticides or metabolites containing a s-triazine ring are derivatives of the s-triazine moiety, manufactured by the reaction of trichlorocyanuric acid (Figure 1) with appropriate intermediates. Two of the three chlorine atoms on cyanuric acid are reactive and easily replaced with other groups to yield a variety of herbicidally active compounds. The third chlorine atom may remain (e.g., in position 2) or be replaced with a methylthio or methoxy group.

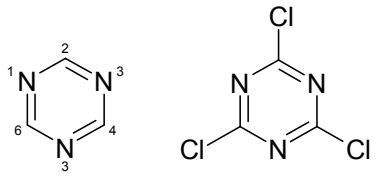


Figure 1. s-Triazine Ring (left) illustrating the ring-numbering convention and 2,4,6-trichlorocyanuric acid (right).

These compounds can be subdivided into several classes, depending on the side chain substitution: the s-triazine herbicides, the sulfonyl urea herbicides with an s-triazine moiety, and miscellaneous s-triazine herbicides. As depicted in Table 1, the s-triazine herbicides may be further subdivided by the presence of a chlorine, a methylthio, or a methoxy group in position 2 of the ring. In particular, the chloro-s-triazines comprise atrazine, simazine, propazine, terbutylazine, and cyanazine. This latter compound differs from the other 2-chloro-s-triazines by the presence of a cyano (CN) group. The methylthio s-triazines comprise ametryn, prometryn and terbutryn. The methoxy-s-triazines include prometon and terbumeton.

As depicted in Table 2, the sulfonyl urea herbicides with an s-triazine moiety comprise metsulfuron methyl, trisulfuron, chlorsulfuron, tribenuron methyl (Express), and DPX-M6316 (Harmony). These compounds differ from the s-triazine herbicides in having a bulky sulfonylurea group attached to carbon 2 of the triazine ring and in possessing an s-triazine ring with only one amino group

attached to it.

Other s-triazine pesticidal compounds considered include cyromazine, melamine, hexamethylmelamine, anilazine, and s-triazines with an aziridine or 5-nitrofuryl moiety.

B. Selection of the Candidate Group

As outlined in the 1999 Common Mechanism Guidance document (US EPA, 1999a), the stepwise process of selecting a candidate group of chemicals starts with an initial grouping of chemicals selected based on having shared structural, toxicological and/or pesticidal properties. HED first examined the triazine pesticides, atrazine, simazine, and cyanazine, for inclusion in the initial common mechanism group. That examination showed that these pesticides shared both structural characteristics and toxicological endpoints. Structurally, all three pesticides contain the s-triazine moiety. Toxicologically, the pesticides are positive for mammary gland tumors in Sprague-Dawley (SD) rats, and atrazine and simazine have data suggesting that they interfere with the LH ovulatory surge.

HED then examined whether any other 1,3,5-triazines or other pesticides or metabolites containing s-triazine rings shared these characteristics. As shown in Table 1, four of the five 2-chloro-s-triazines, atrazine, simazine, propazine, and cyanazine, as well as metabolite DACT (Table 3), are positive for mammary gland tumors in female SD rats. Terbutylazine produced mammary gland tumors in Tif:RAlf female rats in addition to benign testicular tumors in males. Although 2-hydroxyatrazine does not produce mammary gland tumors, it has been found to produce some reproductive developmental effects consistent with atrazine.

Terbutryn produced mammary gland tumors (CR:CD rats), and, in addition, produced statistically significant increases in combined benign and malignant tumors in testis, thyroid, and liver (Table 1). Prometryn and ametryn were negative for rodent mammary gland tumors.

Among the methoxy s-triazines shown in Table 1, terbumeton was positive for mammary gland tumors, but it is not marketed in the United States. Prometon was negative for oncogenicity in rodents.

As shown in Table 2, only one sulfonyl urea herbicide (Express) produced statistically significant incidences of benign and combined benign/malignant mammary gland tumors in female SD rats. The effect was seen at a considerably higher dose (1250 ppm) compared to that observed for other s-triazines and no other tumors were observed. Because metabolism data for Express indicate that cleaved s-triazine products are seen in tissues and excreta of dosed female rats, the sulfonyl urea herbicide is included in the candidate group, based on its capacity to produce mammary gland tumors in female SD rats and evidence that a triazine moiety is released during metabolism of the

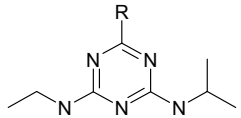
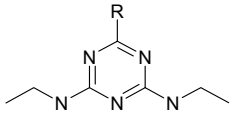
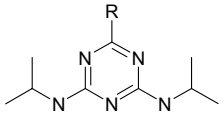
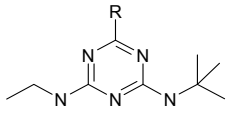
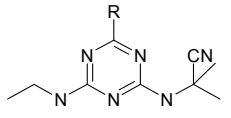
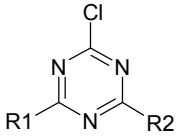
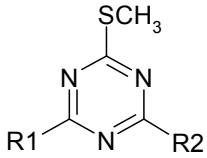
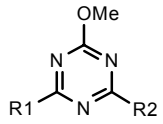
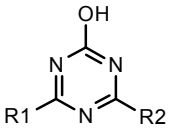
parent compound. No rodent mammary gland tumors were seen for the other four listed sulfonyl urea herbicides, metsulfuronmethyl (SD rats, up to 5000 ppm), trisulfuron (SD rats, up to 6000 ppm), chlorsulfuron (up to 2500 ppm), and harmony (CD rats, up to 2500 ppm).

Based upon OPP's review of the available toxicity information, a subset of eight pesticides containing the triazine moiety – atrazine, simazine, propazine, cyanazine, terbutylazine, terbumeton, terbutryn, tribenuron methyl (Express) – were found to cause the similar toxic effect of inducing mammary gland tumors only in female rats but not male rats or both sexes of mice. The other triazine-containing pesticides – ametryn, prometryn, prometon, metsulfuron methyl, trisulfuron, chlorsulfuron, DPX-M6316 (Harmony), cyromazine, melamine, hexamethylmelamine, anilazine, and s-triazines with an aziridine or 5-nitrofuryl moiety– do not cause that carcinogenic profile or their structures contain moieties that have a confounding effect as to their mechanism of toxicity, and there is no known mechanism of toxicity that would support grouping them by a common mechanism with atrazine, simazine, and cyanazine.

Further, only four of the subset of eight triazine-containing pesticides– atrazine, propazine, simazine, and Express – have uses that result in exposure to the general public; the other four– terbutylazine, terburmeton, cyanazine, and terbutryn – do not have tolerances and either are not registered or have registrations that do not involve exposure to the general public.

Thus, as shown in Table 3, the compounds being considered in making a determination about grouping pesticides via a common mechanism of toxicity are **atrazine, simazine, propazine, tribenuron methyl (Express)** and metabolites, **2-hydroxyatrazine, DACT, DEA, and DIA**, given their structures, ability to induce mammary gland tumors in female SD rats, and/or ability to affect LH-dependent events. This group hereafter will be referred to as the **candidate group**. The metabolites are specifically included because atrazine, simazine, and propazine break down to two or all of them and they are found as residues in drinking water and food. Toxicity data on the chloro-s-triazine metabolites also provide for supporting the common mechanism of toxicity for the parent compounds.

Table 1. SAR and Mammary Gland Tumor Induction by Various s-Triazines Compounds in Rats

					
	Atrazine + Positive for mammary gland tumors at 70 ppm	Simazine + Positive for mammary gland tumors at 100 ppm	Propazine + Positive for mammary gland tumors at 3 ppm	Terbutylazine + Positive for mammary gland tumors at 750 ppm[1]	Cyanazine + Positive for mammary gland tumors at 5 ppm
	Ametryn (–)	NE[3]	Prometryn (–) Negative for oncogenicity up to doses of 3000 ppm	Terbutryn + Positive for mammary gland tumors at 3000 ppm[2]	NE[3]
	NE[3]	NE[3]	Prometon (–) Negative for oncogenicity up to doses of 1000 ppm	Terbumeton +	NE[3]
	OH-Atrazine (–)	NE[3]	NE[3]	NE[3]	NE[3]

[1] Dose considered to be excessive, EPA Classification of D (not classifiable to human carcinogenicity). Study used Tif:RAlf rats . Benign interstitial cell tumors of the testes were also observed.

[2] Also statistically significant ($p \leq 0.03$) increase in combined benign and malignant tumors in testis, thyroid, and liver. [3] NE = not evaluated

Table 2. Mammary Gland Tumor Induction by Sulfonylurea Herbicides Containing the s-Triazine Moiety

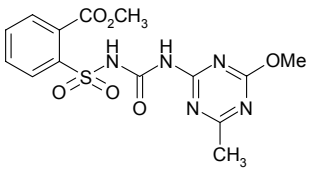
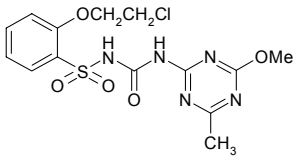
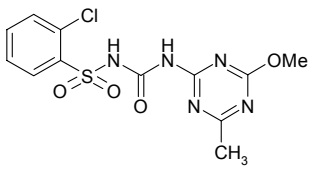
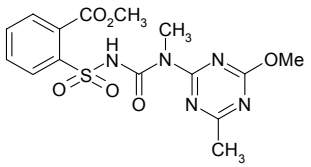
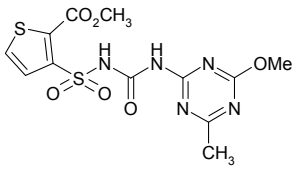
				
Metsulfuronmethyl (–)	Trisulfuron (–)	Chlorsulfuron (–)	DPX-L5300 (Express) + Positive for oncogenicity at 1250 ppm	DPX-M6316 (Harmony) (–)

Table 3. Structures of the Compounds in the Candidate Group

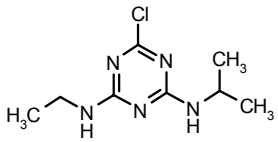
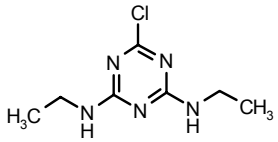
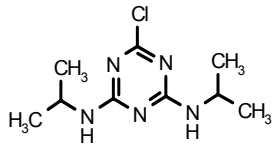
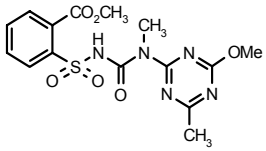
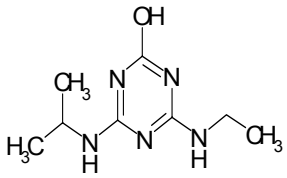
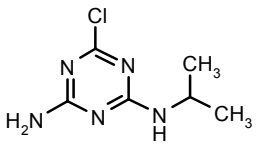
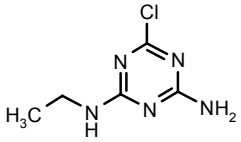
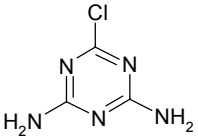
Compound	Structure	CAS No.	PC Code
Atrazine		1912-24-9	080803
Simazine		122-34-9	080807
Propazine		139-40-2	080808
Tribenuron-methyl (Express)		101200-48-0	128887
2-Hydroxyatrazine		2163-68-0	—

Table 3. Structures of the Compounds in the Candidate Group (Continued)

Compound	Structure	CAS No.	PC Code
Desethyl Atrazine (DEA)		6190-65-4	-
Desisopropyl Atrazine (DIA)		1007-28-9	-
Diaminochlorotriazine (DACT)		3397-62-4	-

III. Mechanism of Toxicity

This section describes the proposed mechanism for the common toxic endpoints by which the compounds containing the s-triazine moiety might be grouped. The triazine compound atrazine has been used as a prototype for defining the toxic effects of triazines and elucidating the mechanism of toxicity associated with these effects, given that it is the most extensively studied triazine. Multiple studies on the effects of atrazine have been published in open literature and conducted by registrants and EPA's National Health and Environmental Effects Research Laboratory (NHEERL). These studies demonstrate that the most relevant endpoint selected for intermediate-term and chronic risk assessments is a neuroendocrine effect exemplified in female rats by attenuation of the luteinizing hormone (LH) surge and accompanying disruption of the estrous cycle. In depth reviews and discussions of these studies may be found in the following documents:

1) Revised Preliminary Human Health Risk Assessment (http://www.epa.gov/pesticides/reregistration/atrazine/ revsd_pra.pdf) (US EPA, 2001a).

2) Atrazine: Toxicology Chapter of the Reregistration Eligibility Decision. REVISED, (http://www.epa.gov/pesticides/ reregistration/atrazine/tox_chapter.pdf) (US EPA, 2001b).

3) Hazard and Dose-Response Assessment and Characterization of Atrazine (Part A), Hazard Assessment and Review of Available Studies (Part B), and References, http://www.epa.gov/ scipoly/sap/2000/june27/ finalparta_atz.pdf and http://www.epa.gov/scipoly/sap/2000/june27/ finalpartb_atz.pdf (US EPA, 2000a; US EPA 2000b).

4) SAP Report No. 2000-05, Atrazine: Hazard and Dose-Response Assessment and Characterization, http://www.epa.gov/scipoly/sap/ 2000/ june27/ finalpartc_atz.pdf (US EPA, 2000c).

A few of the more recent and pertinent studies are reviewed in this document in order to establish this neuroendocrine effect as relevant to a toxic effect common among several of the compounds containing the s-triazine moiety and their metabolites, and to establish its mode of action. However, the reader is referred to the citations above for more extensive reviews.

The carcinogenic effects of atrazine have been clearly demonstrated. The earliest published study documenting these effects showed that there were dose-related increases in the incidence and/or early onset of mammary gland tumors (adenomas, adenocarcinomas, and carcinosarcomas combined) in female Sprague-Dawley (SD) rats in a 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 gland adenomas/carcinomas. Less compelling evidence suggests that there is decreased latency for the formation of mammary gland fibroadenomas and pituitary adenomas (Thakur, 1991a and 1992a; Pettersen and Turnier, 1995) and an increased incidence of mammary gland 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 gland 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 gland tumors (Thakur, 1992b). The closely related structural analogues to atrazine (i.e., simazine, propazine, and cyanazine) also produce mammary gland 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.

As a result of the above-mentioned studies with atrazine, a central nervous system (CNS) mechanism of toxicity has been proposed for the increased incidence of mammary gland tumors. It is hypothesized that the carcinogenicity of atrazine is a consequence of the disruption of the normal secretory activity of the hypothalamic-pituitary-ovarian axis. Figure 2 illustrates the proposed mode of action of atrazine in female SD rats on the activity of the hypothalamic-pituitary-ovarian axis and the development of mammary gland and to some extent pituitary neoplasms. As depicted in Figure 2, atrazine exposure affects the hypothalamus, leading to a decreased secretion of hypothalamic norepinephrine (NE) (Cooper 1998). Decreased NE levels result in decreased release of gonadotropin releasing hormone (GnRH) from the hypothalamus (Cooper, 1998). GnRH is the hormone responsible for inducing the pituitary gland to release luteinizing hormone (LH). Thus, a decreased GnRH level leads to an attenuated LH release (Cooper *et al*., 1995, 1996, 2000; Morseth, 1996a, b). LH normally provides a signal to the ovaries promoting ovulation, but under atrazine's exposure serum LH levels are insufficient to stimulate ovulation. Under the tonic secretion of LH and follicle-stimulating hormone (FSH), this feedback mechanism eventually causes the ovarian follicles to continue to secrete estradiol, which in turn leads to the hypertrophy of pituitary lactotrophs and consequently the increase in prolactin secretion. In concert with prolactin, estrogen acts on the mammary gland and increases the risk for mammary gland tumors.

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 estrus. 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*., 1993). Removal of the estrogen stimulus by ovariectomy completely abolishes the formation of mammary gland 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, 1995).

It should be noted, however, that the proposed carcinogenic mode of action for atrazine in rats is not likely to be relevant to humans. As summarized by the FIFRA Scientific Advisory Panel (SAP), "there are considerable differences between hypothalamic-pituitary-ovarian function in rats and humans, and the effects of aging on the function of the axis also is quite dissimilar. Therefore, it is unlikely that the mechanism by which atrazine induces mammary gland tumors in female SD rats could be operational in humans. Nevertheless, it is not unreasonable to expect that atrazine might cause adverse effects on hypothalamic-pituitary function in humans" (US EPA, 2000c). Although the cancer mode of action may not be operative in humans, the SAP went on further to state that "the same endocrine perturbations that induce tumors also appear to play a role in at least some reproductive developmental effects", which may be relevant to humans (See Figure 2).

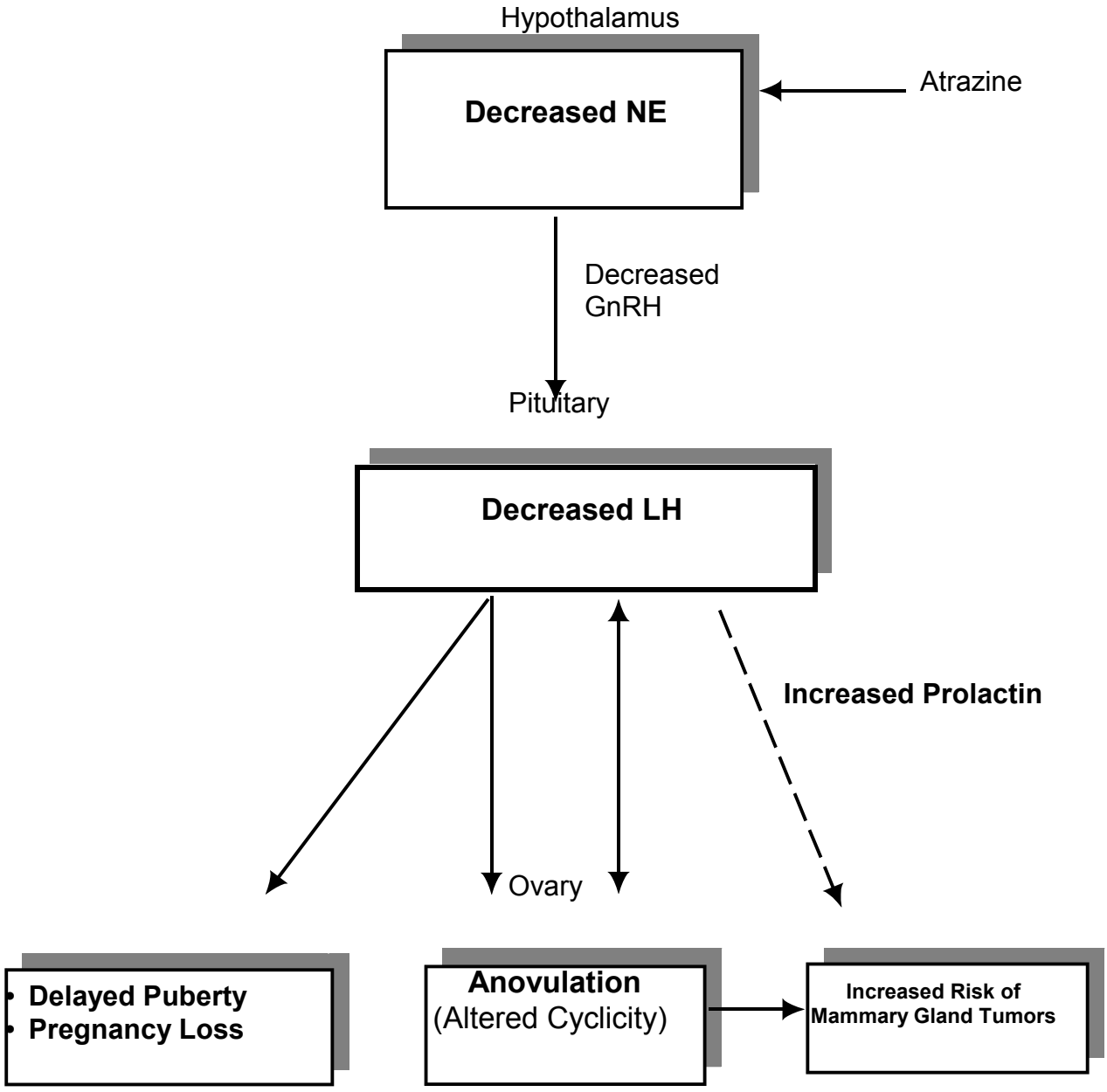
In addition to the disruption of the estrous cycle, the suppression of LH by atrazine has been found to be accompanied by adverse reproductive functions (discussed later in Section IV). Studies designed to evaluate the effect of atrazine on early pregnancy found that atrazine increased pre- and post-implantation loss and delayed parturition in various strains of female rats (Cummings *et al.*, 2000; Narotsky *et al.*, 2001). Further, it has been found that pubertal development is delayed in male and female Wistar rats administered atrazine (Laws *et al.*, 2000; Stoker *et al.* 2000 and in press).

Alternative modes of action for the neuroendocrine effects following exposure to compounds containing the s-triazine moiety have been suggested. Although several studies have found that the estrogenic effects associated with some of the compounds containing the s-triazine moiety *in vivo* are not estrogen receptor-mediated (Tennant *et al.*, 1994a,b; Conner *et al.*, 1996), these effects may be explained partly by their ability to induce aromatase, the enzyme responsible for converting androgens to estrogens. Recent studies demonstrated that atrazine, simazine and propazine, but not metabolites, DEA or DIA, induced aromatase activity in various cell lines (Sanderson *et al.*, 2001, 2000). Further, as raised by Trentacoste (2001) and by one member of the SAP (US EPA 2000c), it has been suggested that the anorexic effects of atrazine could account for most of atrazine's effect on LH since reduced food intake and weight loss is a potent stimulus for reduced LH secretion. However, in pair-fed studies in both males and females, decreased food consumption and body weight could not account for the adverse effects of atrazine on the estrous cycle and pubertal development (Laws *et al*., 2000; Stoker *et al*., 2000) Upon consideration of the SAP comments, OPP's own reviews and the data underlying these reviews, as well as additional information received by the Agency from registrants or presented in open literature, it has been

concluded that the neuroendocrine actions of atrazine are the primary and requisite mode of action for the induction of mammary gland tumors and certain reproductive developmental effects (see Figure 2).

Given the overall consistency and specificity of the evidence for atrazine's proposed mechanism of toxicity, studies on the effects associated with disruption of the hypothalamic-pituitary-gonadal axis have been conducted with the other compounds containing the s-triazine moiety (e.g., propazine, simazine, and certain chloro-s-triazine metabolites). Similar to atrazine, some of these compounds have been found to produce mammary gland tumors only in female SD rats and affect the hormonal control of reproductive functions. The attenuation of the pituitary LH surge and induction of reproductive developmental effects will be the basis upon which a common mechanism of toxicity will be determined for the candidate group, as established in the rest of this document.

Figure 2. Atrazine[1]: Neuroendocrine Mode of Action and Associated Effects Found in Rats.



[1]Atrazine also produced a decrease in pituitary prolactin, which also contributes to effects on reproductive development and affects lactation; NE = norepinephrine; LH=luteinizing hormone; GnRH = gonadotropin releasing hormone.

IV. Lines of Evidence

A. Structure Activity Considerations

In general, based on structure-activity relationships (SAR), the pesticides may be grouped according to their likelihood to generate a common type of toxic molecule or reactive intermediate or their ability to mimic a common biologically active molecule that interferes with the normal homeostasis of the cell (e.g., via receptor binding, enzyme induction, etc.).

As shown in Table 3, all compounds in the candidate group share an s-triazine ring in their structure. All compounds, except tribenuron methyl (Express) and 2-hydroxyatrazine, have a chlorine atom in the 2-position with alkyl amino groups at the 4- and 6- positions (atrazine, simazine, and propazine) or at either the 4- (DEA) or 6- (DIA) positions only. Diaminochlorotriazine (DACT) is the fully dealkylated triazine of the group. This compound is a common metabolite of atrazine, simazine, and propazine in the rat, and can, like its parental precursors, decrease the intensity of the LH surge in female rats.

Based on structure-activity considerations, it is reasonable to expect that three of the candidate herbicides, atrazine, propazine, and simazine may share common toxic effects, metabolic pathways, and mechanism(s) of action. Atrazine and propazine share a common metabolite, desethyl atrazine (DEA), while atrazine and simazine share desisopropyl atrazine (DIA) as a common metabolite. A further dealkylation of the desethyl- or desisopropyl atrazine yields diaminochlorotriazine (DACT), which is thus common to all three chlorotriazines. Atrazine can also lose its chlorine atom to form 2-hydroxyatrazine; however, as shown in Table 1, this compound does not induce mammary gland tumors in SD female rats.

B. Metabolism and Pharmacokinetics Considerations

Metabolism and pharmacokinetics considerations can play an important role in determining common mechanisms of toxicity in a candidate set of chemicals. Information on the disposition of a chemical helps to elucidate issues of target site dose delivery. The study of the biotransformation of the chemicals can determine if a putative common toxic metabolite or its precursor are produced.

As discussed below, the candidate group compounds have many metabolic similarities, as well as some differences.

1. Absorption

Absorption of the candidate group herbicides after oral dosing, as measured indirectly in laboratory studies, is significant and may impact the potential human dose from water and food exposures. Measurement of excretion of radioactivity in urine of rats (an approximate measure of absorption) for (14)-C-labeled atrazine demonstrated 67% of the dose was excreted through the urine (Timchalk *et al.*, 1990). The urinary excretion profiles for the some of the candidate group compounds are listed in Table 4.

The percentage of administered triazine dose excreted is similar for atrazine and propazine (Table 4). Excretion in urine for simazine was slightly smaller, 49.3% of the dose, and that of 2-hydroxyatrazine was slightly higher. Since Table 4 compares data from studies conducted under different protocols, it is difficult to assess the significance of differences in excretion times and profiles; however all of them are consistent with extensive absorption of the test material by the oral route.

Oral administration of the sulfonyl urea Express to Crl:CD:BR rats resulted in urinary excretion of over 60% of the dose.

Table 4. Urinary Excretion for (14)-C -s-Triazines by Orally Dosed Rats

Compound	Oral Dose	% Dose Excreted	Reference
Atrazine	~ 1.5 mg/kg	65.5 (72 hrs)	Bakke et al. (1972)
Atrazine	30 mg/kg	67 (72 hrs.)	Timchalk *et al*. (1990)
Atrazine	Unspecified	65 (72 hrs.)	Trochimowitz *et al*., (1994)
Simazine	1.5 mg/kg	49.3 (96 hrs.)	Simoneaux and Sy (1971)
Propazine	1.0 mg/kg	69.5♂ /68.8♀ (7d)	Krautter (1995)
Propazine	41-56 mg/kg	66 (72 hrs.)	Bakke *et al*. (1967)
2-OH - Atrazine	~1.5 mg/kg	78 (72 hrs.)	Bakke et al. (1972)

2. Tissue Distribution

Tissue residue analysis in rats dosed with radiolabelled atrazine, simazine or propazine indicate extensive tissue distribution of radioactivity from these compounds to sites, including the brain. Paul et al. (1993) administered a single oral dose of ^{14}C -atrazine (1 mg/kg) to male SD rats. At 24 hours after dosing, percent of dose in heart, lungs, brain, liver,

and testes amounted to 0.22, 0.35, 0.49, 3.9, and 0.63 % of the dose, respectively. Radioautography of rats treated with a single oral dose of ^{14}C -atrazine (100 mg/kg) showed extensive distribution of label throughout the body, including the brain and adjacent tissues. Orr and Simoneaux (1986) administered a single oral dose of ^{14}C -simazine (0.5 mg/kg) to CD rats of both sexes. At 7 days after dosing, percent of dose in heart, lungs, brain, liver and uterus in females amounted to 0.04, 0.05, 0.10, 1.26, and 0.01 % of the dose, respectively. Corresponding values for males were 0.04, 0.06, 0.09, 1.08, and 0.01 (for testes), respectively. Bakke et al. (1967) administered a single oral dose of ^{14}C -propazine (~ 49 mg/kg) to male SD rats. At 2 days after dosing tissue residues in heart, lung, brain, liver and spleen amounted to 51, 51, 34, 52 and 47 ppm (as propazine equivalents), respectively.

3. Biotransformation

All of the candidate group compounds undergo extensive biotransformation in rats. As summarized below, numerous metabolites have been detected in both rat and human urine, many of which are the same. As illustrated for atrazine (Figure 3), the main biotransformation pathways for the chloro-s-triazines in rats are N-dealkylation by the hepatic cytochrome P450 system, and glutathione conjugation of either the parent or the N-dealkylated metabolite to the ultimately excreted mercapturic acid conjugate (Figure 3). Express, a sulfonylurea triazine, likewise undergoes extensive biotransformation.

a. Atrazine

The N-dealkylated urinary metabolites of atrazine in rats were quantitated by Bradway and Moseman (1982). As specified in Table 5, the major metabolite was diaminochlorotriazine (DACT). The minor metabolites, (desisopropyl s-triazine (DIA) and desethyl s-triazine (DEA), were detected in the higher dose groups.

Rat metabolism of atrazine was also studied by Timchalk *et al.* (1990). Fischer 344 rats were given a single oral dose of 30 mg (14)C-labeled atrazine per kg of body weight. The atrazine was quickly metabolized as the urine excreted within 24 hours of the dosing contained approximately 57% of the administered radioactivity. As shown in Table 5, the major urinary metabolite was DACT. The other reported urinary metabolites were DACT-mercapturate, DIA, DEA, and DEA-mercapturate. These metabolites were identified based upon similar HPLC retention times as synthesized standards (Timchalk *et al.*, 1990). Paul *et al.* (1993) found levels of DACT up to 25% of the dose in rats (Table 5).

As shown in Figure 4, atrazine, simazine, and propazine share N-dealkylation metabolic pathways and thus these three compounds have the metabolite diaminochlorotriazine (DACT) in common. As will be discussed later, DACT causes a decrease in the LH surge in SD female rats and produces effects on reproduction and development.

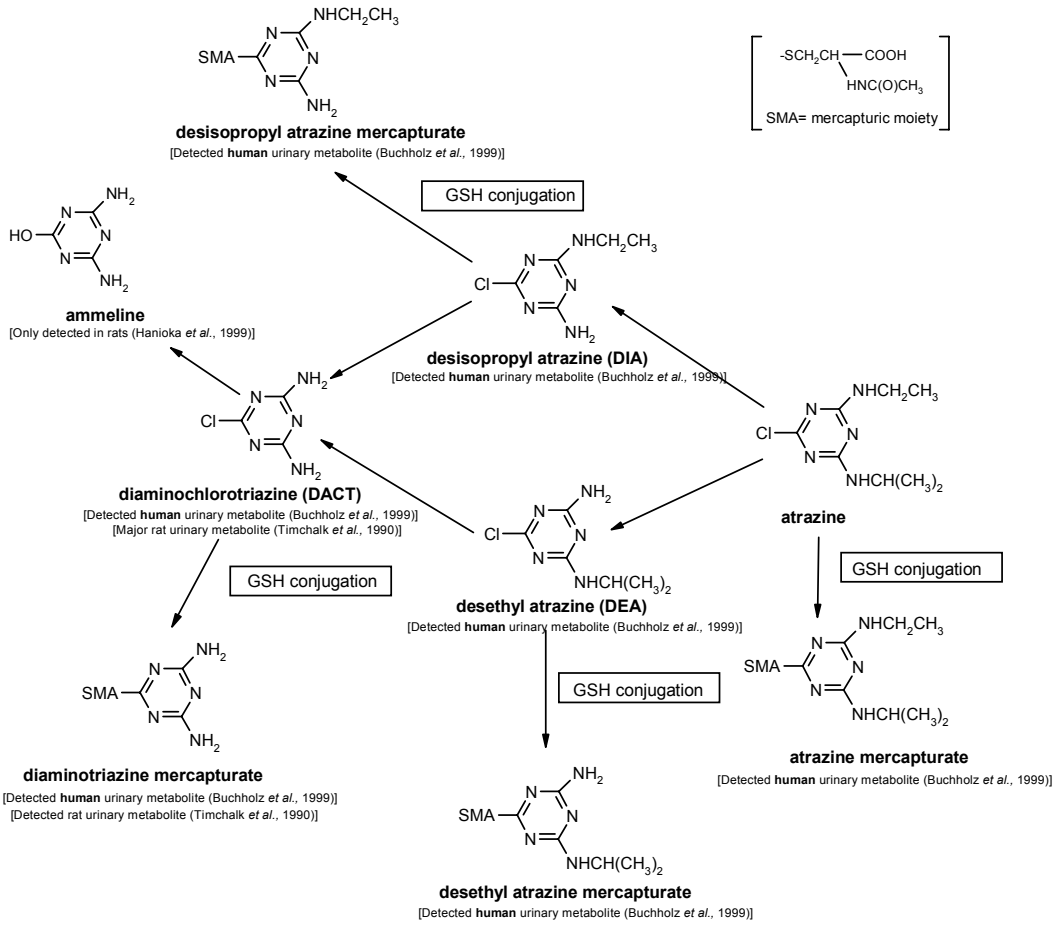


Figure 3. Biotransformation for Atrazine [Adapted from Buchholz *et al*. 1999, Hanioka *et al*. 1999, and Timchalk *et al*. 1990].

Table 5. Relative Percentage(s) of (14)-C Atrazine[2] Urinary Metabolites

Reference	Species	Route	Dose Level	Metabolite(s)[1]	% Administered Dose Excreted in Urine as Metabolite(s)	Notes
Bradway and Moseman, 1982	Rat Males F344	Oral, Single Dose	170 mg/kg	DACT DIA & DEA	Not Measured 3.7%	Results not reported separately for DIA & DEA. DACT not studied.
			17 mg/kg	DACT DIA & DEA	Not Measured 0.3%	
			1.7 mg/kg	DACT DIA & DEA	Not Measured Not Detected	
Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.2% (day 1), 31.9% (day 3)	No DACT recovered at doses of 0.17 & 0.017 mg/kg/day
			1.7 mg/kg/day	DACT	2.9 % (day 1) 4.3 % (day 3)	
Paul et al. 1993 (MRID 44713802)	Rat Males SD	Oral, Single dose	1 mg/kg	DACT DACT-mercapturate DIA DEA	25.8 % 1.1% 0.2 % 0.07 %	
			100 mg/kg	DACT DACT-mercapturate DIA DEA	14.2 % 2.5% 0.8 % 0.2 %	
Timchalk *et al.*, 1990	Rat Males SD	Oral Single dose	30 mg/kg	DACT DACT-mercapturate DIA DEA-mercapturate DEA	39% (67%)[3] 5% (9%)[3] <0.6% (<1%)[3] 8% (13%)[3] 3% (5%)[3]	Data from urine extracts from 0 to 24 hours after dose

[1] See Figures 3 and 4 for structural identity of metabolites
[2] Bradway and Moseman used non-radiolabelled atrazine
[3] Percentage of total urinary radioactivity

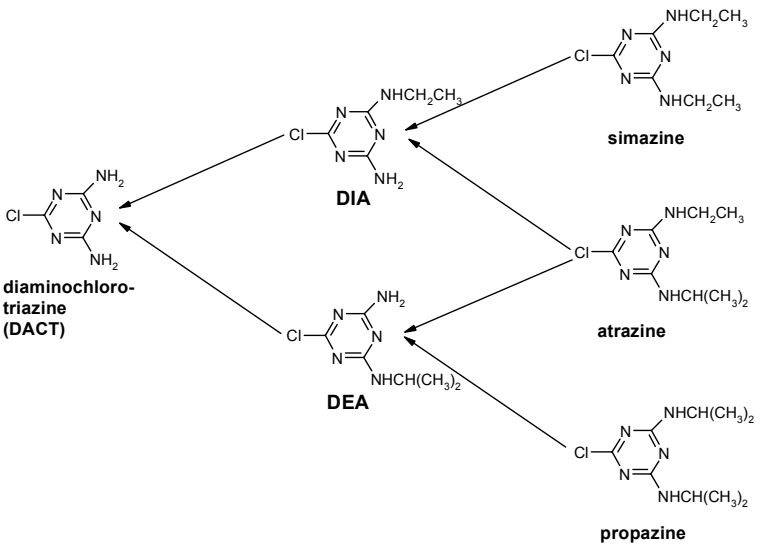


Figure 4. Dealkylation of Simazine, Atrazine, and Propazine

b. Propazine

As summarized in Table 6, the dealkylated urinary metabolites of propazine in rats were quantitated by Bradway and Moseman (1982). The major metabolite was DACT; and a second metabolite, DEA, was detected in the higher dose groups (Bradway and Moseman, 1982).

c. Simazine

The dealkylated urinary metabolites of simazine in rats were quantitated by Bradway and Moseman (1982). As specified in Table 6, the major metabolite was DACT. A second metabolite, DIA, was detected at a lower level (Bradway and Moseman, 1982).

Table 6. Relative Percentage(s) of Propazine and Simazine Urinary Metabolites

Compound	Reference	Species	Route	Dose Level	Metabolite(s)[1]	% Administered Dose Excreted in Urine as Metabolite(s)
Propazine	Krautter, 1995 (MRID 43689801)	Rat Male & Female SD	Oral Single Dose	1 mg/kg	DACT 2-OH-DEA DEA	28.8 % ♂ , 26.9 % ♀ 2.6% ♂ , ND ND ♂ , 0.7% ♀
				100 mg/kg	DACT 2-OH-DEA DEA	28.2 % ♂ , 19.9 % ♀ ND ♂, ND ♀ 0.9% ♂, ND ♀
Propazine	Bradway and Moseman, 1982	Rat Males F344	Oral Single dose	170 mg/kg	DACT DEA	Not Measured 0.5%
				17 mg/kg	DACT DEA	Not Measured 0.08%
Propazine	Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.3% (day 1) 9.6% (day 3)
				1.7 mg/kg/day	DACT	0.34% (day 1) 17% (day 3)
Simazine	Bradway and Moseman, 1982	Rat Males F344	Oral Single dose	170 mg/kg	DACT DIA	Not Measured 2.8%
				17 mg/kg	DACT DIA	Not Measured 0.5%
				1.7 mg/kg	DACT DIA	Not Measured 0.4%
Simazine	Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.9% (day 1) 18.2% (day 3)
				1.7 mg/kg/day	DACT	1.4% (day 1) 1.6% (day3)

[1] See Figures 3 and 4 for structural identity of metabolites

d. Tribenuron methyl (Express)

As shown in Figure 5, Express undergoes extensive biotransformation in rats. In Crl:CD:BR rats dosed orally with ^{14}C-triazine-ring labeled Express recoveries of O-demethyl triazine amine, N-demethyl triazine amine and triazine amine in female urine amounted to 12.2, 3.4 and 3.4 percent of the dose. These values suggest extensive metabolic release of the triazine moiety in the dosed animals.

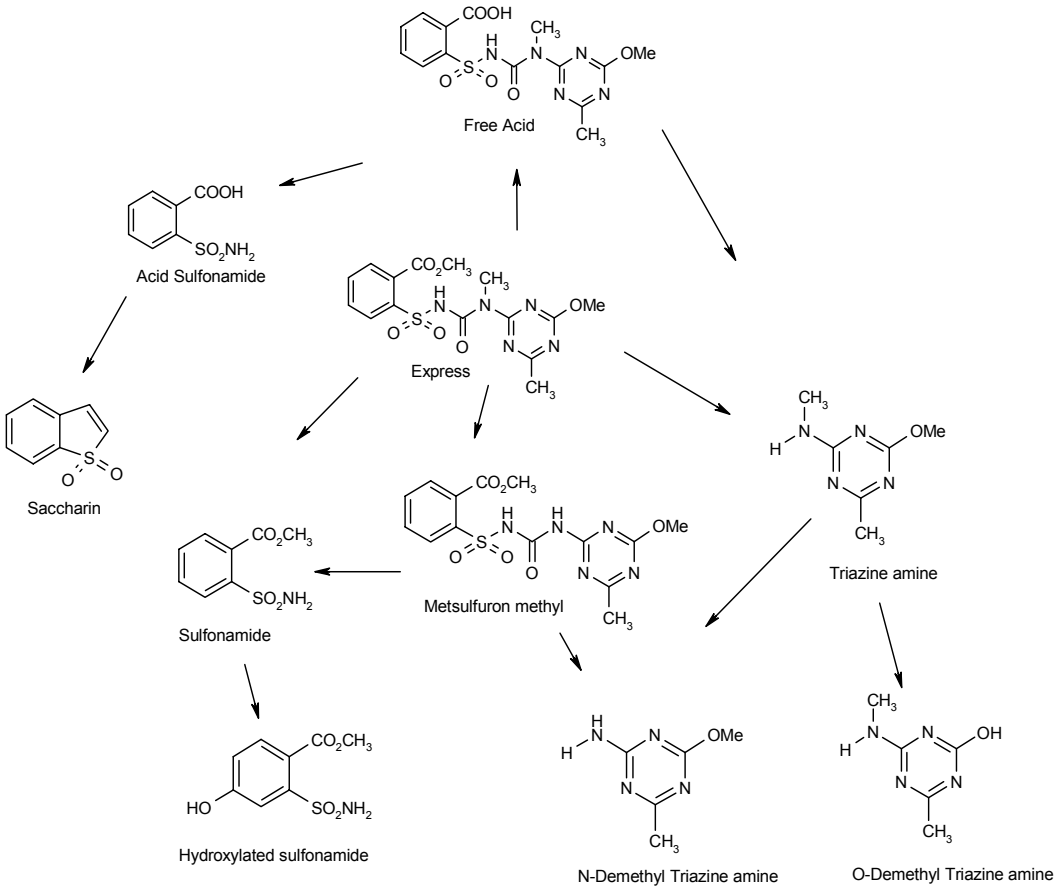


Figure 5. Biotransformation of Express in Rats (Adapted from MRID 40245516)

4. Summary

The previous sections describe the absorption, biotransformation and excretion profiles for the candidate compounds and their metabolites. All of the candidate group compounds are significantly absorbed by rats when administered orally. The main biotransformation pathways identified through excretion profiles involve hepatic cytochrome P450-mediated N-dealkylation and glutathione conjugation of either the dechlorinated or the N-dealkylated metabolite. Biotransformation of atrazine, simazine, and propazine, but not Express, results in the common metabolites DACT and/or DIA and DEA.

C. Toxicological Considerations

The identification of a candidate group of chemicals for a cumulative risk assessment involves an evaluation of effects that may be common to the group of chemicals under review. Following is a discussion of the relevant types of effects reported to be induced by treatment of laboratory animals with compounds containing a triazine moiety and an evaluation of the extent to which the effects are common to this group of chemicals. Hematological and cardiac effects following exposure to compounds containing a triazine moiety are not consistent and not associated with disruption of the hypothalamic-pituitary-gonadal axis, and therefore are not included in this discussion. Furthermore, developmental effects (e.g., incomplete or delayed ossification) are not discussed because there are no data that would suggest that the delays in ossification in fetal animals are due to disruption of the hypothalamic-pituitary-gonadal axis or any other common mechanism by the candidate group compounds. Although the disruption of the hypothalamic-pituitary-axis is plausible in humans, data from human studies are insufficient to rely on for this common mechanism assessment. Thus, this analysis only utilizes data from laboratory animals as a basis of grouping these compounds by a common mechanism of toxicity. The effects likely related to neuroendocrine disruption observed in *in vivo* studies with the candidate group compounds are summarized in Table 7 and discussed below.

Table 7. Neuroendocrine Effects Following Exposure to the Candidate Group

Toxic Effects	Atrazine	Simazine	Propazine	Express	HA[1]	DACT	DIA	DEA
Carcinogenicity								
Increased incidence of mammary gland tumors	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	no data	no data
Reproductive Developmental and Neuroendocrine								
Disruption of the estrous cycle	**Yes**	no data	no data	**Yes**	no data	**Yes**	no data	no data
Attenuation of LH surge	**Yes**	**Yes**	**Yes**	no data	no data	**Yes**	no data	no data
Attenuation of prolactin surge	**Yes**	no data	no data	no data	no data	no data	no data	no data
Delayed vaginal opening	**Yes**	no data	**Yes**	no data	**No**	**Yes**	no data	no data
Altered pregnancy outcome	**Yes**	no data	no data	no data	**Yes**	**Yes**	**Yes**	**Yes**
Delayed preputial separation	**Yes**	no data	no data	no data	**Yes**	**Yes**	**Yes**	**Yes**
Decreased testosterone	**Yes**	no data	no data	no data	no data	**no effect**	**Yes**	**no effect**
Decreased prostate weight	**Yes**	no data	no data	no data	no data	**Yes**	**Yes**	**Yes**
Prostatitis in offspring	**Yes**	no data	no data	no data	no data	no data	no data	no data
Increase or decrease in testes weight	**Yes**	**Yes**	**Yes**	no data	no data	**no effect**	**Yes**	**Yes**

[1] HA = 2-hydroxyatrazine

1. Carcinogenic Effects

Studies submitted to OPP report that mammary gland tumors in female rats are a characteristic common effect in studies conducted with candidate group compounds. The studies provide evidence that administration of these compounds to female SD rats leads to increased incidence and/or early onset of benign and mammary gland carcinomas and adenomas, mammary gland fibroadenomas, and pituitary adenomas (Table 8). As discussed earlier, mammary gland tumors are not likely to be relevant to humans. However, because mammary gland tumors are associated with attenuation of the LH surge they are an indicator of the common mechanism of toxicity.

As shown in Table 8, the carcinogenicity of atrazine in the female Sprague-Dawley (SD) rat has been confirmed in several two-year bioassays. These studies show that atrazine exposure results in an increased incidence and an early onset of mammary gland tumors in female SD rats (Mayhew *et al.*, 1986; Thakur, 1991a, 1992a; Pettersen and Turnier, 1995). However, no tumor response is seen in SD male rats, Fischer 344 rats, or CD-1 mice of either sex (Hazelette and Green, 1987; Thakur, 1991b; Thakur, 1992b). The lowest dose of atrazine associated with an increased incidence in mammary gland carcinomas is 3.5 mg/kg/day (Mayhew *et al*., 1986).

Similar findings are also seen with simazine and propazine. In a combined chronic/carcinogenicity study, simazine at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (45.8 mg/kg/day) resulted in a statistically significant dose-related trend in mammary gland carcinomas (McCormick *et al*, 1988). A higher incidence of mammary gland carcinomas was also seen in the recovery substudy (52 weeks of treatment with 1000 ppm followed by 52 weeks of recovery) for the control and high dose group (1/10 vs 4/10, respectively). Propazine at low and high doses was found to increase the incidence of mammary gland tumors in female SD rats following the administration of propazine in the diet at 0, 3, 100, or 1000 ppm for 2 years (Jessup, 1980).

In a chronic/carcinogenicity study Express was fed to SD rats in the diet at 0, 25, 250, and 1250 ppm for 2 years (Tobia, 1987). A statistically significant increase in mammary gland adenocarcinomas was observed in the highest dose group (1250 ppm) after two years of Express exposure. The LOAEL for this study was 250 ppm, based on body weight gain, and the NOAEL was 25 ppm.

There have been no studies submitted to the Agency on the carcinogenicity of the triazine metabolites DEA and DIA. Recent data submitted to OPP in a draft report indicate that DACT at 200 ppm increased the incidence of mammary gland tumors in female SD rats

(Minnema, 2002). There were no effects of DACT on mammary gland tumor incidence at feeding levels equal to or less than 25, 50, or 70 ppm. There was no increase above control levels in the incidence of mammary gland tumors or tumors of any type in a two-year chronic/carcinogenicity study on 2-hydroxyatrazine (Chow and Hart, 1995).

Table 8. Summary of Female Mammary Gland Tumor Incidence/Onset in Chronic Rat Bioassays Using Candidate Group Compounds

Study	Species/ Strain	Duration	Mammary Gland Tumor Incidence	Mammary Gland Tumor Onset
Mayhew *et al.*, 1986	Rat/SD	2 year (atrazine)	Statistically-significant increase in female carcinomas at 3.5 mg/kg/day when adjusted for survival	Not determined in this study
Thakur, 1991a	Rat/SD	2- year with serial sacrifices (atrazine)	A significant positive trend for fibroadenomas is seen.	The percentage of carcinomas occurring in the first year of the study was 0 in controls, 33% at 4.23 mg/kg/day, and 50% at 26.23 mg/kg/day.
Thakur, 1992a	Rat/SD	2- year (atrazine)	No statistically-significant increases in female fibroadenomas or carcinomas seen at either 3.79 or 24.01 mg/kg/day	The percentage of carcinomas and adenomas occurring in the first year of the study in controls was 0% while at 3.79 mg/kg and 23.01 mg/kg/day 27.3 and 33.3% of the carcinomas appeared in the first year of the study.
Pettersen and Turnier, 1995	Rat/SD	1-year (atrazine)	six carcinomas/adenomas and four fibroadenomas are seen at the 23.9 mg/kg/day group compared to one carcinoma and two fibroadenomas in the control group.	The increased incidence of tumors at one year indicates an earlier onset.
McCormick *et al.*, 1988	Rat/SD	2-year (simazine)	Statistically significant increase in female carcinomas and fibroadenomas at 100 ppm and 1000ppm	A higher incidence of mammary gland carcinomas was observed in the recovery group (52 weeks of treatment, followed by 52 weeks of recovery)
Jessup, 1980	Rat/SD	2-year (propazine)	Mammary gland tumors (adenocarcinomas and adenomas) were increased in low- and high-dose female groups (3 and 1000ppm).	Not altered in propazine exposed animals
Tobia, 1987	Rat/SD	2-year (Express)	Statistically significant increase in mammary gland adenocarcinomas was observed in the highest dose group (1250 ppm)	Not altered in Express exposed animals
Minnema, 2002	Rat/SD	1-year (DACT)	Statistically significant increase in the incidence of mammary gland tumors at 200 ppm.	Not determined in this study

2. Reproductive Developmental and Neuroendocrine Effects

Effects on Females

As shown in Table 9a, the candidate group of compounds have all been found to produce reproductive developmental effects in female rats. Some of these effects include an attenuation of the LH surge and disruption of the estrous cycle. Other effects observed include attenuation of prolactin release, altered pregnancy outcome, and delayed puberty in male and/or female rats. Although Express has been found to act as an estrogen agonist (Cook, 1989), the reproductive developmental effects of atrazine, simazine, and DACT (Tennant *et al.*, 1994a,b; Conner *et al.*, 1996) does not appear to be a result of estrogenic activity. The estrogenic activity of propazine has not been determined.

Evidence for an attenuation of the LH surge following exposure to compounds containing the s-triazine moiety is provided in several studies (Cooper, unpublished data; Minnema, 2001ab; Cooper *et. al.*, 1995,1996, 2000; Cummings *et al*., 2000; Morseth 1996ab). In a recent study submitted to US EPA, Minnema (2001a) compared the effects of simazine, DACT, and atrazine on LH. Simazine, DACT, and atrazine were administered to 20 Sprague-Dawley Crl:CD BR female rats/dose/group by oral gavage at dose levels of 0, 2.5, 5, 40, 200 mg/kg/day once daily for at least 4 weeks. Results showed that all three compounds had similar effects on diminishing the peak LH when the peak for each animal was determined and time axis for the individual animal data was rescaled to zero time. All three compounds at the two highest doses, 40 and 200 mg/kg/day, significantly decreased adjusted peak LH surge. Moreover, unpublished data by US EPA laboratory (Cooper, unpublished data) show that propazine (300.0 mg/kg/day) decreases the LH surge by over 50% of control. However, a pilot study submitted to OPP comparing propazine with atrazine and DACT found that propazine (320.1 mg/kg/day) decreased the peak LH surge to only 78.7% of control, whereas the mean LH peak surges were decreased to 34.5 and 47.6% of control following atrazine and DACT, respectively (Minnema, 2001b).

When the LH surge is analyzed using the maximum increase in plasma LH over baseline level (LHMax), hour at which peak surge of LH occurred (TimeMax), and area under the curve for LH verses time profile (AUC), the effects of the simazine, DACT, and atrazine on LH surge differ. Evaluation of LHMax, TimeMax, and AUC in a 28-day oral gavage study found that simazine at 40 and 200 mg/kg/day and DACT at 200 mg/kg/day significantly decreased LHMax and AUC (Minnema 2001a). Atrazine had no effects on any of the parameters at any dose level. Further, in a one-year chronic study with atrazine, DACT, and

simazine, attenuation of LH surge, as measured by LHMax and AUC, was only observed at the highest dose level (1854 µmoles/kg feed) of DACT (Minnema, 2002). Hence, the use of these parameters as measures of LH surge yield inconsistent data with previous reports and may not represent an accurate measure of the effects of these compounds on LH surge. It should be noted that a recently submitted preliminary report on the effects of atrazine in monkeys on LHMax, rate of rise of LH, AUC, and TimeMax showed inconclusive evidence of atrazine's adverse effects on LH after either 5 or 26 days of treatment due to several confounding factors (e.g., a limited sample size and a higher degree of individual variability in LH measurements than expected) (Parshley, 2001).

The effects of candidate group compounds on reproductive and neuroendocrine functions have been further characterized by cyclicity, pregnancy outcome, and pubertal developmental studies. Atrazine and DACT have been shown to prolong the duration of the estrous cycle at relatively low doses (Morseth, 1996ab; Pettersen *et al.*, 1991). Additionally, Express has been shown to slightly prolong the estrous cycle, in addition to decrease the estrogen-binding affinity of receptors in the uterus and mammary glands, at a very high dose (5000 ppm) (Cook, 1989). It can be expected that, if the LH surge and/or the estrous cycle are affected by exposure to candidate group compounds, then puberty and/or pregnancy may also be affected. In fact, studies conducted by US EPA labs have shown that atrazine at 50, 100, and 200 mg/kg delayed vaginal opening 3.4, 4.5, or greater than 6.8 days and produced irregular cycles in female Wistar rats (Laws *et al.*, 2000). More recently, it was reported that propazine and DACT, but not 2-hydroxyatrazine, delayed vaginal opening by up to 4 and 7 days, respectively (Laws *et al.*, 2002). Although the effects of atrazine and the metabolites (i.e., 2-hydroxyatrazine, DACT, DEA, and DIA) on pregnancy outcome varies considerably based on rat strain, they have been found to induce pre- and post-implantation loss, full litter resorption and delayed parturition (Narotsky *et al.*, 2002, 2001; Cummings *et al.*, 2000). See Table 9a for a summary of these effects.

As previously mentioned, although Express induces mammary gland tumors and appears to affect the ovarian cycle in female rats, limited data suggest that it is an estrogen receptor agonist. In a subchronic study (Cook, 1989), female Crl:CD®BR rats (20/dose level) were fed Express at 0 or 5000 ppm (approximately 390 mg/kg bw/day) for 84 days. At termination, the rats fed 5000 ppm had statistically significantly reduced body weights and body weight gains with respect to controls. Mean relative organ weights for treated rats terminated in estrus were significantly higher than in controls for liver (35% higher), uterus (31%), and ovaries (29%); mean relative organ weights for rats terminated in diestrus were also statistical significantly higher than in

controls for liver and uterus. In addition, the number of rats with a prolonged estrus, number of rats with 2 or more prolonged estrous cycles, and the number of cycles with a prolonged estrus were statistical significantly elevated at 5000 ppm *vs* controls. Furthermore, using radiolabelled thymidine incorporation, it was shown that cell proliferation in the uterus was increased in the rats treated with 5000 ppm. The ability of Express and its metabolites to compete *in vitro* for binding to the estrogen receptors in the uterus was further demonstrated when Express (ester and acid, see Figure 6 for structures) and its metabolites (N-demethyl triazine amine, N-demethyl-6-hydroxymethyl-triazine amine, α-hydroxytriazine amine, sulfonamide urea and metsulfuron methyl; all at 1.0 mM) competed with Diethylestilbestrol (0.125 mM) for binding to the estrogen receptor. None of these competed significantly with R5020 (0.125mM) for binding to the progesterone receptor.

Effects on Males

The candidate group compounds appear to not have a consistent effect on male gonadal weight. Atrazine, simazine, propazine and/or their metabolites, DIA, DACT, and DEA have been shown in different studies to increase, decrease or have no effect at all on testes weight (Mainiero *et al.*, 1987; Tai *et al.*, 1985; Gerspach, 1991; Jessup, 1979; Thompson *et al.*, 1992). However, atrazine and the metabolites have been found to delay the onset of puberty in male rats (Stoker *et al.*, in press; Stoker *et al.*, 2000). Stoker *et al.* (in press) also demonstrated recently that DACT, DIA, and/or DEA reduced ventral and lateral prostate, seminal vesicle, and epididymal weights when administered PND 23 through 53. Furthermore, when atrazine was administered to peripubertal male SD rats (22 to 47 days of age) at doses of 1 to 200 mg/kg/d, serum and intratesticular testosterone levels were reduced in the 100 and 200 mg/kg/d groups, as were seminal vesicle and ventral prostate weights (Trentacoste *et al.*, 2001). In the same study, serum LH was also reduced, suggesting an effect on the hypothalamus, the pituitary gland or both. Deprivation of prolactin during the early postnatal stage in the male offspring of dams receiving >25 mg/kg/d atrazine resulted in an increased incidence and severity of prostate inflammation (Stoker *et al.*, 1999). See Table 9b.

Table 9a. Lowest NOAELs/ LOAELs (mg/kg/day) for Reproductive Developmental Effects Following Exposure to Candidate Group Compounds in Female Rats

Response	Rat Strain	Exposure Period	NOAEL/LOAEL	Reference
FEMALE				
Attenuation of LH surge	LE	Single dose	300 (propazine)	Cooper, unpublished data
	SD	7 single daily doses	320.1 (propazine) 300 (atrazine) 202.4 (DACT)	Minnema, 2001b
	SD	28 daily doses	not determined (simazine)* not determined (DACT)* not determined (atrazine)*	Minnema, 2001a
	LE LE LE SD	1 day dose 3 daily dose 21 daily dose 21 daily dose	200/300(atrazine) <50/50 (atrazine) <75/75 (atrazine) 75/150 (atrazine)	Cooper *et al.,* 2000
	HLZ LE	GD 1-8	50/100 (atrazine)	Cummings *et al*., 2000
	SD	28 days	5/40 (atrazine)	Morseth, 1996a
	SD	6 months	1.8/3.65 (atrazine)	Morseth, 1996b
Altered pregnancy maintenance	F344 SD LE	GD 6-10	25/50 (atrazine) 100/200 (atrazine) 100/200 (atrazine)	Narotsky *et al.,* 2001
	F344 HLZ	GD 1-8 GD 6-10	50/100 (atrazine) 50/100 (atrazine)	Cummings *et al*., 2000
	F344	GD 6-10	25/50 (atrazine) 34/68 (DACT) 87/131 (DEA) 40/80 (DIA) <91/91 (hydroxyatrazine)	Narotsky *et al.,* 2002
Delayed parturition	F344	GD 6-10	50/100 (atrazine) 17/34 (DACT) <44/44 (DEA) 40/80 (DIA) 457/>457 (hydroxyatrazine)	Narotsky *et al.,* 2002
	F344 SD LE	GD 6-10	50/100 (atrazine) 50/100 (atrazine) 200/>200 (atrazine)	Narotsky *et al.,* 2001
Delayed vaginal opening	Wistar	PND 22-41	16.5/33.7 (DACT) 53/107 (propazine)	Laws *et al*., 2002
	Wistar	PND 22-41	25/50 (atrazine)	Laws *et al.,* 2000

* These data are still under review by OPP.

Table 9a continued

Response	Rat Strain	Exposure Period	NOAEL/LOAEL	Reference
Disruption of estrous cycle	Wistar	PND 22-41	25/50 (atrazine)	Laws *et al.,* 2000
	SD	26 weeks	50ppm/400ppm (atrazine)	Eldridge *et al.*, 1999
	SD	28 days	5/40 (atrazine)	Morseth, 1996a
	SD	6 months	1.8/3.65 (atrazine)	Morseth, 1996b
	SD	13 weeks	10/100 (DACT)	Pettersen *et al.*, 1991
	SD	13 weeks	0/5000 ppm (Express)	Cook, 1989
Attenuation of prolactin release	LE LE LE SD	Adult females single dose 3 daily doses 21 daily doses 21 daily doses	atrazine: 200/300 serum <50/50 pituitary <75/75 pituitary <75/75 pituitary	Cooper *et al.,* 2000
Dams prolactin decreased	Wistar	PND 1-4	13/25 (atrazine)	Stoker *et al.,* 1999

Table 9b. Lowest NOAELs/ LOAELs (mg/kg/day) for Reproductive Developmental Effects Following Exposure to Candidate Group Compounds in Male Rats

Response	Rat Strain	Exposure Period	NOAEL/LOAEL (mg/kg/day)	Reference
Males				
Decreased LH	SD	PND 22-47	100/200 (atrazine)	Trentacoste *et al.*, 2001
Decreased testosterone & prostate weight	SD	PND 22-47	50/100 (atrazine)	Trentacoste *et al.*, 2001
Delayed preputial separation	Wistar	PND 23-53	12.5/25 (DEA) 12.5/25 (DIA) 6.25/12.5 (DACT)	Stoker *et al.*, 2002 (in press)
	Wistar	PND 23-53	<12.5/12.5 (atrazine)	Stoker *et al.,* 2000
	Wistar	PND 23-53	<11.4/11.4 (hydroxyatrazine)	Stoker, unpublished data
Increased incidence of prostatitis in offspring	Wistar	PND 1-4	13/25 (atrazine)	Stoker *et al.,* 1999
Increased incidence and severity of prostatitis in offspring	Wistar	PND 1-4	25/50 (atrazine)	Stoker *et al.,* 1999

V. Weight-of-Evidence Evaluation for Grouping the Candidate Group by a Common Mechanism of Toxicity

Table 10 lists the key LH-dependent effects that are considered to be relevant in defining those candidate group compounds that can be considered to have a common mechanism of toxicity due to disruption of the hypothalamic-pituitary-gonadal axis (see Table 7 for additional neuroendocrine toxic effects following exposure to the candidate group compounds). The relevant lines of evidence for grouping are discussed in the following pages. The common toxic effects of the candidate group compounds whose toxic effects have not been fully established is inferred based on metabolism data.

Table 10. Evidence Used in Grouping/Excluding Candidate Group Pesticides by a Common Mechanism of Toxicity[1]

Chemical	Mammary gland tumors	Suppress LH	Alter Cyclicity	Delay puberty	Alter pregnancy maintenance	Estrogen agonist
Atrazine	**Yes**	**Yes male and female**	**Yes**	**Yes male and female**	**Yes**	**No**
Simazine	**Yes**	**Yes**	No data	No data	No data	**No**
Propazine	**Yes**	**Yes**	No data	**Yes**	No data	No data
Express	**Yes**	No data	**Yes**	No data	No data	**Yes**
2-Hydroxyatrazine	**No**	No data	No data	**No (females) Yes (males)**	**Yes**	No data
DACT	**Yes**	**Yes**	**Yes**	**Yes male and female**	**Yes**	**No**
DEA	No data	No data	No data	**Yes male**	**Yes**	No data
DIA	No data	No data	No data	**Yes male**	**Yes**	No data

[1]Effects are observed in females unless otherwise noted.

A. Mammary Gland Tumors

A mechanism for the development of mammary gland tumors in female SD rats treated with **atrazine** has been detailed in this document and in previously cited documents. In summary, mammary gland tumors in the female rat result from a disruption of hypothalamic neurotransmitter and neuropeptide (primarily noradrenergic) regulation of GnRH, and subsequently, LH secretion. The resultant endocrine milieu of enhanced or unopposed estrogen and prolactin secretion provides an environment that is conducive to the development of mammary gland tumors.

Among the compounds listed in Table 10, **atrazine, simazine, propazine, Express**, and the metabolite **DACT** have been found to produce mammary gland tumors in rats.

- ❑ Atrazine, simazine, and propazine are not only structurally very similar, but present the same pattern of species/strain of mammary gland tumors, i.e. all three produce mammary gland tumors in the female SD rat but no other tumors of any type in the female SD rat, male SD rat, or in CD-1 mice of either sex.

- ❑ **DACT,** a metabolite of atrazine, simazine, and propazine, at 200 ppm increased the incidence of mammary gland tumors in female SD rats following one year of exposure (Minnema, 2002).

- ❑ **Express** produced mammary gland tumors in female Tif:RAIf rats. However, it is not clear that the same LH-related mechanism is operative in this compound as it is for atrazine because results from *in vivo* experiments (e.g., increased uterine cell proliferation & increased relative uterine weights) and *in vitro* experiments (e.g., estrogen receptor binding) with Express and its metabolites suggest that Express acts as an estrogen agonist. Thus, given its estrogenic activity, Express **can not** be grouped based on a common mechanism of toxicity and will consequently be excluded from the common mechanism group.

B. Attenuation of LH Surge

Studies have shown that **atrazine, simazine, propazine** and the metabolite **DACT** suppress the LH surge in rats (e.g., SD, Long-Evans). Atrazine suppresses LH in both male and female animals. The proximal effects of atrazine that lead to decreased LH levels outcomes have been identified as decreased hypothalamic norepinephrine levels and diminished ability to release gonadotropin releasing hormone from the hypothalamus (Cooper *et al.*, 1998). Atrazine has also been found by these same authors to increase hypothalamic dopamine and subsequently decrease prolactin secretion. As previously described, these neuroendocrine alterations can produce a cascade of effects which may alter the excitatory and inhibitory pathways and feedback loops essential for hormonal control in the hypothalamic-pituitary-gonadal (HPG) axis. Alteration of these pathways following exposure to compounds containing the triazine moiety have been found to exert effects on hormonal control of the estrus cycle in females, pubertal development in both males and females, pregnancy outcome, and prolactin secretion in laboratory rats. Although there are no direct data indicating that **DEA** and **DIA** attenuate the LH surge, it appears reasonable to expect that they will do so since **DACT**, an LH surge attenuator, is a metabolite common to both DEA and DIA in rats. This contention is supported by data that show that both **DEA** and **DIA** alter pregnancy maintenance (see below), an effect that is attributed to interference with the hypothalamic-pituitary-gonadal axis.

C. Alteration of the Estrous Cycle

Atrazine, **DACT, and Express** have all been shown to disrupt the estrous cycle by prolonging the number of days in estrus. Atrazine can increase the percentages of days in estrus by as much as 70%. Dietary exposure to DACT for 13 weeks induced irregularities of the cycle, which included early, intermittent, or persistent proestrus, estrus, and diestrus. Although **Express** has been shown to slightly prolong the estrous cycle, results of *in vivo* experiments showed that this compound possesses estrogenic activity, and therefore may not be acting by the same mechanism as atrazine, simazine, and propazine, as mentioned earlier in this section

D. Delayed Pubertal Development

Atrazine, **propazine**, and metabolites **2-hydroxyatrazine**, **DACT, DEA,** and **DIA**) have been found to delay pubertal development in rats. Atrazine and DACT delay puberty in both male and female rats, while 2-hydroxyatrazine has been found to delay puberty in males but not females. In females, the administration of propazine from postnatal day 22 through 41 delayed vaginal opening by 4 days. Male rats exposure to DACT, DEA, or DIA during postnatal days 23-53 were found to show preputial separation and decreases in prostate weights.

E. Altered Pregnancy Maintenance

It is well known that the hormonal requirements of the corpus luteum (CL) change as the rat progresses through different stages of gestation. After the first week of gestation, the CL no longer requires prolactin for support and becomes dependent on LH during gestation days (GD) 7-16. During this time as little as 2-4 hours deprivation of LH may be sufficient to terminate pregnancy. **Atrazine** and the metabolites **2-hydroxyatrazine, DACT**, **DEA**, and **DIA** have been reported to alter pregnancy maintenance. These compounds have been found to induce full litter resorption, induce pseudopregnancy, prevent pre or post-implantation, and/or delay parturition. Although **2-hydroxyatrazine** has been shown to alter pregnancy and delay puberty in males, it has not been found to induce mammary gland tumors. Therefore, based on the absence of mammary gland tumor induction by 2-hydroxyatrazine and inconclusive data that show 2-hydroxyatrazine's effect on the LH surge and/or LH-dependent events, the WOE **does not** support including it in the common mechanism group at this time.

VI. Conclusions and Final Grouping of the Candidate Group Compounds Based on a Common Mechanism of Action

To satisfy the requirements of the Food Quality Protection Act of 1996 to assess the cumulative effects of chemicals that have a common mechanism of toxicity, OPP has determined that some of the candidate group compounds can be grouped based on a common mechanism of toxicity. Based upon the weight-of-evidence provided in studies by registrants and EPA laboratories and in studies reported in the literature, the pesticides **atrazine, propazine, simazine, and metabolites diaminochlorotriazine (DACT), desisopropyl s-atrazine (DIA), and desethyl s-atrazine (DEA)** should be considered as a **Common Mechanism Group**, based on suppression of the LH ovulatory surge and the consequent effects on reproductive function and development. Several compounds were excluded (including Express and 2-hydroxyatrazine) from this grouping on the basis of not having sufficient similarity to the remaining compounds with respect to metabolism, pharmacokinetics, and toxic effects (i.e., mammary gland tumors and/or neuroendocrine effects). Others were excluded because they were no longer a registered compound with the US EPA, had minimal human exposure, or were registered outside the United States. Following the initiation of a cumulative risk assessment, the Common Mechanism Group may be modified as a result of the review of new or existing data.

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