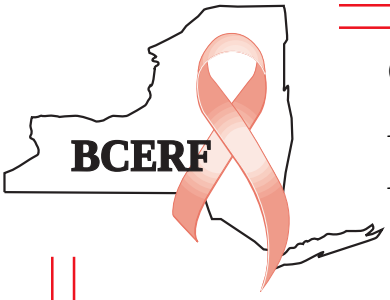


Cornell University
Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)*

Critical Evaluation # 8
April, 1999

Critical Evaluation of Atrazine's Breast Cancer Risk

by

Suzanne M. Snedeker, Ph.D. and Heather Clark, M.S.**

*The institutional home of BCERF is the
Institute for Comparative and Environmental Toxicology (ICET)
in the Cornell Center for the Environment

**** Address correspondence to:**
Dr. Suzanne M. Snedeker
110 Rice Hall
Cornell University
Ithaca, NY 14853
Phone: (607) 254-2893
Fax: (607) 255-8207
email: sms31@cornell.edu

Supported by grants from:
New York State Dept. of Health
USDA-Regional NYC174423

Table of Contents - Critical Evaluation of Atrazine's Breast Cancer Risk

List of Figures

Critical Evaluation of Atrazine's Breast Cancer Risk

Authors' Note: The reader is encouraged to read Appendix B prior to reading this Critical Evaluation. Appendix B includes an explanation of the approach used in writing BCERF Critical Evaluations and an explanation of the BCERF Breast Cancer Risk Classification System.

I. Chemical Information

A. Common Name: Atrazine

B. Chemical Names: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (CAS) 6-chloro-*N*-ethyl-*N'*-isopropyl-1,3,5-triazinediyl-2,4-diamine (IUPAC) (Stevens and Sumner, 1991)

C. Chemical Formula: $C_8H_{14}ClN_5$ (Stevens and Sumner, 1991)

D. CAS Registry Number: 1912-24-9 (Stevens and Sumner, 1991)

E. Chemical Structure of Atrazine: (Ahrens, 1994)

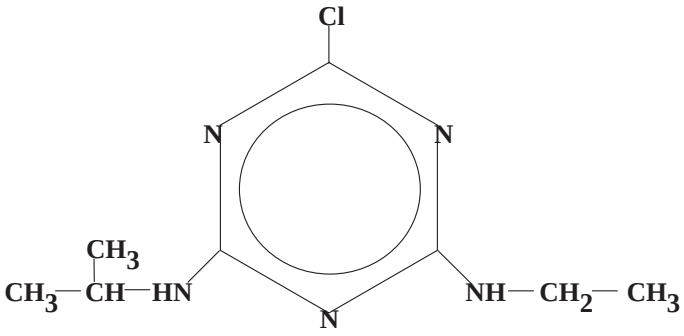


Figure 1. Chemical structure of atrazine

F. Trade Names for Atrazine:*
AAtrex® (Norvartis); Atrachem® (Agrochemical Industries Co. Ltd.); Atranex® (Makhteshim-Agan); Atrasul® (Sulphur Mills Ltd.); Atrataf® (Rallis India, Ltd.); Atratylone® (Chimac-Agriphar S.A.); Atrazin® (Sintagro Ltd.); Azinotox®-500 (Pyosa, S.A. de C.V.); Callizine® (Calliope S.A.); Cat L Siapa® (Industrie Chimiche Caffaro S.p.A.); Crisazina® (Crystal Chemical Inter-America; Dupocsa); Dhanuzine® (Dhanuka Pesticides Ltd.); Drexel® Atrazine (Drexel Chemical Co.); Flotrazine® (Sanachem (Pty) Ltd.); Gesaprim® (Norvartis); Fezprim 500 (Zargo Asia Ltd.); Herbitrin® (Herbitecnica Industria de Defensivos); Limpiamaiz® (Insecticidas Internacionales, C.A.); Malermais® (Diachem S.p.A.); Maizine® (Forward International Ltd.); Sanazine® (Sanachem (Pty) Ltd.); Trac® (Atanor S.A.); Triazol ® (Insecticidas Internacionales, C.A.); Vegfru Solaro® (Pesticides India); Weed Pro® Atrazine (Cornbelt Chemical Co.); Woprazine® (B.V. Industrie- & Handelsonderneming Simonis); X-siprim® (Agsin Pte. Ltd.) (Meister, 1998).

G. Trade Names of Atrazine Pre-Mixes:*
Agimix®, + alachlor (Herbitecnica Industria de Defensivos S/A); Alazine®, + alachlor (Makhteshim-Agan); Amezol®, + ametryn (Insecticidas Internacionales, C.A.); Aterbutex®, + terbutryn (Makhteshim-Agan); Aterbutox®, + terbutryn (Pyosa, S.A. de C.); Athado Winter®, + terbumeton + terbuthylazine (Probelt, S.A.); Atramet Combi®, + ametryn (Makhteshim-Agan); Basis Gold®, + nicosulfuron + rimsulfuron (DuPont Agricultural Products); Bellater®, + cyanazine (American Cyanamid Co.); Bicep® and Bicep Lite II®, both + metolachlor (Norvartis); Buctril®, + bromoxynil (Rhone-Poulenc); Bullet®, + alachlor (Monsanto Co.); Candex®, + asulam (Rhone-Poulenc); Contour®, +imazethapyr (American Cyanamid Co.); Crisazina-Crisatrina Kombi®, + ametryn (Crystal Chemical Inter-America); Cyergy®, + cyanazine (Griffin Corp.); Cy-Pro-AT®, +cyanazine (Griffin Corp.); Erunit®, + acetochlor (Nitrokemia Ltd.); Exatrazine II®, + cyanazine (DuPont Agricultural Products); FulTime®, + acetochlor (ZENECA Ag Products); Galleon®, + sulcotrione (ZENECA Agrochemicals); Guardsman®, + dimethenamid (BASF Corp.); Harness® Xtra, + acetochlor (Monsanto Co.); Headline® B, + bentazone (BASF Corp.); Headline® G, + sethoxydim (BASF Corp.); Herbimix®, + simazine (Herbitecnica Industria de Defensivos S/A); Laddock, Laddock® Neu, Laddock Pro, and Laddock® S-12, all + bentazone (BASF AG); Lariat®, + alachlor (Monsanto Co.); Marksman®, + dicamba (BASF Corp.); Maxipac Trac® 50, + acetochlor (Atanor S.A.); Primagram®, Primextra®, both +*S*-metolachlor (Norvartis); Prompt®, + bentazone (BASF Corp.); Ramrod®/Atrazine, + propachlor (Monsanto Co.); Rastra®, + alachlor (Pyosa, S.A. de C.V.); Shotgun®, + 2,4-D (Platte Chemical Co.); Simatrol® 55, + amitrole + simazine (Industrie Chimiche Caffaro S.p.A.); Simazat®, + simazine (Drexel Chemical Co.); Surpass® 100, + acetochlor (ZENECA Ag Products) (Meister, 1998).

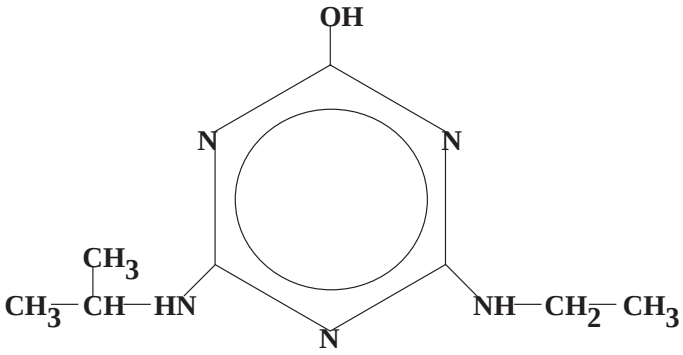
***Note:** Trade names are used herein for convenience and informational purposes only. No endorsement of products is intended and no criticism of unnamed products is implied. Trade names of atrazine and mixtures containing atrazine listed here are those currently in use in 1998 (Meister, 1998).

H. Transformation Products and Metabolites:

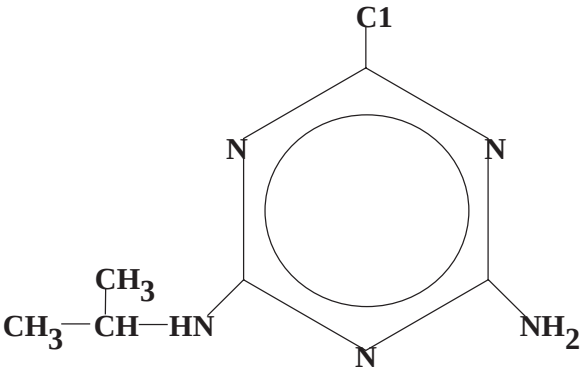
1. Soil:

One of the major degradation pathways of atrazine in surface soils is hydrolysis to hydroxy atrazine (6-hydroxy-*N*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) (Muir and Baker, 1978). The hydrolysis of atrazine in soil is favored by low pH (5.5-6.5), high organic matter content, high clay content, high temperature and low moisture content (Ahrens, 1994; Koskinen and Clay, 1997; Ma and Selim, 1996).

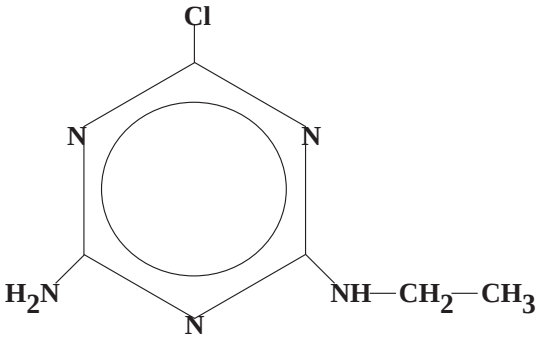
Atrazine can also be biodegraded by microbes in soil. Major biotic degradation pathways include dealkylation to form deethylatrazine (6-chloro-*N*-[1-methylethyl]-1,3,5-triazine-2,4-diamine) and deisopropylatrazine (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine). Atrazine can also be degraded in the presence of sunlight on soil. Major transformation products from photodegradation include deethylated atrazine and *N*-deethyl-*N*-demethylethyl atrazine (also called diaminoatrazine) (Ahrens, 1994; Koskinen and Clay, 1997; Ma and Selim, 1996). The half-life of atrazine and its transformation products are summarized in the environmental fate section of this report (see section VI.B.).



Hydroxyatrazine



Deethylatrazine



Deisopropylatrazine

Figure 2. Major Transformation Products of Atrazine in Soil

2. Plants:

There are three major transformation pathways for atrazine in plants: the *N*-dealkylation of the side chain(s), dechlorination to form hydroxy-forms of atrazine, and the displacement of the chloro-group at position 2 with glutathione (GSH) to form GSH-conjugates (Lamoureux et al., 1998). The type of side chain dealkylation is different in various plant species. For instance, in peas and sorghum, the dealkylation of the ethyl-side chain is favored to form deethlyatrazine. Dealkylated products can be further metabolized to the hydroxy or GSH-conjugated forms of atrazine. The loss of the 2-chloro group of atrazine occurs primarily in corn, wheat and rye, but not in sorghum. In certain tolerant plants, such as corn, atrazine can be conjugated with GSH. The GSH-conjugates of atrazine can be further transformed to gamma-glutamylcysteine, and then to *S*-cysteine. A minor transformation pathway of atrazine in sugarcane is amination, with a substitution of NH_2 for the 2-chloro group. Amino-*s*-triazine transformation products identified in sugarcane include 2-amino-4-ethylamino-6-isopropylamino-*s*-triazine, and its deethylated form, 2,4-diamino-6-isopropylamino-*s*-triazine (Lamoureux et al., 1998).

3. Metabolites Identified in Mammals:

Seventy-two hours after rats were fed an oral dose of 0.53 mg of ^{14}C-labeled atrazine, 65.5% of the parent compound was recovered in the urine, 20.3% in the feces, 15.8% in body tissues and less than 0.1% was detected in the expired air. The four major types of urinary metabolites (47% of the urinary radioactivity) identified were 2-hydroxyatrazine, its two mono-dealkylated analogs 2-chloro-4-amino-6-(ethylamino)-*s*-triazine and 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine, and ammeline (Bakke et al., 1972). A later study identified 2-chloro-4,6-diamino-*s*-triazine as another major atrazine urinary metabolite in adult male Charles River rats (Bradway and Moseman, 1982). In swine, the primary urinary metabolite is deethylatrazine (Erickson et al., 1979). Studies in humans occupationally exposed to atrazine indicate that in addition

to a small amount of atrazine detected in the urine, major urinary metabolites include 2-chloro-4-amino-6-(ethylamino)-*s*-triazine and 2-chloro-4, 6-diamino-*s*-triazine (Catenacci et al., 1990; Ikonen et al., 1988). In *in vitro* studies using livers of rats, mice, goats, sheep, pig, rabbit and chicken, the formation of the mono-dealkyated metabolites of atrazine were found to be cytochrome P-450 mediated (Adams et al., 1990). In addition, GSH conjugates of atrazine and the mono-dealkyated metabolites, have been identified by Adams et al. (1990) and other investigators (Dauterman and Muecke, 1974). The conjugation of atrazine to GSH occurs at the chloro group at the 2-position (Guddewar and Dauterman, 1979).

II. History of Use and Usage

A. History of Use and Uses:

Atrazine is the most widely used herbicide in the United States (US). Atrazine was first registered for use by Ciba Plant Protection in 1959 (USEPA, 1994). Atrazine is a selective pre- and post-emergent herbicide used to control broad leaf and grassy weeds. In the early 1990s, approximately 68% of the corn, 50% of the sweet corn, 65% of the sorghum, and 94% of the sugarcane acreage was treated with atrazine (Gianessi and Anderson, 1995 b). Other crops treated with atrazine include guava and macadamia nuts (Norvartis, 1998). Atrazine is also used for selective weed control in conifers, primarily Christmas trees and ornamentals, and on ecofallow which is land left untilled on a periodic basis for soil and moisture conservation (Meister, 1998; WSSA, 1994). Other non-agricultural uses include weed control in certain turf grasses (Bermudagrass, Centipedegrass, St. Augustinegrass and Zoysia Grass) in Southeastern parts of the US, including use for sod production, on fairways and for home lawn care (HSDB, 1996; Miles and Pfeuffer, 1997; Norvartis, 1998). Atrazine was used industrially to clear right-of-ways, but this use is no longer permitted (Ribaudo and Bouzaher, 1994), except for controlling weeds and grasses along roadsides in certain Western and Midwestern states (Colarado, Kansas, Montana, Nevada, North Dakota, South Dakota and Wyoming) (Norvartis, 1998).

B. Current Usage:

Atrazine was ranked as the most used herbicide in the US during 1990-93. During this time, use for agricultural purposes on cropland was 72 million pounds (lbs) of active ingredient (AI) per year (Gianessi and Anderson, 1995 b). Atrazine was also ranked as the most used herbicide in New York State (NYS), with 1.4 million lbs of AI per year used during the same time period (Gianessi and Anderson, 1995 a). More recent data from the EPA indicates that atrazine still ranks as the most used herbicide in the US. In 1995, 68 to 73 million lbs of atrazine were used on US crops (Aspelin, 1997).

The predominant use of atrazine is to control weeds in production of corn crops in the Midwest; the combined amount of atrazine used on corn crops in Illinois, Iowa, Nebraska, Ohio, Indiana, Missouri, Michigan, Minnesota, Kansas and Wisconsin in 1993 was 47.6 million lbs. Usage on corn crops in Northeastern states was much lower, with Pennsylvania using 1.5 million lbs per year and NYS using 1.16 million lbs/year (Solomon et al., 1996). This is in contrast to other areas of the US where other uses predominate. For instance, in south Florida the predominant use of atrazine in the early 1990s was on sugarcane (2.6 million lbs/year) and on home lawns care and domestic uses (0.23 million lbs/year) (Miles and Pfeuffer, 1997).

C. Registered Application Rates and Actual Field Application Rates of Atrazine on Corn:

Atrazine's registered application rate for weed control on corn was reduced several times in the 1990s. In 1990, application rates were reduced from 5.6 to 3.4 kilograms per hectare (kg/ha). Maximum pre-emergence rates on corn were lowered in 1993 to 1.8 to 2.2 kg/ha, depending on soil type and soil erosion characteristics (Solomon et al., 1996). These application rates are still in force today. Maximum post-emergence application rates for corn are currently 2.2 to 2.8 kg/ha (Norvartis, 1998).

Despite the reduction in permitted application rates, actual usage of atrazine did not dramatically decrease in the late 1980s and early 1990s. This is because the application rates that were being applied to the corn fields in the late 1980s were already far below the permitted application rates. The application rate used on corn fields in 1984 was approximately 2.2 kg/ha; in 1987 the rate was 1.6 kg/ha; it ranged from 1.3 to 1.6 kg/ha in 1994. Some of the reduction of field application rates also has been attributed to the combined use of atrazine and the herbicide metolachlor. The combination of these two herbicides allows for effective weed control on corn at lower rates than if either herbicide is used alone (Solomon et al., 1996).

III. Current Regulatory Status

A. Regulatory Status:

The United States Environmental Protection Agency (EPA) issued a Registration Standard for atrazine in 1983, noting concern that atrazine may pose a potential carcinogenic risk from ingestion of contaminated ground and surface water. A preliminary notification of the EPA's intention to initiate a Special Review was given in 1988 based on carcinogenic potential and possible risks resulting from exposure through the diet from treated food and contaminated water, as well as concerns of potential carcinogenic risk to pesticide mixers and applicators. Additional concerns were raised in 1989 based on studies documenting heart damage in dogs exposed to atrazine (USEPA, 1994).

In 1990, the EPA classified atrazine as a Restricted Use Pesticide (RUP), which included label amendments that reduced application rates for agricultural uses and limited the maximum annual application rate for industrial weed control; prohibited the mixing,

loading or storage of atrazine products within 50 feet of wells; and prohibited applying atrazine through an irrigation system. In April 1992, registrants volunteered to further restrict use including prohibiting industrial use for clearing right-of-ways and establishing buffer zones from wells and surface water for mixing, loading and application (Ribaudo and Bouzaher, 1994; USEPA, 1994).

A Special Review of *s*-triazine herbicides, including atrazine, simazine and cyanazine, was initiated in November of 1994. The review was initiated because experimental animal data indicated these compounds are possible human carcinogens. The EPA determined that exposure to atrazine through food and water, through the use of lawncare products, and by occupational exposures in pesticide applicators, may "pose risks of concern" (USEPA, 1994). A proposed decision on atrazine is not anticipated until sometime in 1999 (BNA, 1997).

B. Drinking Water Standards and Health Advisories:

1. MCL: The EPA has set Maximum Contaminant Level (MCL) for atrazine in drinking water at 0.003 mg/ L (USEPA, 1996). The MCL for atrazine was set in 1991 and was based on adverse effects on the kidney and liver in dog and rat studies (USEPA, 1994; USEPA, 1991). The MCL is an enforceable limit for the maximum allowable concentration of a chemical in public drinking water supplies.

2. HA: Health Advisory (HA)* levels for atrazine in drinking water are as follows:

10 kg child:

- One-day = 0.1 mg/L
- Ten-day = 0.1 mg/L
- Longer term = 0.05 mg/L

70 kg adult:

- Longer term 0.2 mg/L
- Lifetime = 0.003 mg/L

* The HAs are non-enforceable limits of the concentration of the chemical in drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety (USEPA, 1996).

C. Food Residue Tolerances:

The EPA sets tolerances for levels of atrazine residues in food. The Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) are the federal agencies responsible for monitoring the levels of atrazine residues and several metabolites in domestic and imported foods and animal feeds. Because of new legislation set forth in the 1996 Food Quality Protection Act, the tolerances for atrazine may be reset according to the new guidelines. Until the tolerances are reset, current tolerances set by the EPA for atrazine in raw agricultural products are as follows: fodder and forage, 5-25 ppm; eggs, 0.02 ppm; milk, 0.02 ppm; meat or fat from animals, 0.02 ppm (includes goats, hogs, horses, poultry and cattle); sweet corn, 0.25 ppm; rye grass, 15 ppm; sugarcane, 0.25 ppm; and wheat grain, 0.25 ppm (USEPA, 1998).

D. Acceptable Daily Intake, WHO:

The acceptable daily intake (ADI) of atrazine from drinking water has been set at 0.7 mg/kg body weight (bd wt) per day by the World Health Organization (WHO) (Kello, 1989; WHO, 1987).

IV. Summary, Overall Evidence for Carcinogenicity (non-breast sites)

A. Human Studies:

Increased cancer risk at non-breast sites has been documented in agricultural workers exposed to atrazine, including ovarian tumors in female farm workers and some evidence of increased incidence of non-Hodgkin's lymphoma (NHL) in male farm workers.

1. Case-Control Studies in Agricultural Settings:

a. Ovarian Cancer:

Two case control studies have been conducted to determine the relationship between herbicide exposure and ovarian mesothelial tumors in female agricultural workers residing in Northern Italy (Donna et al., 1984; Donna et al., 1989). The first study included 60 cases of primary ovarian mesothelial tumors and 127 hospital-based controls with non-ovarian malignancies matched for year of diagnosis, age and residence. Although actual herbicide exposures were not quantified by measuring blood or urine samples, subjects were divided into three exposure groups: definitely exposed (personal use of herbicide confirmed by subject or next of kin); probably exposed (resided in areas of known usage); and no exposure. In the initial analysis, it was found that the Relative Risk (RR) of ovarian tumors in women with definite exposure to herbicides was 2.2 (95% CI 0.77-6.32); however, this was based on eight cases and no controls. When the definitely and probably exposed cases were combined, a positive association (RR=0.38; 95% CI 1.90-16.07) was found between exposure to herbicides and ovarian mesothelial tumors (Donna et al., 1984). It should be noted that this study did not specifically mention that triazines were among the herbicides the women were exposed to (Donna et al., 1984); however, in the second study conducted by the same investigators (Donna et al., 1989), it was stated that "exposure to triazines was often reported by the interviewed subjects" in the 1984 study.

To further clarify whether triazine exposure affects ovarian cancer risk, a second study using more appropriate population-based controls was conducted (Donna et al., 1989). Cases included 69 women diagnosed between July 1, 1980 and June 30, 1985, with histologically confirmed malignant epithelial ovarian tumors. Cases were identified from the Alessandrian Hospital Cancer

Register. Population controls (two per case) were recruited from electoral rolls of towns in the study area and were matched to cases by 5-year age group. A questionnaire was administered to determine information on occupational history, involvement in agriculture, and personal use of herbicides. From this information, subjects were categorized as being 1) definitely exposed, 2) possibly exposed, or 3) unexposed to triazines. Women "definitely" exposed to triazines had a elevated RR of 2.7 (90% CI 1.0-6.9) for ovarian neoplasms, while those possibly exposed had a RR of 1.8 (90% CI 0.9-3.5) (Donna et al., 1989). Although the methods used to categorize exposure were relatively crude, these preliminary findings should be confirmed or refuted by a study which more carefully quantifies exposure to triazine herbicides.

b. Non-Hodgkin's Lymphoma (NHL):
Epidemiological case-control studies of male farmers exposed to atrazine conducted in Kansas (Hoar et al., 1986), and eastern Nebraska (Weisenburger, 1990 a; Zahm et al., 1988), found an increased risk of NHL associated with atrazine or triazine use, while an association was not found in a study conducted in men from Iowa and Minnesota (Cantor et al., 1992).

In the Kansas study, newly diagnosed cases of NHL in white men, from 1976 through 1982, were identified through the University of Kansas Cancer Data Service (Hoar et al., 1986). Population controls (three per case) were selected by random digit dialing and from Medicare files. Controls were matched to cases according to age ($\pm$ 2 years) and vital status. History of pesticide use, types of crops farmed (wheat, corn, sorghum or pasture), number of days per year exposed to specific pesticides, type of protective equipment used, and number of acres treated were obtained by telephone interview. The OR for NHL was elevated in those that ever used triazine herbicides (OR=2.5; 95% CI 1.2-5.4), based on 14 cases and 43 controls. When use of 2,4-D and uracils was controlled for in triazine users, the OR decreased to 2.2 (95% CI 0.4-9.1), though this was based on only three cases and 11 controls. The authors acknowledged certain limitations of this study, including recall biases and possible inaccuracies in assessing pesticide exposure by questionnaire; however, the authors suggest this may have lead to an underestimate, rather than to an over estimate of exposure (Hoar et al., 1986).

In the Nebraska study, cases of NHL diagnosed during 1983-86 in whites aged 21-years or older, were identified using the University of Nebraska Lymphoma Study Group Registry and area physician records. The study included 201 men with histologically confirmed NHL. 725 population-based controls (three per case) were matched for age ($\pm$ 2 years), sex, race and vital status. Atrazine use was associated with an OR of 1.4 (95% CI 0.8-2.2) for developing NHL, and when duration of use was greater than 15 years, the OR increased to 2.0 (Weisenburger, 1990 a; Zahm et al., 1988).

A case-control study conducted in Iowa and Minnesota determined the risk of NHL in 622 men with newly diagnosed NHL. Eligible cases included male residents of Iowa 30 years or older with NHL diagnosed between 1981 and 1983 (all cases in state eligible) and Minnesota men (cases did not reside in major cities in Minnesota) diagnosed with NHL between 1980 and 1982. Diagnoses were confirmed by pathologists. Population based-controls (n=1245) were matched by 5-year age group, vital status at time of interview, and state of residence. Information on medical, occupational and residential history; known or suspected cancer risk factors; history of farming practices, type of livestock, crops grown, and length of residing on farm; and detailed history of pesticide use was obtained by a trained interviewer. This was one of the few studies that controlled for confounding variables, including vital status, cigarette use, family history of lymphopoietic cancer, and nonfarming jobs related to NHL. This study did not find a significant association between atrazine use and NHL (OR = 1.1; 95% CI 0.8-1.6), based on 64 cases and 133 controls (Cantor et al., 1992).

A study by Zahm et al. (1993 a) evaluated the role of different herbicides in the development of NHL by pooling the data from studies conducted in Kansas (Hoar et al., 1986), Nebraska (Weisenburger, 1990 a; Zahm et al., 1988; Zahm et al., 1990), and Iowa and Minnesota (Cantor et al., 1992). Each study used questionnaires to obtain information on agricultural practices, though the instruments were not the same in all studies. When the data from the studies were combined, atrazine use was associated with an OR of 1.4 (95% CI 1.1-1.8) for developing NHL. However, when the use of the phenoxy herbicide, 2,4-D, and organophosphate (OP) insecticides was controlled for, the association between NHL and atrazine was reduced (OR=1.2; 95% CI 0.9-1.7). The state with the most dramatic reduction in OR was Nebraska. Without adjustment for 2,4-D and OP, the OR for NHL in triazine users was 1.7; with adjustment for OP and 2,4-D use the OR dropped to 0.7. It should be noted that this state had the most detailed information on atrazine usage. The authors concluded that there was little to no increased risk of NHL in white men exposed to atrazine (Zahm et al., 1993 a). Questions about the analysis of the four studies were raised by Crosignanni and Berrino (1994), and these concerns were answered in an accompanying letter by Zahm and Blair (Zahm and Blair, 1994).

Only one study has evaluated the role of agricultural pesticide use on the development of NHL in women residing in Eastern Nebraska (Zahm et al., 1993 b). Methods for selecting cases and controls were the same as specified previously (Weisenburger, 1990 a; Zahm et al., 1988). The risk of developing NHL in women who had used triazines on farms was low (OR = 1.2; 95% CI 0.6-2.6), based on 12 cases and 38 controls. The risk of NHL was elevated in women who had personally handled triazines (OR = 2.2; 95% CI 0.1-21.5), but this was only based on one case and two controls. However, as mentioned previously, this study and the study on eastern Nebraska men was reanalyzed controlling for use of OP insecticides and the herbicide 2,4-D. When these pesticides were

controlled for, the effect of atrazine on NHL risk was greatly diminished (Zahm et al., 1993 a).

An international cohort of 21,183 workers from 11 countries was followed for the development of NHL in workers exposed to various herbicides and other environmental contaminants (Kogevinas et al., 1995). Exposures were assessed by a panel of industrial hygienists, using company exposure questionnaires. There was no excess risk of NHL in workers exposed to triazines (OR = 0.7; 95% CI 0.1-3.1). The specific type of triazine exposure was not identified.

c. Multiple Myeloma (MM):
Two small case control studies did not find an association between triazine exposure and the risk of developing MM in men (Brown et al., 1993; Burmeister, 1990). A case-control study was conducted in Iowa to look at the relationship between MM and exposure to a variety of herbicides, including triazine herbicides (Burmeister, 1990). Cases of MM diagnosed between January 1982 through March 1984 were identified using the Iowa State Health Registry records (n=198). Controls were selected using random digit dialing and were matched for sex and age $\pm$ 5 years (n not specified). Exposure to any of the herbicides evaluated in this study did not significantly increase the risk of MM. The OR for developing MM with exposure to triazines was slightly elevated (OR=1.29; CI not specified).

In another population-based, case-control study, Brown et al. (1993) examined the risk of MM in men who had mixed, handled or applied specific pesticides, including atrazine. Cases were identified from Iowa Health Registry Records and included all men over the age of 30 diagnosed with MM during the years 1981 to 1984. Controls did not have lymphatic or hematopoitic cancer and were identified using random digit dialing, Medicare records and state death certificates. Cases were matched to controls by age ($\pm$ 5 years) and vital status at the time of the interview. A questionnaire was used to obtain information handling and use, and duration of use of specific pesticides. There was not a relationship between exposure to atrazine and the risk of MM (OR =0.8; 95% CI 0.4-1.6) in this study. However, this conclusion was based on a small number of cases (n=13) and controls (n =73).

d. Colon Cancer:
A case-control study conducted in Kansas did not find a relationship between the incidence of colon cancer and use of triazine herbicides (Hoar et al., 1986). The type of triazine herbicide used was not specified. In this study, the cases were obtained from all colon cancer cases diagnosed in Kansas from 1976 to 1982, while controls were obtained from the general population by using random digit dialing and from Medicare and State mortality files. No information was available on methods used to obtain information on pesticide use practices, nor did the study state if cases and controls were matched for age or vital status. The OR for developing colon cancer in those who had used triazines was 1.4 (95% CI 0.2-7.9), based on two cases and 43 controls. While this study does not support a relationship between triazine exposure and cancer risk, the very small number of subjects limits the statistical power of this study to detect an association.

e. Leukemia:
The risk of leukemia in men exposed to specific types of pesticides, including triazines, was investigated in a population case-control study in Iowa and Minnesota (Brown et al., 1990). White men with newly diagnosed cases of leukemia were determined from March 1981 to October 1983 through the Iowa Tumor Registry, and in Minnesota leukemia cases that occurred between October 1980 and September 1982 were identified through a network of hospital and pathology laboratories located in non-urban areas of the state. Population-based controls were selected by using a combination of random-digit dialing, Medicare records and state death certificate files. Cases and controls were matched by 5-year age group, vital status at the time of the interview, and state of residence. A questionnaire was administered via an interview to subjects, or to close relatives if cases where deceased, to obtain information on residential history, non-farm occupational history, smoking and alcohol use, medical records, family history of cancer, farm activities regarding crops and livestock raised, and the types and usage of insecticides, herbicides and fungicides. A supplemental questionnaire was administered to obtain additional information on the number of days per year that pesticides had been handled. Potential confounders, including vital status, age, state, tobacco use, family history of leukemia, non-farming occupation, and exposure to chemical agents known to affect leukemia risk were adjusted for in statistical analysis of the data. As a class, use of triazine herbicides was found not to be associated with increased risk of leukemia (OR=1.1; 95% CI 0.8-1.5), based on 67 cases and 172 controls. Risk according to use of specific types of triazine herbicides was also evaluated, but neither use of atrazine (OR=1.0; 95% CI 0.6-1.5, 38 cases and 108 controls) nor the use of cyanazine (OR=0.9; 95% CI 0.5-1.6, 21 cases and 64 controls) was associated with increased risk of leukemia. Although this study does not support a relationship between triazine exposure and risk of leukemia, risk was based on a relatively small number of individuals, and since exposure was assessed by questionnaire, recall bias may have affected the accuracy of the estimated exposures.

2. Cohort Mortality Studies, Triazine Manufacturing Workers:
Causes of mortality have been evaluated in a series of studies in a cohort of men employed at a Alabama plant that manufactured agricultural chemicals, including organochlorines, OPs, fungicides, mitocides and triazine herbicides (Sathiakumar et al., 1992; Sathiakumar et al., 1996).

In the first study mortality rates of the 4323 men employed at the manufacturing facility were compared to mortality rates of US or Alabama men in order to calculate Standardized Mortality Ratios

(SMR). SMR is defined at the ratio between observed and expected deaths divided by 100. To be included in the cohort, men had to be employed in production-related jobs for at least one month between January 1, 1951, and January 1, 1987. It should be noted that only 39% of the cohort was followed for at least 20 years and 85% of the cohort was less than 45 years of age. The young age of the cohort limits the usefulness of these data in determining the relationship between mortality from cancer and chemical exposures. Mortality from cancer was elevated in those employed less than five years (SMR=148; 95% 103-206), but was lower than expected in those employed greater than five years (SMR=89; 95% CI 54-139). Among those employed for less than five years, elevated mortality rates were reported for cancer of the bucal cavity (SMR= 451; 95% CI 91-1318), esophagus (SMR=812; 95% CI 218-2079), lung (SMR=173; 95% CI 92-295), the central nervous system (SMR 310; 95% CI 83-793) and NHL (SMR=280; 95% CI 58-819). However, among those employed for greater than five years, mortality rates were slightly, but not significantly elevated for only lung cancer (SMR=126; 95% CI 57-239); and all cancers of the lymphatic and hematopoietic tissue (SMR=142; 95% CI 38-364). In this study, there was no control for tobacco use, so increases in mortality of the bucal cavity, esophagus and lung cannot be interpreted and may not necessarily be due to occupational exposures to chemicals. This study made no attempt to estimate exposures to specific classes of chemicals, so this study is of limited usefulness in determining if exposure to a specific triazine affected cancer mortality in this cohort (Sathiakumar et al., 1992).

In a later study, an attempt was made to see if there was relationship between cancer mortality and possible exposure to triazine herbicides in a cohort drawn from two chemical manufacturing plants (Sathiakumar et al., 1996). Employment records were used to obtain work histories and job titles to determine those with definite (group 1), probable (group 2), or possible (group 3) triazine-related work histories. No actual quantification of triazine exposures was done, nor were the types of triazine herbicides specified. Of the 4388 men employed at plant 1 (same cohort as Sathiakumar et al., 1992), 3676 men were included who had potential exposure to triazines. From plant 2, subjects were employed for at least six months from 1970 to 1986 in production-related jobs. Of the 1472 men in the original cohort, 1269 were determined to have potential exposure to triazines. Therefore, the total number of men included was 4917 subjects (28 worked in both plants).

Of the 4917 subjects, 55% (n=2683) had definite or probable exposure to triazines. Expected mortality for any cancer in the definite/probable group was lower than expected (SMR=72; 95% CI 58-89), which may suggest a healthy worker effect. The only cancer that had an elevated mortality rate was NHL (SMR=385; 95% CI 79-1124), based on three observed and 0.78 expected deaths. However, two of the NHL cases had worked at the manufacturing plant for less than one year in triazine-related positions. The definite/probable triazine exposure group had, on average, 18 years of follow-up, which may not be a sufficient time to allow for long-latency period between an exposure and development of cancer. Also, duration of exposure to triazines was relatively short, since 85% of the definite/probable group had been exposed to triazines for less than four years (Sathiakumar et al., 1996). While this study did not find any convincing evidence of a causal relationship between triazine exposure and any type of cancer, further studies are needed with longer monitoring periods. Future studies also need to include women in the cohorts.

3. Summary, Human Carcinogenicity Studies (non-breast sites):

One study has shown an increased risk of ovarian cancer in women exposed to an herbicide containing atrazine (Donna et al., 1989). Exposure to and/or use of triazine herbicides in agricultural settings does not appear to affect the risk of NHL in women (Zahm et al., 1993 b) or in men when the confounding effects of using other herbicides such as 2,4 -D and OP are controlled for (Zahm et al., 1993 a). Studies have not shown an association between exposure to triazine herbicides and the risk of MM (Brown et al., 1993; Burnmeister, 1990), leukemia (Brown et al., 1990), or colon cancer (Hoar et al., 1986) in men. However, these studies were based on a small number of cases and therefore had a limited power to detect an effect of triazine exposure on cancer risk.

B. Experimental Animal Studies (non-mammary sites):

Studies in mice have not shown an oncogenic effect of long-term administration of atrazine in the diet, while atrazine administered by injection has been found to induce lymphomas in male mice. Some long-term animal feeding studies with atrazine have found treatment-related tumors in the uterus and lymphatic system and in the pituitary in female rats. These studies are summarized below.

1. Mice:

An unpublished mouse study (Hazelette and Green, 1988) was located in summary form in the Special Review Document on atrazine (USEPA, 1994), a Drinking Water Criteria Document (USEPA, 1989) and in the EPA Integrated Risk Assessment System (IRIS) Database (IRIS, 1998). Male and female CD-1 mice (60 per dose per sex) were fed 0, 10, 300, 1500, or 3:000 ppm atrazine starting at five weeks of age for 91 weeks. While cardiac thrombi was observed in the animals fed 1500 and 3000 ppm atrazine and contributed to unscheduled deaths before the end of the study, there were no treatment-related effects of atrazine on the incidence of any tumors (USEPA, 1989). The number of animals per group, and the duration of treatment were adequate for assessing the oncogenicity of atrazine. However, other aspects of this cancer bioassay could not be evaluated because of the limited amount of information available in the summaries. For instance, no information was available on how the histopathological evaluation of the tissues was conducted. There did appear to be an effect on

body weight that was treatment related. Body weights after three months of treatment were reduced 33% in males and 14% in females receiving 3000 ppm atrazine, while body weights in the 1500 ppm group were reduced by 40.6% for males and 12.1% in females, respectively. This suggests that the Maximum Tolerated Dose (MTD) was exceeded in the females receiving 3000 ppm atrazine, and in the males receiving 1500 ppm atrazine. Body weight changes observed in the 1500 ppm and 3000 ppm atrazine-treated groups may have altered the sensitivity of the bioassay to detect an oncogenic effect. Weight reduction data in the control group and in animals fed the lower doses of atrazine were not available in the summary (IRIS, 1998).

The effect of triazines on tumor induction in mice was evaluated by injecting Fogard S (a formulation composed of 25% atrazine and 37.5% simazine) into 25 female Swiss albino mice at three-day intervals over a 39-day period for a total of 6.5 mg active principal per mouse (Donna et al., 1981). The control group of 50 animals received an equivalent amount of saline by subcutaneous injection. Animals were killed at timed intervals over a seven month period, and it was determined that a significantly ($p < 0.01$) higher number of lymphomas were detected in the Forgard S treated animals (3/24) compared to the controls (0/50). This study did have some limitations as a cancer bioassay, including an inappropriate route of administration by subcutaneous injection, limited duration of treatment, limited observation time (seven months), inadequate number of animals per group (only 25) and the loss of animals due to disease (black scours).

Donna et al. (1986) conducted a second study evaluating the oncogenic effect of atrazine in mice. Thirty four-week-old male Swiss albino mice received 13 injections (i.p.) of a 0.25 ml atrazine solution every three days (total dose of atrazine 0.26 mg/kg bd wt) during the 39-day dosing period. Control groups consisted of 50 mice injected with 0.25 ml of saline and 50 untreated mice. Surviving animals were killed after 375 days of observation. The incidence of lymphomas was significantly increased ($p < 0.001$) in the atrazine-treated mice (6/30) compared to saline-treated (0/50) and (1/50) untreated controls.

2. Rats :

A life-time carcinogenicity bioassay of technical grade atrazine (98.9% purity) was performed on female and male Fischer 344 / LATI rats (Pinter et al., 1990). Male and female rats weighing 150 to 180 grams ($n = 50$-56 per dose per group per sex) were fed 0, 500 and 1000 ppm atrazine in the diet for the first eight weeks of treatment, but because of toxicity symptoms, including changes in body weight and water consumption, mid- and high-doses were lowered to 375 and 750 ppm for the duration of the 126 week dosing period. While survival rate was similar in the females in the atrazine-treated and control groups, in male rats survival rate was significantly higher in the 375 ppm ($p = 0.019$) and 750 ppm ($p < 0.0001$) atrazine-fed groups compared to controls. Complete autopsies were performed on all animals who died during the course of the experiment and all tissues were examined histopathologically.

In the female rats there was a significant trend (Cochran-Armitage trend test, $p < 0.05$) for a higher incidence of the total number of malignant uterine tumors in the high-dose 750 ppm (14/45) atrazine-treated group compared to controls (7/45). The authors noted that the incidence of endometrial carcinomas is generally low in F344 rats (1.4%). Even with the higher rate of uterine adenocarcinomas in the control group of 13.3% (6/45), the incidence of 28.8% (13/4) in the high dose 750 ppm atrazine group was still significant ($p < 0.05$). However, there were no treatment related differences in the total number of benign and malignant uterine tumors in the 750 ppm (19/52) and 375 ppm (17/45) atrazine-treated groups compared to controls (16/45). The combined incidence of leukemias and lymphomas was significantly higher (Cohran-Armitage trend test, $p < 0.05$) in 750 ppm atrazine-treated females (22/51) compared to controls (12/44). However, there were no significant-treatment related differences when the incidence of leukemia and lymphomas were evaluated separately in the females. There was no treatment-related effect on leukemias or lymphomas in male rats. While there was an increased incidence in pituitary adenomas in the males fed 375 ppm (21/41) and 700 ppm atrazine diets (17/43), compared to controls (9/42), this effect was not statistically significant (Pinter et al., 1990).

A 104-week atrazine oncogenicity study, conducted in male and female Fischer 344 (F344) fed 0, 10, 70, 200 and 400 ppm atrazine, did not find an increased incidence of uterine tumors, or lymphomas and / or leukemias in atrazine-treated animals (Thakur et al., 1998). Treatment and control groups consisted of 60 animals per dose per sex. Complete autopsies were performed on all animals at the end of the 104 week study, and a histopathological evaluation was performed on all tissues. Differences in the results of this study and the Pinter study may be due to the differences in atrazine dose levels used in these studies. Pinter observed treatment-related effects only at the high dose level (750 ppm) and not at the lower dose (375 ppm) of atrazine. The 375 ppm level of atrazine used in the Pinter study is similar to the highest dose (400 ppm) of atrazine used in the Thakur study.

In another study, female Sprague-Dawley (SD) rats fed 0, 10, 70, 500, and 1000 ppm atrazine (96% pure) for their lifetimes developed a significantly higher incidence ($p \leq 0.05$) of pituitary tumors (combined adenomas and carcinomas) in the 500 ppm group (61/70) compared to controls (47/70) (Stevens et al., 1994). There were no significant treatment-related differences when the pituitary tumor incidence was reported separately for adenomas and carcinomas, nor were treatment differences observed at the 1000 ppm dose level. This study was only available in summary form, and full details of the experimental design and histopathological evaluation were not available.

3. Summary, Experimental Animal Studies (non-mammary sites):

An oncogenic effect of atrazine has not been observed in long-term feeding studies in mice (Hazelette and Green, 1988). An increase in the incidence of lymphomas has been observed in male Swiss albino mice that were treated with atrazine by injection (Donna et al., 1986). An increased incidence of uterine tumors, and combined incidence of leukemia and lymphomas, has been observed in F344/LATI female rats fed up to 750 ppm atrazine during their lifetimes (Pinter et al., 1990). Other two-year cancer bioassays have not observed an increased incidence in uterine or lymphatic system tumors in F344 male or female rats (Thakur et al., 1998) or in SD female rats (Stevens et al., 1994). The combined incidence of benign and malignant pituitary tumors was significantly increased in female SD rats fed 500 ppm atrazine compared to controls. However, there was no treatment effect when the incidences of pituitary adenomas and carcinomas were analyzed separately (Stevens et al., 1994).

C. Classification of Carcinogenicity by Other Agencies:

1. IARC Classification: Group 2B, possible carcinogen (IARC, 1991). The IARC Working Group concluded that there was inadequate evidence to classify atrazine as a human carcinogen and limited evidence of experimental carcinogenicity in animals. The classification took into account the observation that atrazine has been found to disrupt endocrine pathways along the hypothalamic-pituitary-gonadal axis. Studies in humans and animals indicated sites of carcinogenicity in tissues known to be affected by hormonal factors, including ovarian cancer in women (Donna et al., 1984; Donna et al., 1989) and an increased ncidence of mammary cancer in male rats (Pinter et al., 1990). Note that the IARC bases its carcinogenicity evaluations on studies available in the open-peer reviewed literature. In 1991, none of the Ciba-Geigy studies on the oncogenicity of atrazine in rats or mice had been published (Stevens et al., 1994; Thakur et al., 1992; Thakur et al., 1998; Wetzel et al., 1994).

2. NTP Classification: Not listed in the 8th edition of the *Report on Carcinogens* (USDHHS, 1998).

3. EPA Classification: The EPA's IRIS database indicated the carcinogenicity assessment of atrazine as "pending" (IRIS, 1998). The USEPA had previously classified atrazine as a class C, possible carcinogen (USEPA,1994).

V. Critical Evaluation of Evidence for Breast Cancer Risk

A. Human Breast Cancer Studies:

While an epidemiology survey of female farmers in the US did find an excess mortality associated with diseases of the genital-urinary system, this study did not find an excess mortality from breast cancer (Blair et al., 1993). However, since this occupational information was obtained from death certificates, no pesticide exposure-related information was available on these subjects. Another study of 50,682 Swedish women farmers did not find an excess of breast cancer incidence (Wiklund and Dich, 1994). Although no information was available on the specific types of pesticides used by this population, the authors did note that the most common herbicides used in Sweden have been phenoxy herbicides. It is not known if this population was exposed to triazines. Although a study of Danish women employed in herbicide manufacture (Lynge, 1985) and Japanese study of women rice workers (Vineis et al., 1986) are commonly cited as evidence for a lack of a relationship between pesticide exposure and breast cancer risk, it should be noted that these studies focused on whether exposure to phenoxy herbicides affected cancer incidence. No mention is made of atrazine use in these studies.

An ecological study in Kentucky has recently compared breast cancer rates by county to an index of estimated triazine exposure (Kettles et al., 1997). Age, race, age at first birth, income, and level of education were controlled for using regression analysis. The OR for breast cancer risk with "medium" or "high" levels of triazine exposure was significantly elevated (OR = 1.14, $p < 0.0001$; OR = 1.2, $p < 0.0001$, respectively). However, this study does not provide evidence for a causal relationship between triazine exposure and breast cancer risk, because individual exposures were not determined, and many assumptions were made in calculating the triazine exposure index. Some of these assumptions were not validated. For instance, estimates of levels of triazines in surface water were obtained from reports in the Division of Water of the Kentucky Environmental Protection Cabinet. The results were reported as being "positive or negative;" actual levels of triazines in the surface water were not available. The levels of atrazine, simazine and cyanazine as separate compounds were not available. Triazine use was estimated from figures on the total amount of pesticide used by applicators in each county; specific breakdown of the use of triazine pesticides was not available. A surrogate measure of pesticide exposure, acres of corn planted for the years 1970, 1980, and 1990, was used to estimate atrazine use. However, such data would be expected to also predict use of other corn herbicides, cyanazine (also a triazine) and non-triazines, metolachlor and alachlor. The assumptions made in estimating triazine exposure severely limit the usefulness of the study's index of atrazine exposure and, therefore, limit the usefulness of the results of this study in predicting whether atrazine exposure affects breast cancer risk.

A prospective epidemiology study is currently underway (Agricultural Health Study) in Iowa and North Carolina that will evaluate whether exposures to pesticides, including atrazine, affect the rate of breast cancer in female agricultural workers, pesticide applicators and women who live on farms. It is expected that over 30,000 women will be enrolled in this study (Alavanja et al., 1996).

B. Experimental Animal Studies of Mammary Carcinogenicity:
1. Mice:
Studies in mice have not demonstrated a oncogenic effect of atrazine in the mammary gland. A unpublished 91-week cancer bioassay in CD-1 mice (Hazelette and Green, 1988) was available in summary form in an EPA Drinking Water Criteria document (USEPA, 1989) and in the Special Review document on triazines (USEPA, 1994). In this study, five-week old CD-1 mice (60 animals per sex per dose) received 0, 10, 300, 1500 and 3000 ppm atrazine in the diet for 91 weeks. There were no treatment-related effects on the incidence of mammary tumors. Due to limited information on the experimental design of this study, we could not evaluate if it was an adequate cancer bioassay.

Flaws in the experimental design of several mouse cancer bioassays make them inappropriate to include in an evaluation of atrazine's oncogenic potential. Innes, et al. (1969) found no evidence of treatment-related tumors at any site after oral administration at 21.5 mg/kg (82 ppm) atrazine for 18 months via stomach tube in two strains of mice. This study only administered one dose of atrazine, so a dose-response could not be evaluated. Also, an insufficient number of animals was used to evaluate the effect of long-term atrazine administration on tumorigenesis. A study by Weisenburger et al. (1990 b) on the carcinogenicity of atrazine and *N*-nitrosatrazine in mice also can not be used in this evaluation because of lack of multiple doses, short duration of follow-up, and problems with toxicity and morbidity of the animals.

2. Rats :
The mammary tumorigenesis of atrazine has been evaluated in several strains of rats, including the Fischer 344/LATI, F344, and the SD.. Critiques of these studies are presented below.

Life-time carcinogenicity bioassay of atrazine was performed on female and male F344 /LATI rats (50 animals per dose per sex) (Pinter et al., 1990). Rats (150 to 180 grams bd wt) received 0, 500 and 1000 ppm atrazine in the diet for the first eight weeks of treatment, and because of toxicity symptoms, mid- and high-doses were lowered to 375 and 750 ppm for the duration of the 126 week dosing period. This life-time carcinogenicity study, which was conducted in Europe, had a longer duration than the 104-week cancer bioassay, which is more commonly the duration of long-term assays conducted in the US. While survival rate was similar in the females between treated and control groups, survival rate in the atrazine-treated males was significantly higher than controls. A significantly higher incidence of benign mammary tumors (fibroma, fibroadenoma and adenoma combined) were reported in the high-dose male group (9/53) compared to controls (1/48, $p < 0.01$ by Fischer's exact test; $p < 0.05$ by Peto's incidental tumor test). When the incidences of these tumors were reported separately, there were no significant differences between control and atrazine-treated male F344/LATI rats. In the females, there was a high background incidence of benign mammary tumors in control and both treated groups, but no treatment-related effects were reported (Pinter et al., 1990).

This result is in contrast to an atrazine cancer bioassay conducted in F344 rats reported by Thakur et al. (1998). Male and female F344 rats (60 per dose per sex) were fed 0, 10, 70, 200 or 400 ppm atrazine for 104 weeks. Incidence of mammary tumors in female rats were not reported. There were no treatment-related effects on the incidence of either benign mammary fibroadenomas or malignant mammary adenocarcinomas in the male F344 rats. It has been argued that the difference in the Pinter et al. (1990) and Thakur et al. (1998) studies may be due to differences in their experimental design. The Pinter et al. (1990) study was a life-time cancer bioassay which treated the animals for up to 126 weeks, with some animals surviving in the treated groups as long as 136 weeks. The Thakur et al. (1998) study terminated the experiment 104 weeks after initiating treatment. The longer survival time of the male atrazine-treated groups compared to controls has been cited as a problem in interpreting the results of Pinter et al. (1990) in regard to the development of late-stage tumors in F344 male rats (Thakur et al., 1998). For example, by 110 weeks, about 15% of the controls, 30% of the 375 ppm, and 50% of the 700 ppm male animals survived in the Pinter study. None of the control rats survived beyond 113 weeks, while 10% of the 700 ppm atrazine treated males survived to 136 weeks (Pinter et al., 1990). Thakur et al. (1998) noted in their critique of the Pinter study that six out of the eight mammary gland tumors observed in the 750 ppm atrazine treated male F344 rats occurred after the last male control rat had died. They further argued that these differences in survival rate makes a statistical comparison of tumor incidence between the treated and control groups invalid (Thakur et al., 1998). Another possible explanation may be in differences used in the pathological classification of the tumors. In Pinter's study benign mammary gland tumors were classified as fibromas, fibroadenomas, or adenomas, in contrast to Thakur who used only the fibroadenoma classification to describe benign mammary gland tumors. It is also possible that the European F344/LATI rat has a different responsiveness to atrazine-induction of mammary tumors than the American-bred F344 rats.

Several studies have been conducted evaluating the oncogenicity of atrazine in the SD rat. A two-year chronic feeding study was conducted by Ciba-Geigy in 1986 (Mayhew et al., 1986) and was available in summary form in two EPA publications (USEPA, 1989; USEPA, 1994). Some of the results of this study have been published in the peer-reviewed literature (Stevens et al., 1994). Male and female SD rats at 37-38 days of age (50 per sex per dose), were fed 0, 10, 70, 500 or 1000 ppm technical atrazine (96% pure) for two years. Ten additional rats per sex were fed the high-dose (1000 ppm) atrazine diet for a 12-month interim sacrifice. In the female SD rats, a significant increase ($p < 0.01$) in mammary

fibroadenomas was observed in rats fed the 1000 ppm atrazine diet (46/89) compared to controls (29/88). There was also a significant increase in the incidence of malignant mammary adenocarcinomas in females fed 70 ppm (27/69, $p < 0.05$), 500 ppm (27/70, $p < 0.01$), and 1000 ppm atrazine (45/89, $p< 0.01$), compared to 0 ppm controls (15/88). The incidence of the mammary fibroadenomas and adenocarinomas was outside the historical control range. The incidence of mammary tumors in male SD rats was not reported by Stevens et al. (1994).

Stevens et al. (1994) also reported the incidence of mammary tumors in two other long-term carcinogenicity studies conducted in SD female rats. In a cancer bioassay called "Study 1", female SD rats were fed atrazine at 0, 10, 100, and 1000 ppm for two years. A significant increase in mammary fibroadenomas was observed in treated groups fed atrazine diets at 10 ppm (20/52, $p < 0.05$), and 1000 ppm (22/49, $p <0.01$), but not at 100 ppm (14/54), compared to controls (11/54). However, interpretation of this study is difficult, since complete details on the experimental design were not available in the Stevens et al. (1994) article. A review of this study by the EPA indicated that there was high mortality due to infections in all treatment groups and the control group during the last six months of the study (USEPA, 1989). While these unscheduled deaths compromise the usefulness of this study, it does demonstrate the appearance of mammary tumors even at low levels (10 ppm) of atrazine in the diet in female SD rats.

Another study reported by Stevens et al. (1994), called "Study 3", fed 0, 10, 50 or 500 ppm atrazine in the diet over two years to SD female rats. This study did not find a significant increase in either fibroadenomas or in adenocarcinomas when the incidences of these tumors were reported separately. However, the combined incidence of these mammary tumors was higher in the 10 ppm (10/40, $p < 0.01$) and 50 ppm (13/40, $p < 0.01$), but not the 500 ppm (11/29), atrazine-fed female SD rats compared to controls (11/30).

Effects of feeding atrazine in the diet on mammary tumor incidence in SD and F344 female rats were compared in a two-year cancer bioassay (Wetzel et al., 1994). The treatment and histopathological analysis of the tissues were conducted in this Ciba-sponsored study by Hazelton Laboratories. Starting at six to eight weeks of age, SD rats were fed 0, 70 or 400 ppm atrazine, while F344 females were fed 0, 10, 70, 200, and 400 ppm atrazine. The SD rats were not fed all the same doses as the F344 rats, since previous studies had conducted cancer bioassays at these doses in the SD rat (personal communication with Kerry Miller of Ciba-Geigy). At the termination of the study at 104 weeks, full necropsies were performed on all animals. All tissues were retained from the F344 rats for histopathology, while the pituitary, mammary gland, uterus and ovaries were processed from the SD animals. There were no treatment-related effects on mammary gland tumor incidence in the F344 rats. In the female SD rats, the incidence of mammary gland tumors was significantly elevated in the 400 ppm group (10/45, $p \leq 0.05$), but not in the 70 ppm atrazine group (1/40) compared to controls (1/40). It should be noted that the type (benign/malignant) and the histopathological classification (i.e., adenoma, adenocarcinoma, fibroadenoma, etc.) was not specified. An earlier abstract of this study (Thakur et al., 1992) gave additional information on the onset and types of tumors observed. There was a significant positive trend for mammary fibroadenomas and/or carcinomas and a significant increase in the rate of onset of the mammary gland tumors in the SD rats that received the diet containing 400 ppm atrazine (Thakur et al., 1992). Thakur et al. (1992; 1998) and Wetzel et al. (1994) noted the SD rats fed 400 ppm atrazine were at, or may have exceeded, the MTD as evidenced by loss of body weight.

Because of the differences in the responsiveness, onset, and incidence of mammary tumors in the female SD and F344 rat, considerable attention has been given to determining the mechanism of mammary tumor induction in the SD rat and determining if this mechanism is relevant to atrazine carcinogenicity in humans. Therefore, in Section V. C., "Other Relevant Data on Breast Cancer Risk," we have devoted considerable attention to critiquing the hypotheses that have been put forth to explaining the differences in atrazine-induced mammary tumors in these two rat strains.

Long-term cancer bioassays usually evaluate oncogenic potential of a chemical in both sexes of two species, typically the laboratory mouse and rat. It has been argued that the evidence of atrazine oncogenicity is not strong because it has been identified as a mammary carcinogen only in one strain of female rats and not in either gender of tested mouse strains (Chapin et al., 1996). However, evidence of mammary carcinogenicity only in the female of one species is fairly typical of the chemicals tested for their oncogenic potential by the National Toxicology Program (Dunnick et al., 1995). For instance, as of 1995, 34 chemicals had tested positive for mammary carcinogenicity. Evidence for mammary carcinogenicity according to gender and species was as follows; 62% (21/34) of the chemicals tested positive only in female rats; 15% (5/34) tested positive only in the female mouse; 15% tested positive in both the female mouse and the female rat; 9% (3/34) of the chemicals tested positive for mammary carcinogenicity in both the female and male rat (Dunnick et al., 1995). Therefore, it is not unusual, but actually common, for chemically-induced mammary tumors to be observed only in the females of one species.

3. Summary, Experimental Animal Studies of Mammary Carcinogenicity:

These studies in experimental animals indicate that atrazine did not induce mammary tumors in mice (Hazelette and Green, 1988; USEPA, 1994), or in female F344 rats (Pinter et al, 1991; Wetzel et al, 1994). While an increased incidence in mammary tumors has been reported male F344 rats treated with 750 ppm atrazine in a life-time feeding study (Pinter et al., 1991), the significance of

these results have been called into question because of the different survival rates of controls and treated animals (Thakur et al., 1998). Long-term chronic administration of atrazine in the diet in two-year cancer bioassays induced an increased incidence in malignant mammary tumors in female SD rats fed diets containing 70 ppm to 1000 ppm atrazine and benign mammary tumors in rats receiving 1000 ppm atrazine (Mayhew et al., 1986; Stevens et al., 1994). Other long-term feeding studies in the SD rat which we could not fully evaluate because of a lack of information on the experimental design of the studies, reported an increased incidence of mammary tumors in SD females fed 10 ppm and 1000 ppm atrazine (Study 1 as cited in Stevens et al., 1994) and combined incidence of benign and malignant mammary tumors in SD rats fed 10 or 50 ppm atrazine compared to controls (Study 3 as cited in Stevens et al., 1994). Other long-term atrazine feeding studies in the SD female rat have reported a decreased latency in appearance of mammary gland tumors in the high-dose group (1000 ppm) (Thakur et al., 1992; Wetzel et al., 1994). While results have not been consistent study to study, in the SD rat dietary administration of atrazine has resulted in an increase incidence or earlier appearance of mammary tumors over a wide dose range (10 to 1000 ppm).

C. Other Relevant Data on Breast Cancer Risk:

1. Evidence of Estrogenicity:

Atrazine does not appear to be estrogenic when tested either *in vivo* or *in vitro*. In *in vivo* assays using immature rat uteri or the uteri from ovariectomized (bilateral removal of ovaries) rats, atrazine did not stimulate an increase in uterine wet weight gain (Connor et al., 1998; Eldridge et al., 1994 b). A uterotropic response is considered to be an indication of a chemical's estrogenicity. The ability of atrazine to suppress estrogen-stimulated uterine weight gain was evaluated by Tennant et al. (1994). Ovariectomized female rats were gavaged daily with vehicle or 20, 100 or 300 mg atrazine/kg for three days; 2 mg of estradiol was administered on day two and three. Estrogen-dependent uterine wet weight was significantly depressed ($p < 0.05$) in animals receiving 100 and 300 mg atrazine/kg, but not in animals receiving 10 mg atrazine/kg, compared to controls that received vehicle (Tennant et al., 1994). Atrazine's effect on estrogen-stimulated cell proliferation in the uterus was also evaluated. Uterine cell proliferation was measured by tritiated thymidine incorporation in the uteri of ovariectomized rats treated with atrazine (1, 10, 50, 100, 300 mg/kg) or vehicle for two days, followed by injection of 0.15 μg of estradiol on day two. Atrazine significantly suppressed estrogen-stimulated uterine thymidine incorporation in rats that received 50 to 300 mg atrazine/kg ($p < 0.05$), but not in animals that received the 1 or 10 mg/kg dose of atrazine. The authors suggest that the suppression of estrogen-dependent uterine weight gain and thymidine incorporation indicates that atrazine may have anti-estrogenic properties when administered at higher doses (Tennant et al., 1994).

In vitro studies have also shown a lack of atrazine estrogenicity. In the E-SCREEN test for estrogenicity, atrazine did not stimulate cell proliferation in an estrogen-dependent MCF-7 human breast tumor cell line (Soto et al., 1995). A lack of an estrogenic response was also observed in atrazine-treated HeLa cells transfected with a Gal4-human estrogen receptor (hER) chimeric construct and a luciferase reporter gene. The lack of induction in receptor gene activity in atrazine-treated cells suggests that atrazine does not interact with the estrogen receptor (Balaguer et al., 1996). A lack of an estrogenic response was similarly observed in atrazine-treated yeast transfected with hER and an estrogen-sensitive reporter. Competition binding assays demonstrated that atrazine displaced radiolabeled estradiol from the recombinant hER, suggesting that atrazine may have some receptor-mediated anti-estrogenic activity (Tran et al., 1996). These *in vivo* and *in vitro* assays demonstrate that atrazine is not estrogenic and may, at certain doses, have an anti-estrogenic effect.

2. Evidence of Hormone Disruption:

Atrazine has been shown to effect endocrine pathways, including gonadal steroids, in both female and male animals. Some of these effects appear to be at the hormone receptor level, or by directly affecting the activity of enzymes involved with steroidogenesis.

Atrazine has the capacity to inhibit the formation of the estrogen-receptor complex (Tezak et al., 1992). *In vitro* studies indicated that the number of binding sites for estradiol in the rat uterus cytosol decreased as the concentration of atrazine in the incubation media increased. The decrease in number of free binding sites on the estrogen receptor molecules was the same at each of the dosing levels; i.e. not dose dependent.

In another study, daily injections of atrazine and the atrazine metabolite deethylatrazine to rat dams during pregnancy and lactation resulted in slow maturation of the pups' gonadotropic system as a consequence of modified male and female pituitary 5-alpha-reductase activity (Kniewald et al., 1987). In 28-day old female progeny from dams treated with atrazine during gestation, anterior pituitary 5-alpha dehydrotestosterone (DHT) activity was significantly elevated in atrazine-treated animals compared to controls (Kniewald et al., 1987). In other studies, atrazine or deethylatrazine administration inhibited anterior pituitary 5-alpha reductase and 17β–estradiol activity (Babic-Gojmerac et al., 1989). What effects these changes in steroid metabolism may have in terms of effects on subsequent sexual differentiation and reproductive tract development in male or female animals, or mammary gland development, have not been evaluated. However, these results do show that atrazine can affect hormonal receptor levels and pathways in endocrine tissues.

Other researches have investigated whether atrazine can affect the metabolism of estradiol (Bradlow et al., 1995). Estradiol can be

hydroxylated at either the C-2 or C-16-alpha position. The 2-hydroxyestrone (2-OHE1) metabolite is weakly estrogenic, and there is some evidence that 2-OHE1 may inhibit breast cell proliferation. Hydroxylation to form 16-alpha hydroxyestrone (16-OHE1) yields a metabolite that has been associated with enhanced breast cell growth, increased unscheduled DNA synthesis, and anchorage-independent growth. This suggests that the 16-OHE1 pathway may be associated with genotoxic events that may increase breast cancer risk. To determine if environmental contaminants influence the hydroxylation pathway of estradiol, MCF-7 estrogen-receptor positive human breast cancer cells were grown in the presence of different pesticides, including atrazine. The ratio of 2-OHE1 to 16-OHE1 was determined after a 48 hour incubation period. The ratio was increased from control values of 1 to approximately 12 in the atrazine treated cells. The authors suggest that this indicates atrazine can have an effect on the endogenous production of estrogen metabolites, and that by altering the ratio of 16-OHE1 to 2-OHE1, atrazine may increase breast cancer risk. Further research will be needed to confirm these results. The authors are investigating whether the ratio of 16-OHE1 to 2-OHE1 in the urine can be used to predict breast cancer risk in humans as a part of the Long Island Breast Cancer Study (personal communication, L. Bradlow). Others have not been able to demonstrate that atrazine elevates the ratio of 16-OHE1 / 2-OHE1 in MCF-7 breast cancer cells. Unlike the Bradlow study which demonstrated a ratio of approximately 12, McDougal and Safe (1998) reported a mean 16-OHE1 / 2-OHE1 ratio of only 0.71 in atrazine-treated MCF-7 cells. Further studies will be needed to resolve the different results observed by these two laboratories.

3. Commentary on Cycling and Hormonal Changes in the Aging SD Rat:

There has been considerable debate on whether the induction of a higher incidence and/or earlier appearance of mammary tumors in the female SD rat, but not the female F344 rat, is solely due to an induction of "premature reproductive aging" in the female SD rat. A number of papers and presentations at the 1995 Society of Toxicology (Chapin et al., 1996) and the 1996 American Chemical Society meetings (Eldridge et al., 1998; Simpkins et al., 1998; Thakur et al., 1998) have been devoted to exploring this hypothesis. The hypothesis suggests that atrazine is not a direct-acting mammary carcinogen, but that promotion of mammary tumor development in atrazine-treated SD female rats is dependent on induction of endocrine-related events, and that these endocrine changes only occur after a certain threshold of exposure to atrazine is exceeded. It is hypothesized that when the threshold of exposure is exceeded, neuroendocrine events are affected that result in a hormonal environment which would support mammary tumor growth in the female SD rat. This hypothesis suggests that long term atrazine administration in the SD female rat: 1) lengthens the reproductive estrous cycle, 2) increases the number of days in estrus, and 3) thereby increases exposure to estrogen (Wetzel et al., 1994). In the aging female F344 rat, a different hormonal environment is present, and it is suggested that this is one reason why the female F344 rat has a lower rate of spontaneous mammary gland tumors and a lack of responsiveness to mammary tumor induction when treated with atrazine. We will critique the hypothesis and the strength of the evidence supporting the "atrazine-reproductive aging" theory. First, it is necessary to briefly review the reproductive cycle of the rat and hormonal changes associated with changes in the cycle and during aging.

a. Hormonal Events in the Normal Cycling and Aging Rodent:
The reproductive cycle in rodents is characterized by a four to five day cycle called the estrous cycle (Cooper et al., 1986; Nelson et al., 1982). In the rat, phases of the cycle include one to two days of diestrus, proestrus, and estrus (Nequin et al., 1979). The phases of the cycle can be characterized by obtaining vaginal smears of cells, and predominance of certain cell types corresponds to the stage of the cycle. These changes in vaginal cytology are in response to cyclic changes in the levels of ovarian and pituitary hormones (Cooper et al., 1986). Sequential rise of the ovarian steroid estrogen, followed by a rise in progesterone in the blood during proestrus affects the timing and amplitude of the pre-ovulatory surge of luteinizing hormone (LH) from the pituitary. The LH surge affects other gonadotropins which regulate ovulation (Cooper et al., 1980; Cooper et al., 1986). During mid-life in 8-12 month old rats, changes occur in the amplitude and timing of the LH surge (Wise, 1987; Wise et al., 1991). Cycles become irregular during this transitional period. In most strains of rats prolonged periods estrus or constant estrus (CE) and/or periods of persistent diestrus and a pseudopregnant state are observed as the animals age (Cooper and Walker, 1979; Huang and Meites, 1975; Huang et al., 1978). In very old animals (greater than 24 months) ovaries become non-functional, and the animals enter an anestrus state characterized by low levels of serum estrogen (Cooper et al., 1986; Huang et al., 1978). Reproductive aging in rodents is under complex neuroendocrine control, and the possible mechanisms which may be responsible for the aging process have been the subject of several reviews (Cooper et al., 1986; Finch et al., 1984; Wise et al., 1991).

b. Discussion of the Atrazine-Induced Premature Reproductive Aging Hypothesis:
The SD female rat has a high incidence of mammary gland tumors, and in several studies atrazine-treated SD rats display periods of prolonged estrus (Stevens et al., 1994; Wetzel et al., 1994). It was originally hypothesized that the atrazine-treated SD rat has an accelerated onset of reproductive aging, characterized by periods of CE, and it was suggested that periods of CE would result in periods of elevated serum estradiol (Stevens et al., 1994). These elevations in serum estrogen would then provide the stimulus to support the growth of mammary tumors, resulting in the early onset and greater number of mammary tumors in atrazine-treated female SD rats. This hypothesis was developed further to include possible changes in neuroendocrine control in the atrazine-treated female

SD rat (Chapin et al., 1996). It was suggested that an unresponsiveness of noradrenerigic neurons in the hypothalamus to rising levels of estrogen and resulting failure in the release of adequate amounts of gonadotropin releasing factor hormone (GnRH) would result in delayed or absent LH release with no ovulation. It was suggested that the ovarian follicles would then maintain estrogen secretion, resulting in a state of prolonged estrus. The elevated estrogen levels may then provide the stimulus for supporting the growth of mammary tumors. This is in contrast to the aging atrazine-treated F344 rat, which does not show periods of prolonged estrus, but instead displays periods of prolonged diestrus and pseudopregnancy, which is associated with higher levels of progesterone and moderate to low levels of circulating estrogen. It was also hypothesized that the elevation of progesterone in the F344 rats creates an environment that is less conducive to the stimulation of the mammary gland and the formation of mammary gland tumors. Differences in the pattern of prolactin secretion in the two rat strains were also suggested as an explanation for the differences in the rates of spontaneous mammary tumors (Chapin et al., 1996).

If this theory is correct, the following types of evidence would be needed to support it: CE should be the prevalent vaginal cytology pattern observed in aging, non-cycling SD rats; a state of CE should consistently be associated with elevated blood estradiol levels; atrazine-treated SD rats should have an earlier appearance of irregular ovulatory cycles than control animals; atrazine-treated SD rats should have an earlier onset of CE compared with untreated control animals; atrazine-treated F344 rats would not be expected to display a pattern of CE as they age; prolactin levels would be expected to be elevated in atrazine-treated SD rats compared to F344 rats; and atrazine-treated SD rats should show a change in neuroendocrine control during early phases of the reproductive aging process. We will explore the evidence in support of the atrazine-reproductive aging hypotheses.

- **Is CE the prevalent vaginal cytology pattern observed in aging, non-cycling SD rats?**

The studies most frequently cited to describe the changes in cyclicity and circulating hormone levels in the aging rat are the studies of Lu et al. (1979) and Huang and Meites (1975). These studies were conducted using the Long-Evans rat strain. Long-Evans female rats display a pattern of irregular cycling starting at 10 to 12 months of age, which progresses to a state of CE. An animal is considered to be in CE when cells from vaginal smears show a cornified appearance for at least 15 consecutive days. As the animal ages, many in CE enter a state of persistent diestrus starting at 20 months of age. In the female SD rat, CE is not necessarily the only vaginal cytology pattern observed in an aging, non-cycling animal. LeVever and McClintock (1988) reported several different reproductive cyclicity patterns in aging SD rats. Some of the SD rats showed a pattern similar to the Long-Evans rats, with cycling animals progressing through phases of irregular cycles, then CE, followed by irregular cycles, and then persistent diestrus. Other groups of SD animals skipped the CE phase, and maintained irregular cycles until they entered persistent diestrus. Therefore, it would appear that there is a wide range of changes in the reproductive cycle of the aging SD rat.

- **Is a state of prolonged estrus or CE always associated with elevated blood estradiol levels?**

One of the incorrect assumptions of the atrazine-reproductive aging theory is that a state of CE is always associated with "elevated" levels of circulating estradiol. The hormonal profile of rats in CE is variable and apparently changes as the animal ages. In CE rats that are middle-aged (8 to 14 months) levels of blood estradiol are similar to levels observed during estrus and diestrus in the young four to five month old cycling animal (Lu et al., 1979). Elevated serum estradiol levels are usually not observed in CE rats until they are greater than 20 months of age, where the levels approach the levels observed in proestrus in the young cycling animal (Huang and Meites, 1975; Lu et al., 1979). Therefore, it is not correct to assume that a state of CE is automatically associated with elevated serum estradiol levels.

Whether blood estradiol levels are elevated in atrazine-treated SD rats with prolonged periods of estrus cannot be determined from the published literature. This is because of a flaw in the experimental design of the studies. The blood samples obtained during the course of a 24-month study in SD and F344 female rats fed up to 400 ppm atrazine were obtained from animals killed during the proestrus phase of the cycle (Wetzel et al., 1994). The method section states that if the animal was not killed on proestrus, it was sacrificed regardless of cycle stage after 21 days of obtaining vaginal smears to determine stage of cycle. No experiments were located in the published literature that specifically determined the level of estradiol in blood samples obtained from atrazine-treated SD rats or F344 rats during estrus in the young cycling animal or during prolonged estrus, CE or prolonged diestrus. Therefore, there are no data that support the hypothesis that atrazine-treated animals that are in a state of prolonged estrus or CE have elevated estradiol levels compared to hormone levels observed in young cycling animals.

This brings up important questions regarding both the cyclicity data and the hormone data in these studies. Since the animals were primarily killed during proestrus, this suggests that the majority of the animals were cycling and were not in either of the non-cycling patterns associated with reproductive aging, i.e. CE (at least 15 days with cornified vaginal smears) in SD rats, or persistent diestrus (at least 15 days of smears of primarily leukocytes) in F344 rats. Estradiol values for blood samples obtained at proestrus and other phases of the cycle were not reported separately, so the exact stage of the cycle for the hormonal data points is not known.

- **Do atrazine-treated SD rats have an earlier appearance of irregular ovulatory cycles, and earlier onset of prolonged or constant estrus compared to untreated control SD rats? Are there differences in the timing of the onset and patterns of reproductive aging in atrazine-treated SD animals compared to treated F344 rats?**

The chronic exposure studies that have evaluated cyclicity changes with time in atrazine-treated rats have not determined when irregular cycles have started. Instead, the percent time in different phases of the cycle was determined. The percent days in estrus was determined in SD and F344 female rats by rating vaginal smears taken for 14 to 21 days after 1, 3, 9, 12, 15, 18 or 24 months of treatment with up to 400 ppm atrazine (Wetzel et al., 1994). Percent days spent in estrus for F344 rats were similar in control and atrazine-treated rats for the one through 15 month time periods, with means in the range of 20 to 28 percent days. In SD rats, percent days spent in estrus was significantly greater ($p \leq 0.05$) after one month of atrazine treatment in the 70 ppm group (23.5 ± 5.0) compared to controls (19.0 ± 3.9). No atrazine effect was observed at three months, but at nine months, percent days in estrus was significantly greater in both 70 ppm (34.3 ± 9.0; $p \leq 0.05$) and 400 ppm atrazine-treated groups (44.8 ± 11.5; $p \leq 0.01$) compared to controls (24.2 ± 7.6). The proportion of time spent in estrus increased with age after 15 and 18 months in SD rats, while the percent time spent in estrus decreased in the F344 rats.

Short-term studies in SD-rats evaluating the effect of atrazine treatment on cyclicity have yielded similar results. Eldridge et al. (1994 a) treated SD and F344 female rats by gavage with 100 or 300 mg/kg/day of atrazine for two weeks to evaluate effects on changes in the reproductive cycle. They reported a slight, but significant lengthening of the estrous cycle in the SD atrazine-treated animals compared to controls, with a significant increase in time spent in estrus and a reduction in the time spent in diestrus. Atrazine-treated F344 rats spent less time in estrus and more time in diestrus than the SD atrazine-treated rats.

In a study of similar design, Cooper et al. (1996) administered atrazine by gavage for 21 days to cycling female SD rats at 75, 150, and 300 mg/kg/day. Although a disruption of the reproductive cycle was observed, a pattern of CE was not observed in atrazine treated animals. At the 75 mg/kg/day dose, irregular cycles with no consistent pattern in the cycle were observed, while at the two higher doses of atrazine, repetitive pseudopregnancies characterized by persistent diestrus was observed. There was also no evidence of elevated serum estradiol levels at any of the atrazine doses tested. The authors concluded that although atrazine changed the endocrine profile of these animals, there was no evidence of a hormonal pattern, such as elevated estradiol levels, that would support the theory that atrazine creates a hormonal environment that would promote mammary tumor growth.

These studies indicate that while atrazine treatment does cause an early disruption of the reproductive cycle in both long-term and short-term feeding studies, a variety of patterns are observed. In the atrazine treated-SD female rat prolonged estrus was observed (Eldridge et al., 1994 a; Wetzel et al., 1994) while in another study, irregular cycles and persistent diestrus was observed (Cooper et al., 1996). In F344 rats, atrazine did not induce prolonged estrus. Increased time was spent in proestrus in a chronic feeding study as the female F344 rats aged, but there was no treatment-related effect, while increased time was spent in diestrus in atrazine-treated female F344 rats in a short-term, high-dose study (Eldridge et al., 1994 a; Wetzel et al., 1994).

- **Are estradiol levels in aging atrazine-treated female SD rats in CE higher than levels in SD or F344 rats in a state of prolonged diestrus?**

As was mentioned previously, in both long- and short-term atrazine bioassays, hormone analysis was conducted on blood samples obtained from rats during the proestrus phase of the reproductive cycle. This makes it very difficult to interpret the significance of the ovarian steroid hormone levels or the estradiol/progesterone ratios, reported in the studies published to date (Chapin et al., 1996; Eldridge et al., 1994 a; Wetzel et al., 1994). Studies need to be conducted that analyze the ovarian hormone levels of SD rats in prolonged and constant estrus compared to levels observed during persistent diestrus in the F344 atrazine-treated rats.

The reproductive cycles and the ovulatory state of the animals in short-term and long-term atrazine cancer bioassays need to be better characterized. Instead of reporting percent time in certain phases of the cycle, vaginal cytology should be used to stage the animal using more conventional ratings. This would include four-day estrous cycle, five-day estrous cycle, irregular cycles, prolonged phase of estrus (greater than four consecutive days in estrus), constant estrus (greater than 15 days in estrus), persistent diestrus (greater than 15 days in dietrus), persistent diestrus with pseudopregnancy (persistent diestrus with corpus luteum present in histological sections of the ovary), or anestrus (small, nonfunctioning ovaries).

- **Are blood prolactin levels elevated in atrazine-treated SD rats compared to F344 rats?**

The atrazine-reproductive aging hypothesis also suggests that differences in the pattern of prolactin secretion may explain the higher incidence of spontaneous mammary tumors in the SD rat compared to the F344 rat (Chapin et al., 1996; Wetzel et al., 1994). Elevations in serum prolactin levels induced by hypothalamic lesions had been shown to increase the number of spontaneous mammary tumors observed in the female SD rat, suggesting a role of prolactin in mammary tumorigenesis (Meites, 1972). However,

when prolactin levels were monitored during the course of a 24-month chronic feeding study in F344 and SD female rats fed 0 to 400 ppm atrazine, with the exception of one time point, there were few differences in the magnitude or pattern of serum prolactin levels in the two strains of animals (Wetzel et al., 1994). Prolactin levels were monitored after 1, 3, 9, 12, 15, 18, and 24 months of atrazine treatment. Blood samples from early time points were hemolyzed and unavailable for hormone analysis. Serum prolactin levels were very high in F344 rats after nine months of treatment (means ranged from 55 to 86 pg/ml, no treatment effect) and were significantly higher ($p < 0.01$) in 400 ppm atrazine treated SD female rats (45.8 ± 20.0 pg/ml, $p < 0.01$) compared to controls (17.8 ± 12.4 pg/ml). However, for all the remaining time points from 12 months to 24 months mean plasma prolactin levels were very similar in the F344 and SD rats, in the range of 10 to 22 pg/ml. There were no treatment-related effects, nor trends with aging in either rat strain with regard to prolactin levels. Therefore, in the study by Wetzel et al. (1994) there were no patterns in blood prolactin levels in SD and F344 control or atrazine-treated rats receiving up to 400 ppm that would explain differences in the earlier appearance of mammary tumors in the SD rats compared to the F344 rats (Note: the units of prolactin in the Wetzel study may not be correct, since prolactin is usually reported in ng/ml and not as pg/ml). In a recently reported abstract, Cooper et al. (1998) have demonstrated that atrazine can depress the estrogen-induced prolactin surge in ovarectomized female rats. These studies suggest that atrazine does not consistently induce elevated prolactin levels in rats, and in some studies atrazine has been shown to depress estrogen-dependent surges of prolactin.

Others have found that elevated prolactin levels in rats are associated with specific pathologies in the pituitary. Lu et al. (1979) found that serum prolactin levels were low (below 50 ng/ml) in CE Long-Evans rats aged up to 16 months and prolactin levels did not rise until the animals were 25 to 30 months of age. The highest levels of prolactin in CE rats (greater than 475 ng/ml) were associated with animals greater than 25 months of age that had hemorrhagic pituitaries.

There is some indication that extremely high doses of triazines over long periods of time can induce pituitary tumors associated with elevations in serum prolactin levels. Serum prolactin levels were significantly higher ($p \leq 0.01$) in female SD rats treated with 1000 ppm simazine for two years (204 ± 147 ng/ml) compared to controls (29 ± 18 ng/ml). Prolactin levels were not significantly elevated in rats receiving 10 or 100 ppm simazine compared to controls. The incidence of mammary gland fibroadenomas and adenomas was also significantly increased ($p < 0.01$) only in the rats receiving the 1000 ppm simazine diet.

Unfortunately, serum prolactin levels were not reported in a similar study which fed atrazine at 0, 10, 70, 500, and 1000 ppm to female SD rats for 24 months (Stevens et al., 1994). However, while there was a significant elevation in mammary adenocarcinomas in the 70 to 1000 ppm atrazine-treated groups, the incidence of pituitary tumors was significantly elevated in only the 500 ppm atrazine treated rats, and only when the pituitary tumors were reported as the combined incidence of malignant and benign tumors. There were no treatment-related effects when pituitary adenomas and carcinomas were reported separately. This suggests that incidence of mammary tumors and pituitary lesions is not necessarily related in atrazine-treated SD rats. Further analysis of the pathology of the study of Stevens et al. (1994) should determine if the animals with mammary gland tumors also had tumors or other lesions of the pituitary.

- **Do atrazine-treated SD rats show a change in neuroendocrine control during early phases of the reproductive aging process ?**

Decreased amplitude and delayed timing of the LH surge during proestrus has been observed in mid-aged cycling SD animals just prior to when the mid-aged rat starts showing a pattern of irregular cycles (Wise, 1987). It has been hypothesized that atrazine may induce premature reproductive aging by affecting the amplitude of the LH surge. In a series of three experiments reported by Simpkins et al. (1998), studies were conducted to evaluate the effect of atrazine treatment on the amplitude and timing of the LH surge in ovariectomized female SD rats implanted with a sustained-release capsule of estradiol. In the first experiment rats were ovariectomized and implanted with a capsule containing 4 mg/ml estradiol in sesame oil and then gavaged with vehicle or with atrazine at 300 mg/kg bd wt for three days. On the third day of treatment, animals were killed at 11:00, 13:00, 15:00, and 22:00 hours to obtain trunk blood samples for determination of plasma LH levels. Vehicle-treated animals displayed an estrogen-induced LH surge at 18:00 hours, while atrazine-treated animals did not show an LH surge at 18:00 hours and only showed a blunted rise in LH levels at 22:00 hours. This result indicates that the estrogen-induced LH surge was delayed and blunted in the atrazine-treated animals.

A second longer term study was conducted to determine the dose-response effects of atrazine treatment on the LH surge (Simpkins et al., 1998). Female SD rats were gavaged with 0, 2, 5, 40 or 200 mg/kg/day of atrazine for 28 days, were ovariectomized on day 28 and implanted with an estradiol-containing capsule, and were continued on the atrazine treatments for three additional days. Animals were killed at 11:00, 14:00, 16:00, 18:00, 20:00, 22:00 and 24:00 hours. Vehicle-treated animals showed an LH surge that peaked at 16:00 to 18:00 hours. The LH surge was delayed in some of the atrazine-treated animals. The rise in LH was significantly less at 16 hours in the animals receiving 200 mg/kg bd wt atrazine compared to vehicle controls. Patterns of plasma LH with the lower doses of atrazine (2.5 to 40 mg/kg bd wt) were similar to LH levels in vehicle-dosed animals.

Another experiment of the same design as the second study was conducted, except sequential blood samples were obtained from the same animals for each time point. While all of the atrazine-treated SD rats had an LH peak at 18 hours, the amplitude of the peak was diminished in the 200 mg atrazine/kg dose and to a lesser degree in the 40 mg/kg atrazine-treated animals compared to vehicle controls. Statistical analysis on the differences in LH amplitude in the atrazine and vehicle-treated rats was not available.

The authors suggested that the depressed amplitude in the LH surge in SD rats is consistent with the hypothesis that atrazine can cause premature aging of the SD rat and reduce the age at which CE occurs (Simpkins et al., 1998). While the study does demonstrate that high doses of atrazine have the capacity to affect the amplitude and timing of the LH surge in estradiol-implanted ovariectomized animals, it is not known if this effect is directly responsible for the earlier onset of prolonged estrus in the atrazine-treated SD rat.

The LH surge has been shown to be altered in amplitude and timing during middle age in both cycling and irregularly cycling rodents (Cooper et al., 1980; Wise, 1984; Wise et al., 1991). Cooper et al. (1980) suggested that a delay in the secretion of LH in the middle-aged female may be due to changes in neural timing mechanisms that control the release of GnRH. Others have shown that middle aged rodents have an altered response to estradiol and a decreased pituitary responsiveness to GnRH stimulation (Wise, 1984). Ovariectomized middle-aged rats required a longer exposure to estradiol than younger rats in order for an LH surge to occur, and even when the LH surge occurred, it was delayed by one hour and had a lower amplitude than LH surges observed in younger rats. Pituitary responsiveness to injections of GnRH were blunted in middle-aged rats compared to younger rats (Wise, 1984). It is not known if the atrazine-induced delay in the LH surge similarly affects responsiveness to GnRH. The neuroendocrine events controlling reproductive aging in the rat are extremely complex, making it difficult to know whether events in the pituitary or changes in levels of gonadotropins or ovarian steroids are responsible for the changes in the aging process. It is not known whether the anti-estrogenic properties of atrazine may contribute to a lack of responsiveness to estradiol. This is another possible mechanism by which atrazine could disrupt endocrine events leading to a depressed amplitude in the LH surge.

c. Summary:

There are some aspects of experimental studies which support the hypothesis that atrazine causes premature reproductive aging in the SD rat and that the resulting hormonal environment may support the development of mammary tumors, while other studies do not support the reproductive aging hypothesis. There are also assumptions made in the original hypothesis which may not be correct. Atrazine does appear to cause an earlier onset of periods of prolonged estrus in the SD rat and not in the F344 rat in chronic feeding studies. However, whether differences in reproductive aging in the two strains result in different levels or ratios of ovarian hormones could not be determined, because blood samples were obtained during the period of proestrus and not during the predominant pattern of cycling in the respective strains of aging rats, e.g. persistent diestrus in the F344 rat and prolonged estrus in the SD rat. There are also inconsistencies in short-term dosing studies, since some have shown that high-doses of atrazine induce a pattern of prolonged estrus, while others have demonstrated that atrazine induces a state of persistent diestrus. Neither of the short term dosing studies demonstrated an elevation in serum estradiol levels with atrazine dosing. There does not appear to be evidence that blood prolactin levels are affected by atrazine treatment in either rat strain in animals dosed with up to 400 ppm atrazine in chronic two-year feeding studies. Studies have shown that high doses of atrazine administered for 3 to 28 days to female SD rats can reduce the amplitude of the LH surge in estrogen-implanted, ovariectomized SD rats. It has been hypothesized that this may contribute to accelerated reproductive aging in the SD rat.

It should be noted that the weakest link in the atrazine-reproductive aging hypothesis is the assumption that when the female atrazine-treated SD rat is in prolonged estrus or CE, it will have elevated serum estradiol levels. Further data are needed on the levels of serum estradiol and progesterone during normal cycles, when atrazine-induced irregular reproductive cycles begin, and during periods of prolonged estrus, CE, and persistent diestrus in the SD and F344 aging female rat. At the present time, there is insufficient evidence to conclude that accelerated reproductive aging is the sole cause of the increased incidence or decreased latency of mammary tumors observed in atrazine-treated female SD rats.

4. Reproductive Toxicity:

Reproductive toxicity of atrazine has been evaluated in rats and rabbits, with few or no adverse effects on reproduction reported. One ecological study has examined the relationship between atrazine levels in the water supply in Iowa and the incidence of intrauterine growth retardation. These studies are summarized below.

Following mating, atrazine was administered orally by gavage at 0, 10, 70 or 700 mg/kg/day on gestational days 6 to 15 to female Charles River CD rats [Crl: COBS CD (SD) BR]. At the high dose, 700 mg/kg, atrazine was toxic to the dams, and 21/28 of the dams died. At the low and mid-doses, there was no effect on number of animals implanted, percent pregnant, number of implantations per litter, resorptions per litter, fetal sex ratio or fetal weight of pups in either gender. There were no treatment-related visceral or skeletal malformations. Minor skeletal variations, including incomplete ossification of the skull, hypoid, teeth, forepaw metacarpals, and hind paw distal phalanges, were significantly elevated in the mid-dose 70 mg/kg atrazine-treated group compared to controls (Infurna et al., 1988).

The effects of a pesticide/fertilizer mixture based on levels of pesticides found in California and Iowa groundwater were used to evaluate the reproductive toxicity of these mixtures administered in drinking water to COBS Crl:CD-1 (ICR) BR VAF/Plus outbred mice (Heindel et al., 1994). Both the fertilizer mixture (California mix) and the pesticide mixture (Iowa mix) contained atrazine (1X=0.05 mg/L) and other pesticides at concentrations of 1X, 10X and 100X of median groundwater concentrations. This study was conducted using a modification of the National Toxicology Program's Reproductive Assessment by Continuous Breeding (RACB) protocol. Breeding pairs in the F_0 generation (40 breeding pairs for controls; 20 breeding pairs for each dose) were continuously exposed to the pesticide mixture in drinking water for 98 days. Selected pups from the F_1 litters were maintained on the treatments until 74$\pm$10 days of age, when the males and females from different litters were bred by cohabitation for seven days and then were housed separately until the litters were delivered. There were no treatment-related effects on fertility or any measures of reproductive performance over the two generations of animals.

In a reproductive toxicity study in rabbits treated with 0, 1, 5 or 75 atrazine mg/kg bd wt, maternal toxicity was noted at the 75 ppm dose level. However, reproductive parameters were not affected at the low and mid-dose range compared to controls. There was no effect on the number of pups per litter or weaning weight per pup in dams fed up to 1000 ppm atrazine from day one of gestation. However, when atrazine was administered by injection, atrazine was embryotoxic at 800 or 2000 mg/kg bd wt (Peters and Cook, 1973).

An ecological study conducted in Iowa has examined whether there is an association between levels of herbicides in drinking water and the incidence of intrauterine growth retardation (IUGR), birth weight and prematurity (Munger et al., 1997). It has been previously shown in a 1986-87 state-wide survey of herbicides in drinking water, that levels of atrazine were elevated in the area served by the Rathburn Rural Water System (RRWS) in southern Iowa. This study compared the 1984-1989 drinking water levels of a variety of herbicides from the RRWS to levels in other surface water suppliers, and groundwater sources serving the southern tier of Iowa. The RRWS had the highest levels of atrazine (mean = 2.2 µg/L) compared to other surface water suppliers (0.7 µg/L), and groundwater sources (0 µg/L). The area served by the RRWS had a greater risk of IUGR (RR= 1.8; 95% CI 1.3-2.7) than other southern Iowa communities. To examine the relationship between IUGR and levels of herbicides in the water supplies of southern Iowa, Spearman rank-order correlation coefficients were calculated between age-adjusted community rates of IUGR and levels of water contaminates. Correlations between IUGR and levels of herbicide residues in water supplies were reported were as follows: atrazine ($r = 0.31$, $p=0.001$), metolachlor ($r = 0.28$, $p = 0.004$), and cyanazine ($r = 0.24$, $p = 0.02$). Though these results are suggestive of a relationship, these results do not provide evidence for a causal relationship between IUGR and levels of these herbicides in the water supplies. Since this was an ecological study, there were no individual estimates made of pesticide exposures. No information was available on whether water treatment was used in the home, or if bottled water was consumed during the pregnancy. Also, some of these associations may be due to the socioeconomic characteristics of the communities. For instance, the Rathburn community had higher rates of indicators that predict IUGR and prematurity, including poor prenatal care, less education, lower median income, less participation in the workforce, and higher maternal smoking rates. The results of this study should be regarded as preliminary, and should be followed-up by case control studies which assess individual exposure to environmental contaminants and control for confounding factors which may affect the incidence of IUGR.

5. Mutagenicity and Genotoxicity:

Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides found an increase in chromosomal gaps and 25 fold increase in chromatid breaks in the lymphocytes of workers during mid-season exposure to herbicides, but the usefulness of this information is limited because the workers were exposed to other herbicides besides atrazine (Yoder et al., 1973).

Neither atrazine, hydroxyatrazine, nor AAtrex® (commercial formulation of atrazine) was found to be mutagenic in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1538) in the presence or absence of archlor-induced rat liver extracts (Lusby et al., 1979). A lack of mutagenicity of atrazine or commercial formulations containing atrazine has been observed by others using strains of *Salmonella typhimurium* with and without liver activation (Eisenbeis et al., 1981; Kappas, 1988; Plewa et al., 1984). In contrast, atrazine has been found to increase the rate of reverse mutations at the *wx* locus in maize pollen grains under field conditions (Plewa et al., 1984).

The results of over 50 tests of atrazine's genotoxicity were recently evaluated using a computerized weight-of-evidence scheme (Brusick, 1994). This included tests of atrazine's genotoxicity conducted in bacteria host-mediated assays in yeast and *E. coli*; Sister Chromatid Exchanges (SCE) in rodent cell lines and in human lymphocytes; unscheduled DNA synthesis in rodent and human cell cultures; the mouse dominant lethal assay; and chromosome aberrations in Chinese hamster ovary cells (CHO) and human lymphocytes. The majority of these tests of atrazine's genetic toxicity were negative. The author concluded that atrazine "does not pose a genetic risk for humans under general conditions of use" (Brusick, 1994).

Since the publication of Brusick's evaluation in 1994, there have been both positive and negative reports of atrazine's genotoxicity. Atrazine's ability to induce DNA damage in human lymphocytes was evaluated by the single-cell gel electrophoresis (SCGE) assay

(Ribas et al., 1995). Atrazine tested positive in treatments with and without S9 liver activation. Others have determined that chromosomal damage can be induced in CHO cells by atrazine at concentrations at levels observed in public water supplies (Biradar and Rayburn, 1995). DNA damage was also observed in erythrocytes obtained from frog tadpoles after exposure to atrazine or the commercial formulation AAtrex® using the alkaline SCGE assay (Clements et al., 1997). Atrazine tested positive for genotoxicity in the *in vivo* mouse-bone-marrow micronucleus test in female mice treated with 1400 mg atrazine/kg bd wt (Gebel et al., 1997). However, this was the highest dose tolerated by the animals, and similar effects were not observed in male mice treated with 1750 mg atrazine per kg bd wt.

Others have not observed genotoxic effects of atrazine when evaluated by *in vitro* SCE tests with human lymphocytes or alkaline elution assay with rat hepatocytes, V79 cells, or human lymphocytes (Dunkelberg et al., 1994). Ruiz and Marzin (1997) also failed to observe positive results in mutagenicity tests using bacteria assays (*Salmonella*/microsome mutagenicity assay) or the SOS chromotest (using *E. coli).* Therefore, there is still some debate as to the genotoxicity of atrazine, though the majority of the evidence would indicate this compound is not genotoxic.

6. *N*-Nitrosoatrazine Formation:
Some researchers have indicated concern that farmers who ingest water contaminated with atrazine and nitrate may form *N*-nitrosoatrazine in the presence of acid in the stomach. Nitrosomines are known stomach carcinogens (Janzowski et al., 1980; Meisner et al., 1993) . Studies conducted by the EPA indicated that triazine compounds were free from nitrosomine contamination (Bontoyan et al., 1979). However, few studies have evaluated the distribution, movement, or persistence of *N*-nitrosoatrazine in soils (Kearney et al., 1977). The studies by Kearney, et al. (1977) which examined the possible formation and stability of nitrosoatrazine under natural and artificial conditions indicated that synthetically formed nitrosoatrazine is unstable and is degraded to atrazine and polar products. In most agricultural soils of basic to neutral pH, the formation of nitrosoatrazine is unlikely, even with high rates of nitrogen fertilizer. Under the conditions of acidic soil, nitrosoatrazine formation was small and transient. However, given the presence of acid rain in some parts of the country, and the high use of atrazine as an herbicide, further research is needed to determine the extent and persistence of nitrosoatrazine compounds in soils and water supplies in agricultural areas with high atrazine use and nitrate contamination. This is important in light of recent evidence that human lymphocyte cultures exposed to *N*-nitrosoatrazine as low as 0.0001 µg/ml showed significant elevations in chromosome breakage. Levels of comparable chromosomal breakage with atrazine alone were achieved at concentrations 10,000 fold greater at 1 µg/ml (Meisner et al., 1993).

7. Tumor Promotion:
One study using the F344 rat leukemia transplant model suggested that atrazine may be a potential leukomogen as evidenced by enhanced neoplastic progression in atrazine-treated leukemia transplant recipients (Dieter and Garnett, 1993). It is not known if atrazine can act as a tumor promoter of breast neoplasms. Animal studies are needed to assess atrazine's ability to promote mammary tumors induced by known mammary carcinogens such as dimethyl[*a*]benzanthracene (DMBA).

VI. Other Relevant Information

A. Biomarkers:
Several studies have measured the levels of free atrazine or atrazine metabolites in the urine of animals and humans to assess atrazine exposure. Researchers measured free atrazine in male workers at an atrazine production facility (Catenacci et al., 1990). The authors concluded that while atrazine measured in the urine did reflect the pattern of exposure, the free atrazine only represented a small fraction of the total amount absorbed. Therefore, the measurement of free atrazine in the urine is more appropriately used for a qualitative confirmation rather than a quantitative assessment of recent exposure in humans occupationally exposed to atrazine (Catenacci et al., 1990).

There is some evidence that the sum of two urinary metabolites, the *N*-dealkylated atrazine form, and 2-chloro-4-ethylamino-6-amino-*s*-atrazine (deisopropylatrazine) quantitatively reflected exposure in railway workers exposed to atrazine. Rats given atrazine in drinking water similarly excreted 2-chloro-4-ethylamino-6-amino-*s*-atrazine in a dose dependent fashion (Ikonen et al., 1988)

As a part of the Agricultural Health Study, biological methods are being evaluated to measure atrazine residues in serum and blood in farm families. This includes using mass spectrometric methods to measure atrazine in serum and atrazine, deethylatrazine and atrazine mercapturate in urine (Hill et al., 1996). Pilot studies have been recently conducted to validate methods for assessing atrazine levels in the environment and occupational exposures in agricultural settings. Environmental samples were collected from the air, house dust, dermal patches, hand wipes, indoor surface and equipment wipes, drinking water, solid food, and biological samples (urine and blood) to evaluate methods for determining potential for exposure of farmers to pesticides. This abstract did not provide the results of this study (Hill et al., 1996).

B. Environmental Fate:
The environmental fate of atrazine has been the subject of intensive investigation over the last 35 years, and its retention and transport in soils have been the subject of recent reviews (Flury, 1996; Koskinen and Clay, 1997; Ma and Selim, 1996). The purpose of discussing the environmental fate of atrazine in this Critical

Evaluation is to give the reader an overview of its fate, persistence, and trends in the detections of atrazine and its degradation products in surface water, groundwater and in precipitation. The environmental fate of atrazine can affect the potential for exposure to atrazine and its breakdown products to humans.

1. Persistence and Half-lives of Atrazine and Its Transformation Products:

It has been estimated that the surface run-off of atrazine usually ranges from 0.5% to 3% of the applied atrazine. The highest amount of surface run-off, up to 9% of applied atrazine, has been reported under conditions of intense rainfall one week after application. Whether rainfall or tillage practices has the greatest impact on surface run-off is currently being debated. Some investigators conclude that rainfall pattern, rather than tillage practices, has the greatest impact on atrazine in surface run-off. The potential for atrazine to contaminate surface water is less than its potential to leach into groundwater (Koskinen and Clay, 1997; Ma and Selim, 1996).

In surface soils atrazine is hydrolyzed to form hydroxyatrazine (Harris, 1967; Muir and Baker, 1978). Soils adsorb hydroxyatrazine more strongly than atrazine, and most studies have not found that hydroxyatrazine leaches into sub-soils (Ma and Selim, 1996). In a study of ^{14}C-labeled atrazine degradation products, Sorenson et al. (1993), observed that hydroxyatrazine was the predominant transformation product in the upper 10 cm of sandy loam. The microbial transformation product, deethylatrazine, was found to be the predominant transformation product at lower soil depths of 10-30 cm. Both deethylatrazine and deisopropylatrazine were the more predominant transformation products as soil depth increased, while the proportion of hydroxyatrazine decreased with soil depth. Therefore, the microbial degradation products of atrazine have higher mobility and greater potential to contaminant groundwater than hydroxyatrazine, which is strongly adsorbed onto surface soils.

Degradation of atrazine in soil appears to be bi-phasic. In Midwestern states atrazine rapidly degrades during the first two months after application, followed by a slower degradation during the dry summer months and the cold fall and winter months (Koskinen and Clay, 1997). In Alabama the rate of degradation of atrazine during the warm growing season is relatively fast, with a half-life of 20 days (Buchanan and Hiltbold, 1973). The half-life of atrazine is also affected by soil moisture. Degradation of atrazine was monitored in sandy soil with water holding capacity of 4% (air dried), 35% and 70%. Corresponding half-lives were 151, 37, and 36 days, respectively (Hurle and Kibler, 1976).

Degradation of atrazine in aqueous systems is affected by pH and by temperature. Under laboratory conditions, at 25^{o}C the half-life of atrazine hydrolysis was 34.8 days at pH 2.9, 174 days at pH 4.5, 398 days at pH 6.0, and as long as 742 days at pH 7.0. The half-lives decreased dramatically as the temperature increased. For example, at pH 6.0, the half-life at 40^{o} C was 95.5 days, and at 60^{o} C the half-life of atrazine was only 21.9 days (Khan, 1978).

While most studies have reported half-life for atrazine in soil in the range of 11 to 150 days (Koskinen and Clay, 1997; Muir and Baker, 1978; Redondo, 1997) other studies have reported a considerably longer half-life under certain conditions. The half-life of atrazine in subsurface soil samples is considerably longer than in surface soil samples. In anaerobic soil, the half-life of atrazine has been reported to be 1.8 years in clay sediments (Ribaudo and Bouzaher, 1994). In sub-soil samples from agricultural areas in Wisconsin, the half-life of atrazine in silt loam was 1.4 years, and in sandy soil it was 5.4 years (Rodriguez and Harkin, 1997). Degradation products of atrazine, especially deethylatrazine and to a lesser extent deisopropylatrazine, deethyl-deisopropylatrazine (diaminoatrazine) and hydroxyatrazine, were found to accumulate in the sub-soil samples. A few studies have determined the half-lives of these atrazine transformation products; we have summarized these studies below.

Winkelmann and Klaine (1991) followed the concentrations of ^{14}C-labeled atrazine transformation products over 180 days in soil samples from western Tennessee. All transformation products were applied at a rate of 0.5 kg/ha. They reported half-lives for deethylatrazine (26 days), deisopropylatrazine (17 days) and hydroxyatrazine (121 days). The degradation of atrazine and its degradation products is slower once these compounds reach an aquifer. The degradation of atrazine has been monitored in aquifer slurries in Wisconsin (Rodriguez and Harkin, 1997). No significant degradation of atrazine was observed after 270 days in aquifer slurrys, indicating that atrazine is resistant to degradation in groundwater and aquifer sediments. Others have also reported that the degradation of atrazine and its dealkyated products is very slow once they reach an aquifer. The degradation half-life of atrazine and deethylatrazine in a sandy-till aquifer has been estimated to be 3400 and 2700 days, respectively (Levy and Chesters, 1995).

2. Levels in Groundwater:

One of the reasons the EPA initiated the Special Review of atrazine was concern about health risks from consuming drinking water contaminated with atrazine, especially in the Midwestern states where atrazine is widely used as a corn herbicide (USEPA, 1994). Levels of atrazine regularly exceeded 1-2 μg/L in Midwestern groundwater supplies in the 1980s, with maximum levels reported several fold above the atrazine MCL of 3 μg/L. More recent studies have found a high rate of detections of low levels of atrazine, with few samples exceeding the MCL. There is concern, however, that levels of atrazine plus its degradation products may exceed 3 μg/L. Though there is not an MCL established for atrazine degradation products, levels of atrazine and its degradation products in groundwater supplies are important to monitor because the microbial transformation products deethylatrazine and

deisoproplyatrazine do not adsorb as strongly to the soil and can leach into groundwater because of their greater mobility and appear in water supplies. In the following section, we have summarized the results of several studies and reviews which represent trends in atrazine levels in groundwater during the 1980s through the mid-1990s. We also have included studies which determine levels of atrazine transformation products.

Ritter (1990) reviewed the extent of pesticide contamination in US groundwater during the 1980s. Atrazine was the most widely detected pesticide, and levels in groundwater were correlated with high nitrate concentrations. In the early 1980s atrazine had been detected as high as 88 μg/L in Nebraska in shallow wells near irrigated fields. Atrazine has been reported in the groundwater of other Midwestern and Eastern states, including 1 to 45 μg/L in the Appoquinmink watershed of Delaware, 2.5 to 10 μg/L in the Big Springs watershed of Iowa, 0 to 25.6 μg/L in an agricultural watershed in Virginia, and 0.13 to 1.1 μg/L in rural wells in a agricultural area of Pennsylvania.

Low levels of atrazine were detected in wells sampled during the summer of 1986 in agricultural areas of Pennsylvania (Pionke et al., 1988). Of the 20 wells that were monitored, 14 of the wells had detections, ranging from 0.013 to 1.11 μg/L. The extent of contamination was associated with agricultural practices, with groundwater near fields under continuous corn production having a higher frequency of detections. Other studies have reported atrazine in groundwater in Western states. Levels of atrazine were monitored in four wells located in the Platte River Valley of Weld County, Colorado (Wilson et al., 1987). Samples were collected at two week intervals between July 31, 1985 and November, 1985. While two of the wells had atrazine levels that were below the detection limit (0.8 μg/L), the other two wells showed atrazine residues in the range of 1.1 to 2.3 μg/L.

One study conducted in upstate NY by the Cornell Water Resources Institute did find several sites with atrazine detections in groundwater (WRI, 1989). Seventy-three samples were obtained from 29 sites in 19 counties of upstate NY. There were three detections of atrazine, including a detection associated with a stock-watering operation, and a unconfirmed detection in a temporary well. Of the three atrazine detections, two were within the field and one was in a well within 50 feet from reported atrazine use site. The authors concluded that "....site-specific and localized contamination may occur in groundwater recharged from immediately adjacent treated areas. In essence, those most at risk from contamination are likely to be rural residents whose shallow domestic wells pump water from formations underlying areas treated with pesticides. These residents are often the same people who have a direct interest in the treated fields—in that they are part of the farming operation producing crops on adjacent lands" (WRI, 1989).

The US Geological Survey (USGS) has monitored levels of pesticides in wells in the Hudson River Basin during 1992-95 (Wall et al., 1998). They determined the level and frequency of detection of atrazine in 16 wells in agricultural regions of the basin, 26 urban/residential wells, and 49 wells that were only used as a domestic water supply. Atrazine was detected in approximately 70% of the wells in agricultural areas, in none of the urban/residential wells, and in 5% of the samples from domestic wells. None of the levels detected exceeded the atrazine MCL of 3.0 μg/L.

The "National Pesticide Survey", which included a summary of pesticides in community water systems and drinking water wells, was published in 1990 (USEPA, 1990). EPA sampled 1300 community water system wells and rural domestic wells for the presence of 101 pesticides and nitrates. The survey was designed to give information on the frequency and levels of pesticides on a nationwide basis but was not designed to give an assessment of pesticide contamination in drinking water wells at the local, county or State levels. From the survey it was estimated that 0.7% of rural domestic wells (70,800) may contain atrazine.

There is evidence that atrazine persists in soil well after application ceases and has the potential to contaminate tile drainage water. In one study conducted in Minnesota, atrazine had been applied to a field starting in 1974. During its last season of application in 1988, atrazine was detected in the tile drainage system at 0.84 μg/L. During the next 12 to 18 months, levels in the tile water did not decline, while 24 to 30 months after the final application levels declined to 0.42 μg/L. This result indicates that low levels of atrazine can contaminate tile drain water several years after use is discontinued (Buhler et al., 1993).

There is some indication that there may be an association between lower levels of atrazine in Iowa groundwater and decreased application rates (Kolpin et al., 1997 b). As a part of the Iowa Groundwater Monitoring Program, staff from the USGS sampled untreated groundwater from 1019 municipal wells from 1982 to 1995. Average application rates for atrazine in Iowa were 1.64 kg/ha in 1982-86; 1.06 kg/ha from 1987-91; and 0.98 kg/ha from 1992-95. The median frequency of detection of atrazine was 15.4% from 1982-86; 19.3% from 1987-91; and 13.2 % from 1992-95 samples. A subset of the wells (n=89) that were sampled repeatedly from 1982-95 were examined, and a significant temporal trend for decreasing median concentrations of atrazine was observed in the 1987-91 samples compared to the 1992-95 samples ($p < 0.021$). The authors noted that the decreasing trend in median atrazine concentrations is consistent with results of other long-term groundwater studies in the Midwest.

Frequency of detection of atrazine and its degradation products in groundwater is also related to land use. For example, in a 1995 report released by the California EPA (Bartowiak et al., 1995)

atrazine, which is primarily a corn herbicide and is not used extensively in California, had only 19 verified detections in 213 sampled wells in 17 California counties. Another triazine herbicide, simazine, which has a wider use on fruit crops, had 142 verified detentions (Bartowiak et al., 1995). Atrazine and deethylatrazine were reported as the compounds most frequently detected in a nation-wide monitoring study of pesticides in shallow groundwater of the US (Kolpin et al., 1998). Groundwater was sampled from 20 major hydrologic basins during 1993-1995 as a part of the National Water Quality Assessment Program. The frequency of detections were related to land use. The percentage of samples with atrazine were high in areas where corn and alfalfa (78.2%), corn (55.4%), or wheat (60.4%) were grown, compared to lower frequency detections in non-agricultural urban areas (18.6%). The detection limit in this study was 1 ng/L, which is lower than detection limits of analytical methods used in the 1980s. Patterns and magnitude of frequency of detections for deethylatrazine were similar to those of atrazine. The maximum detected in this study was 3.60 µg/L for atrazine and 2.60 µg/L for deethylatrazine. Mean and median levels were not reported.

A study conducted much earlier in the mid-1970s reported that levels of deethylatrazine were frequently greater than levels of atrazine residues in water samples collected from tile drain outlets located underneath atrazine-treated corn fields (Muir and Baker, 1976). Of the 23 samples taken over a two year period, 16 of the 23 tile-drain water samples had higher levels for deethylatrazine than atrazine. Average residue levels were 1.20 µg/L for atrazine and 1.34 µg/L of deethylatrazine. Levels of deisopropylatrazine were lower, averaging 0.17 µg/L. The levels of atrazine (AAtrex® 90W) applied to the corn plots were 2.8 kg/ha, which is the maximal rate currently allowed for atrazine in corn and sorghum preemergent applications (Norvartis, 1998).

Extensive studies on the transport of atrazine and its major degradation products from corn fields to the groundwater of the Midwestern states have been conducted by the USGS (Thurman et al., 1998). These studies were conducted during the spring and summer of 1991 in nine states that make up the "corn belt." Because of the mobility of the degradation product deethylatrazine in soil, the ratio of deethylatrazine to atrazine (called DAR) was found to be an indicator of transport from soil to groundwater, with the lowest ratios indicating the most rapid rates of transport. In this study, the most frequently detected compound was deethylatrazine (15.4%), followed by atrazine (14.7%) and deisopropylatrazine (4%). The maximum level of atrazine detected was 2.3 mg/L, and for deethylatrazine the maximum level was 2.1 mg/L.

In a more recent study conducted in Iowa, levels of atrazine, deethylatrazine and deisopropylatrazine were monitored in tile drainage water from experimental corn plots treated with 1.68 kg atrazine/ha (Jayachandran et al., 1994). Levels of atrazine in tile drain water samples ranged from 1.3 to 5.1 µg/L, while levels of deethylatrazine ranged from 0.9 µg/L to 3.2 µg/L, and levels of deisopropylatrazine from 0.3 to 0.9 µg/L. The additive concentrations of atrazine plus the two degradates averaged from 2.5 mg/L to 8 µg/L. This result suggests that the transformation products by themselves or in combination with atrazine can exceed the atrazine MCL of 3 µg/L.

Two recent studies conducted by the USGS have evaluated the prevalence of atrazine and atrazine degradation products in groundwater samples from the Midwest. In the first study, 106 municipal wells in Iowa were sampled during the summer of 1995 (Kolpin et al., 1997 a). The frequency of detection was 40.6% for atrazine, 34.9% for deethylatrazine, and 15.1% for deisopropylatrazine. The only herbicide compound with a higher level of detection was alachlor ethanesulfonic acid, with a frequency of detection of 65.1%. None of the herbicides exceeded established MCLs. The maximum levels detected were 2.13 µg/L for atrazine, 0.59 µg/L for deethylatrazine and 0.44 µg/L for deisopropylatrazine. Mean and median levels were not reported in this study. The second study monitored the frequency of herbicide detections in 303 wells that were a part of a groundwater reconnaissance network in 12 Midwestern states (Kolpin et al., 1996). The frequency of deethylatrazine detections (22.8%) was similar to frequency of atrazine detections (22.4%). Prevalence of deisopropylatrazine was not evaluated. None of the samples tested exceeded the MCL for atrazine. Maximum levels reported were 2.09 µg/L for atrazine and 2.2 µg/L for deethylatrazine.

These studies suggest that while levels of atrazine that exceeded the MCL were frequently reported in Midwestern, Western, and Eastern states in the 1980s, there appears to be a trend toward decreasing levels in groundwater in the mid-1990s. Atrazine remains as one of the most frequently detected herbicides in Midwestern groundwater, though the higher frequency of detection is partially attributed to an increase in the sensitivity of analytical methods available. The samples with positive detections of deethylatrazine were sometimes comparable to levels of detections observed for atrazine. Since deethylatrazine is mobile and can reach groundwater, it will be important in the future to obtain mean and median levels of this transformation product to better understand the prevalence of its contamination of water supplies. Additional data are presented on levels of atrazine and its degradation products in drinking water from rural wells in section IV.B. 5.

3. Levels in Surface Water:

As a part of its Special Review of atrazine, the EPA summarized a dozen published and unpublished studies which monitored levels of triazines in the Midwestern corn belt during the mid- to late 1980s (USEPA, 1994). The percentage of samples with positive detections for atrazine ranged from 75.1% to 100% in these studies. Most of these studies have found that atrazine levels correspond

to time of application and spring run-off events. Concentrations were usually less than 1 µg/L prior to atrazine field applications and rose to at least several µg/L and even exceeded 20 µg/L post-application in May and early June, corresponding to post-application spring rainfall events. Ten of these studies reported median concentrations of maximum detections of atrazine in the range of 3.3 to 22 µg/L. The agency concluded that "a number of surface source drinking water supply systems within the corn belt will have annual average atrazine concentrations exceeding the atrazine MCL of 3 µg/L" (USEPA, 1994).

As a part of a study assessing the ecological risk of atrazine residues in aquifers in Northern states, Solomon et al. (1996) presented previously unpublished data on levels of atrazine in 14 Midwestern watersheds in Ohio, Iowa, Kansas, Illinois, and Nebraska collected from 1989 to 1992. In order to estimate exposure frequency, they calculated the percentage of time atrazine residue levels exceeded 2, 5 and 20 µg/L, based on 4 and 21 day averages. Atrazine levels rarely exceeded 20 µg/L. However, the percentage of time atrazine levels exceeded 2 µg/L ranged from 5 to 32% in these Midwestern watersheds. Twelve of the fourteen watersheds exceeded the 5 µg/L level for 6 to 20% of the time. While levels of atrazine exceeding 20 µg/L were rare and were associated with transient rainfall events in the spring after atrazine application, these data suggest that surface water levels of atrazine in the Midwest were frequently in the range of 2 to 5 µg/L in the late 1980s and early 1990s.

Data were also presented on levels of atrazine in Midwestern reservoirs (Solomon et al., 1996), including USGS monitoring data of atrazine residues in 76 Midwestern reservoirs collected from 1992-93. Detection of low levels of atrazine in Midwestern reservoirs was widespread. Atrazine was detected in 92% of the reservoirs. The 90th percentile for levels observed for early June to July was 4.6 µg/L, while median levels were 1.23 µg/L during this same time period. During the rest of the year, median atrazine levels were in the range of 0.37 to 1.23 µg/L, and 90th percentile values ranged from 2.5 to 2.7 µg/L. This result suggests that surface runoff of atrazine during spring rains after herbicide application results in the largest inputs into reservoir systems.

Under controlled conditions using a rain simulator, the concentration of atrazine in runoff and in sediment was monitored over time in soil from the Blackland Prairie of Texas (Pantone et al., 1996). Peak atrazine levels in run-off were detected at the five minute time point (220 µg/L) and rapidly dropped to approximately 85 µg/L ten minutes after runoff initiation. Levels of atrazine in sediment were simultaneously monitored. Levels of atrazine were highest at the five minute time point in sediments, and the levels were an order of magnitude higher at approximately 2750 µg/kg than the level of atrazine in water runoff samples. Levels of atrazine in sediment dropped to 200 to 400 µg/kg at the 15 minute time point. This study also demonstrated that different types of tillage affected atrazine losses. No-tillage soil management practices reduced atrazine load in the runoff by 45% and decreased atrazine load in sediment by 77% as compared to chisel-tillage system of soil management.

There are several recent studies which have monitored atrazine levels in surface water of Northeastern states and Canada. Both application rates and rainfall patterns have been found to affect the level of atrazine residues detected in Vermont streams (Gruessner and Watzin, 1995). Water samples were collected following the first rain event after atrazine application, and this pattern was repeated for the entire growing season for two successive years, 1992 and 1993. After the first rainfall (3.5 cm) in May 1992, levels of atrazine in the streams ranged from 1 to 5 µg/L and peaked again with a third rain event at 1 to 7 µg/L after rainfall (2.5 cm) in June, 1992. In contrast, in 1993 after the first rain event (1 cm), stream levels of atrazine were between 0.05 and 0.1 µg/L, nearly 50 fold lower than the first rainfall event in 1993. This result may have been due to two factors: lower atrazine application rates in 1993 compared to 1992 and a lighter initial rainfall in 1993 compared to 1992. In 1992 the maximum allowable application rate was 6.6 kg atrazine AI/acre/year. By 1993, the application rate had been lowered to 3.5 to 4.4 kg AI/acre/year. In most cases, after a rainfall event levels of atrazine in the streams declined within several days after the rainfall.

In several recent studies, the USGS has monitored surface water levels of atrazine and other herbicides in NYS. Although atrazine residues have been detected widely, most detections have been below the MCL of 3 µg/L. Detected levels of atrazine were usually related to land-use and pesticide application patterns. Three of these studies are summarized below.

USGS researchers (Phillips et al., 1998) determined the level of pesticides in water samples taken in June of 1997 from 64 NYS rivers. Atrazine was detected in 97% of the samples analyzed. Levels of atrazine ranged from 0.001µg/L to 0.8 µg/L. None of the samples exceeded the MCL of 3 µg/L or NYS water-quality criteria. Concentrations that exceeded 0.1 µg/L were detected in streams from the western regions of NYS where corn production is the greatest.

Atrazine levels in surface waters of the Hudson River Basin were monitored by the USGS in base-flow samples from a network of 46 sites on 42 streams and rivers during May through late June of 1994 (Wall and Phillips, 1997 a). Atrazine was detected in 84.8% of the samples, but the levels detected were low. Only four of the samples had atrazine residue levels that exceeded 0.05 µg/L. The highest level detected was 0.38 µg/L, nearly 8-fold below the MCL for atrazine.

The same researchers monitored levels of atrazine and deethylatrazine in three sites in the Mohawk River subbasin at least monthly from March 1994 to September 1995 (Wall and

Phillips, 1997 b). The streams monitored had different land-use patterns. Canajorharie Creek drains from a predominately agricultural watershed; the Mohawk River at Cohes drains from a mixed land use watershed with urban, forested, and agricultural land uses; and Lisha Kill drains from a predominately urban watershed. Detections of atrazine in watersheds with agricultural land use were greatest in samples collected from May through August and peaked after the first major rain-fall after spring application of atrazine. In Canajorhaire Creek samples, atrazine levels ranged from 0.04 to a maximum of 4.3 μg/L during July and August ,while they were between 0.02 and 0.04 μg/L during the rest of the year. Levels of atrazine from the Mohawk River at Cohes in the spring and summer growing season ranged from 0.04 to 0.37 μg/L and were less than 0.04 μg/L during the fall. In contrast, peak levels of atrazine from Lisha Kill were not associated with spring run-off, which is consistent with its predominately urban land use. The maximum level of atrazine from Lisha Kill in May-August samples was 0.13 μg/L, which is several fold lower than maximum levels observed in the two other sites with agricultural land-use.

The levels of atrazine and deethylatrazine, and other herbicides, were determined from samples obtained at the stream flow-gauging stations located close to the mouth of three Canadian rivers: the Grand, Saugeen and Thames River (Frank et al., 1991). Samples were taken during base-flow and storm-flow conditions year-round from 1986 to 1990. A total of 474 samples were collected during this time period. Atrazine was the most frequently detected herbicide, with detections in 73% of the samples from the Grand River, 43% from the Saugeen River, and 98.6% from the Thames River. Percent detections and mean annual concentrations were related to land use in each water shed. For instance, annual mean concentrations of atrazine plus deethylatrazine ranged from 1013 to 2550 ng/L in the Thames River basin which had over 601 thousand acres of farm land while the Saugeen River basin had 292 thousand acres devoted to farm land, and had comparatively lower annual mean levels of atrazine plus deethylatrazine residues, at 152 to 319 ng/L, respectively.

Most of the studies evaluating contamination of atrazine in surface water have been done in the Midwestern and Northeastern corn producing states. Relatively little information is available on atrazine contamination in Southern states, where its primary use is to control weeds on turf and in sugarcane. A recently published study has monitored the levels of atrazine and other pesticides in surface water samples from canals in south Florida (Miles and Pfeuffer, 1997). Water samples were collected from November 1991 to June of 1995 at 27 stations. The maximum detection limit for atrazine was 0.01 μg/L. Atrazine was the most frequently detected pesticide with 274 positive detections. The number of samples with positive detections of atrazine varied seasonally, with the maximum number of detections occurring in the winter to late spring. This pattern corresponded to use of atrazine in controlling weeds on turf and on sugarcane. Median and mean levels of atrazine were not provided; the highest concentration reported in canal surface water was 18 μg/L.

4. Removal of Atrazine from Tap Water:
Levels of atrazine in finished tap water and in untreated samples from rivers which served as source water were monitored from late May through the end of July, 1983, in Northwestern Ohio (Baker, 1983). At all sites levels of atrazine in river samples were related to rain fall events. For example, the high levels of atrazine in the Sandusky River in late June through early July (7 to 8 μg/L) were related to a rainfall event. At other times, atrazine levels in river samples were in the 1 to 4 μg /L range. Concentrations of atrazine in the corresponding finished tap water at the Tifflin, Ohio water supply were very similar to the levels observed in the Sandusky River samples. This is in contrast to finished water samples from the Fremont, Ohio water supply where the water was treated with granulated activated charcoal. The levels of atrazine in the Fremont finished water samples were at or below 1 μg/L during the entire sampling period. The levels from the untreated source water were as high as 8 μg/L during early July. Activated charcoal treatment of water supplies during the spring and early summer, after application of atrazine and when spring rains are most likely to result in run-off, appears to be an effective means of reducing levels of atrazine in source water supplies. Activated charcoal was also used effectively to remove atrazine from contaminated wells in the Lombardia region of Italy in the late 1980s (Funari et al., 1988).

5. Estimates of Levels in Community Water Systems (CWS):
As part of a Ciba-sponsored assessment of human exposure to atrazine and simazine via ground- and surface water, a database was constructed to estimate exposures to these herbicides from the Safe Drinking Water Act monitoring data collected from 21 states which represent the major triazine using states (Clarkson et al., 1996; Tierney et al., 1998). The data set is largely based on information obtained from states and large municipalities with regulated CWSs. Data were collected between January 1993 and July 1995. In some cases, monitoring data from the peer-reviewed literature were used to estimate drinking water levels when primary CWS data were not available. Since not all CWSs provided data to Ciba, and other CWSs did not collect data during 1993 to 1995, the data set is not complete for all CWSs in the states studied. For instance, only 42% of the groundwater CWSs and 41% of the surface water CWSs had reportable levels of atrazine that were entered into the database (Tierney et al., 1998). The database did not include information from non-regulated water supplies, including private and rural wells. In rural states, those without access to CWSs may represent a significant proportion of the population. For instance, in Nebraska 23.8% of the population is not served by a CWS, and another 17.3% of the population is served by CWSs not included in the Ciba database (Clarkson et al., 1996). Various techniques were used to estimate non-detects, including a

simple substitution method which replaces numerical values for one-half the detection limit (detection limits ranged from 0.1 to 1.2 ppb) or a "Robust Method" which used a statistical model to assign a concentration to non-detects.

Atrazine residue values were tied to populations served by the CWS included in the Ciba database to estimate human exposures. Of the CWS-served populations included in the database, it was estimated that 9.06% were exposed to atrazine concentrations in the drinking water greater than 0.5 μg/L and 4.11% greater than 1.0 μg/L. Only 0.4% were exposed to concentration greater than 3 μg/L, which is the MCL for atrazine. However, the incompleteness of the database can be illustrated by the data presented for NYS. For NYS 37.5% of the population was served by CWS that were not included in the Ciba database, and an additional 9.2% of the population was not served by a CWS. This means there were no estimates of exposure for 46.7% of the population of NYS. Of the NYS population that was included in the database, 0.2% were exposed to less than 0.05 μg /L; 5.1% to 0.1 μg/L; and 48.1% from 0.1 to 0.5 μg/L. None of the water samples from surface or groundwater CWS from NYS included in the Ciba database had atrazine residue levels averaging above 1.0 μg/L (Clarkson et al., 1996).

The major limitations of these estimates of atrazine exposure through drinking water include: 1) relatively high minimum detection levels used by state monitoring programs (up to 1.2 ppb) and subsequent possible errors in estimating residue levels of non-detects; 2) lack of data for many CWS; 3) lack of information on exposures in populations not served by CWS (i.e. rural wells), which may make up significant portions of the population in rural states; and 4) lack of information on levels of major atrazine degradation products, such as deethylatrazine and deisopropylatrazine. Despite these limitations, it should be noted that this was one of the only sources of information compiled on drinking water levels of atrazine supplied through CWS at a national level, and efforts should be put forth to improve and maintain such a database through cooperation with state and federal agencies.

A study recently conducted by Ciba-Crop Protection has addressed some of the data gaps present in the CWS study, including the monitoring of atrazine residues in private and rural wells (Balu et al., 1998). Atrazine and its degradation products were monitored in private wells in 19 states with high atrazine use or in areas with groundwater that may be vulnerable to atrazine contamination. Water from 1505 wells, representing 30 to 200 wells per state, was analyzed for atrazine and its major degradation products. The limit of detection was 0.10 ppb. The year and time of year the wells were sampled were not stated in the report. Atrazine was detected in 23.9% of the wells, with similar percent detects for deethylatrazine (28.8%) and diaminochlorotriazine (24.1%). Lower percent detects were observed for deisopropylatrazine (14.9%), and hydroxyatrazine (4.5%), deethylhydroxyatrazine (2.8%), and ammeline (0.5%). This is one of the only studies that has monitored hydroxyatrazine degradation products in groundwater or drinking water. While the MCL for atrazine (3 μg/ml) was only exceeded in 0.5% of the wells, 11.83% of the wells had atrazine from 0.10 to 0.29 μg/ml and 8.37% had levels in the range of 0.3 to 0.99 μg/ml. Over 20% of the wells had levels of deethylatrazine or diaminochloroatrazine in the range of 0.1 to 1.0 μg/ml (Balu et al., 1998). This study indicates that while the level of atrazine seldom exceeds 3 μg/ml, many wells contained atrazine and degradation products in the range of 0.1 to 1 μg/ml.

6. Atmospheric Deposition and Levels in Precipitation:
Atmospheric transport and deposition of atrazine is of concern because of impacts on surface drinking water sources and potential effects on sensitive ecosystems. Several studies on atmospheric deposition of atrazine via rainfall are presented below.

Rainfall levels of atrazine were determined from samples taken near the Rhode River on the western shore of the Chesapeake Bay during 1977 and 1978 (Wu, 1981). Samples were analyzed for atrazine residues twice a week during this time period. Levels of atrazine in rain water were highly variable, in the range of 0.003 to 2.2 μg/L. The sample with the highest level of atrazine, 2.2 μg/L, was associated with a moderate rainfall (0.75 cm) in May of 1977. The authors noted that levels of atrazine in the winter months were "unexpectedly high," in the range of 0.003-0.97 μg/L.

Investigators with the Iowa Department of Natural Resources, Geological Survey Bureau, monitored the concentrations of herbicides in rainfall in rural and urban areas in Iowa from October 1987 to September 1990 (Nations and Hallberg, 1992). They found that atrazine, alachlor, cyanazine and metolachlor were the most common pesticides detected in rainfall during the growing season. Atrazine was detected in 28.7% of the precipitation samples, with mean levels at 1.08 μg/L and median levels at 0.58 μg /L; however, one sample did have a value as high as 40 μg/L. Samples from urban sites (Iowa City) had similar but lower detections of the same pesticides detected at rural sites. In the agricultural areas concentrations of herbicides were greater in the sites where pesticides are applied, suggesting that distant transport and volatilization of pesticides may affect concentrations in rainfall. The authors concluded that deposition in rainfall may be contributing to pesticide residues at non-target sites such as sensitive ecosytems and organic croplands (Nations and Hallberg, 1992).

Atrazine has also been detected in rain samples collected at the Blue Earth River near Frost, Minnesota (Capel et al., 1995). This watershed drains from an agricultural area of intensive corn and soybean production. Weekly composited rain samples were collected from April through July, 1994. The two highest levels of atrazine were reported in rain collected during the last week in

April (0.68 µg/L) and the third week in June (1.6 µg/L). These levels are in the same range as those reported in Iowa precipitation by Nations and Hallberg (1992). Atrazine levels for other weeks were either zero (no rain), or had positive detections in the to 0.1 to 0.2 µg/L range.

These studies indicate that atrazine is detected in precipitation in high-use states in the Midwest and that these levels can exceed 1 µg/L.

C. Atrazine Residues in Food and Dietary Cancer Risk Estimates:

There is relatively little information on residue levels of atrazine or its transformation products in crops, such as corn, where it is used for weed control as a pre-emergent herbicide. In one study atrazine (AATREX® 4L) was applied at 26 g AI per 10 x 10 meter plot and corn plants were sampled at two week intervals until harvest (Pylypiw et al., 1993). The highest level of atrazine detected was 0.48 ppm in the first two week interval. By eight weeks, the level of atrazine in the corn plant was undetectable (below detection limit of 0.002 ppm). Atrazine was not detected in corn ear samples at 12 weeks post-application.

The FDA recently released the 1996 results of its pesticide residue monitoring in domestically produced and imported foods (FDA, 1998). In 1996, 5062 domestic food samples and 5312 imported food samples were analyzed for 392 pesticide residues, including triazine herbicides. Atrazine was not detected in any of the analyzed samples. A special study was also conducted by the FDA in 1995 and 1996 to develop methods for determining residues of triazine herbicides and several metabolites. These methods were used to analyze 56 domestic samples and 47 imported food samples for triazine residues. Atrazine residues were not detected in the targeted commodities (apples, bananas, grapes, oranges, pears and plums). Simazine, another *s*-triazine herbicide, was detected in six domestic samples of oranges, though none exceeded established tolerances. Triazine residues were not detected in the imported food samples.

The EPA, in its Special Review of atrazine, estimated dietary cancer risk from atrazine residues of treated crops and animal products from livestock that consume atrazine-treated feeds (USEPA, 1994). Dietary cancer risk estimates were based on atrazine residues from corn, sorghum, eggs, milk, poultry and red meat, and on atrazine residues, or atrazine plus chloro- metabolite residues for other treated crops, including guava, macadamia nuts, millet, pineapple, sugarcane and wheat. The percent crop treated was considered in calculating estimated exposures from these foods. The upper bound cancer risk estimates for atrazine was calculated to be 4.4×10^{-5} (USEPA, 1994).

Ciba-Geigy scientists have prepared a report on the magnitude and nature of *s*-triazine residues (simazine and atrazine) in food (Simoneaux et al., 1998). The principal field crops (corn, sorghum, sugarcane, wheat grain, fodder crops, macadamia nuts, and guava) were grown in field plots treated with the maximum registered use rates of atrazine or simazine for the given year the experiment was conducted (i.e. in 1992, atrazine use rates for corn and sorghum were 2.5 lbs AI per acre per calendar year) to determine the level of different transformation products in the raw agricultural products. Crops were also grown with ^{14}C- atrazine to determine the types and levels of residues. Residues in cattle tissues, milk, and eggs were estimated from feeding studies of ^{14}C-atrazine or ^{14}C-hydroxy-*s*-triazine to poultry and lactating and non-lactating goats. Residues were reported according to class of major transformation product, i.e. chloro-*s*-triazines (atrazine, simazine, deethylatrazine, deisopropylatrazine, diaminochloro-*s*-triazine), hydroxyatrazines (hydroxyatrazine, deethylhydroxyatrazine, deethylhydroxysimazine), and amino-*s*-triazines.

A dietary exposure assessment of atrazine was then conducted for the US population and sensitive sub-groups based on FDA food 1977-78 food consumption data. The anticipated residues were also compared to the reference dose (RfD) which is based on the no effect level obtained from rat feeding studies with a 100-fold safety factor. The dietary exposure to total chloro-*s*-triazines was 0.000007 mg/kg/bd wt/day for the general US population, 0.000031 mg/kg/bd wt/day for non-nursing infants less than one years old, and 0.000019 mg/kg/bd wt/day for children one to six years of age. For total free hydroxy-*s*-triazines the dietary exposure was 0.000039 mg/kg/bd wt/day for the general US population and 0.000162 mg/kg/bd wt/day for non-nursing infants less than one year old. For total triazines residues minus hydroxy-*s*-triazines, dietary exposure was 0.000229 mg/kg/bd wt/day for the general US population, and 0.000583 mg/kg/bd wt/day for children one to six years old; levels for non-nursing infants less than one year were not calculated. The % of the RfD for the triazine residues exceeded 1% in two cases; for total free hydroxy-*s*-triazines in non-nursing infants (% of RfD was 1.62) and for total triazines minus hydroxy-*s*-triazines for children one to six years of age (% of RfD was 1.67). The authors concluded that the "total dietary exposure represented a very small percentage of the reference dose which is based on a no effect level and a wide margin of safety" (Simoneaux et al., 1998).

D. Toxicity of Mixtures Containing Atrazine:

Toxicity studies were conducted with pesticide and fertilizer mixtures representative of groundwater contamination found in Iowa. The "Iowa mixture" included atrazine, alachlor, cyanazine, metolachlor, metribuzin and ammonium nitrate. Mixes were administered via drinking water for up to six months to female and male F344/N rats and B6C3F1 mice. No significant adverse effects were observed in the animals receiving concentrations in the drinking water up to 100 times the median concentrations of the individual chemicals as determined from agricultural groundwater contamination studies (NTP, 1996). The 100X concentration of atrazine in this study was 50 ng/ml (50 ppb).

Levels in contaminated water have been detected in excess of 50 ppb, as high as 80 ppb in Nebraska (Ritter, 1990). Therefore, the choice of 50 ppb for that atrazine concentration of the mixture underestimated actual levels of atrazine contamination observed in the Midwest. There are no published carcinogenicity studies conducted on these mixtures.

E. Occupational Exposures:

The EPA, in its initiation of the Special Review of atrazine, expressed concern that agricultural workers handling atrazine may be exposed to levels that represented an unacceptable cancer risk (USEPA, 1994). In response to this concern, Norvartis Crop Protection has recently published an assessment of worker exposure to atrazine (Lunchick and Selman, 1998). A Pesticides Handlers Exposure Database (PHED), developed through a joint effort of the EPA, Health Canada, and the American Crop Protection Association, was used to estimate exposures to mixer/loaders, applicators and flaggers. EPA had calculated cancer risk estimates before this database was developed. This review also critiques the assumptions made in EPA's earlier risk assessment of agricultural workers' exposure to atrazine. EPA's original assessment of atrazine exposure and Norvartis' more recent assessment of worker exposure were compared. Exposure estimates calculated by Norvartis were usually 10 to 100 fold lower than that EPA's estimates. The highest level of exposure was to mixer/loader/applicators applying atrazine to corn, and to sugarcane (Florida). In the sugarcane mixers/loaders, conditions included using an open pour method of mixing and an open cab. Atrazine exposure was estimated to be 1.2×10^{-3} mg/kg/day using the PHED database; previous EPA estimates of exposure were 5.4×10^{-2} mg/kg/day. Among corn growers, applicators using an open-cab system had estimated exposures of 3.0×10^{-2} mg/kg/day using PHED database; EPA estimates of exposure were 1.8×10^{-4} mg/kg/day. Exposures when workers used closed cab systems were reduced by ten-fold. Norvartis scientists suggest the new exposure estimates represent a more accurate risk assessment. EPA comment on this report and assumptions made in the risk assessment were not located.

VII. Recommendations for Breast Cancer Risk Classification:

Because of the lack of case-control studies evaluating the effects of atrazine exposure on breast cancer incidence and mortality in human populations, there is insufficient evidence to conclude that atrazine is a human carcinogen. However, evidence of increased incidence and/or decreased latency of malignant and/or benign mammary tumors over a wide range of dose levels in female SD rats is sufficient evidence to conclude that atrazine is a **2B possible breast carcinogen**. Although atrazine is not an estrogen mimic, there is evidence that it can affect hormones along the hypothalamic pituitary gonadal axis. Changes in the levels of hormones in this pathway may affect levels of estradiol or estradiol metabolites such as 16-OHE1, which may affect breast cancer risk.

In our Critical Evaluation, we have carefully considered and evaluated the hypothesis that atrazine-induced mammary tumors in female SD-rats are due to premature reproductive aging. The reader is referred to section V.C.3. entitled "Commentary on Cycling and Hormonal Changes in the Aging SD Rat" for a detailed evaluation of this hypothesis. As we have previously noted, there are inconsistencies in data or lack of data which do not support this hypothesis. This includes the observation that estradiol levels were not elevated by treatment with atrazine in female SD rats in a study conducted by Cooper et al. (1994). There is also a lack of data demonstrating that estradiol levels are significantly elevated in atrazine-treated SD rats in prolonged or constant estrus.

It should also be noted that while most cancer research has been devoted to determining the carcinogenicity of compounds that are genotoxic and that are capable of inducing mutations or other damage to DNA that can increase the progression of a cell to a cancerous state, chemicals that may affect cancer risk by non-genotoxic mechanisms are as important to identify.

The difficulty in this cancer assessment is whether the low levels of atrazine in food and water are sufficient to affect breast cancer risk in the general population. Case control studies evaluating cancer risk in women exposed to atrazine are in progress (Agricultural Health Study), but the results are not yet available. Other information that is not available is whether atrazine transformation products, which can be persistent in the environment and contaminate water supplies, also affect breast cancer risk. Given the wide-spread and long-term use of atrazine for agricultural crop protection, it is important to resolve these issues. Recommendations for further research are listed below in the next section.

VIII. Research Gaps and Recommendations for Future Research:

- Human epidemiological case-control studies are needed to assess the risk of breast, ovarian and uterine cancer in women exposed to atrazine in the work place, including agricultural workers, farm women who live near atrazine-treated fields and female manufacturing workers.
- Further studies are needed to determine the mechanism by which atrazine increases the rate and earlier appearance of mammary tumors in SD female rats. This includes determining the extent of elevations in serum estrogen levels in atrazine-treated SD rats in prolonged or constant estrus compared to estrogen levels in atrazine-treated F344 rats in prolonged diestrus.
- The presence and persistence of atrazine-transformation products, including deethylatrazine, deisopropylatrazine, diaminoatrazine, and *N*-nitrosoatrazines in the soil, surface water, groundwater and finished tap water, should be monitored. Such studies could be used to identify areas where

unacceptable levels might suggest limiting the use of atrazine-containing products. Monitoring studies should include both Midwestern and Northeastern corn and sorghum producing states and Southeastern states that use atrazine primarily on turf and sugarcane.

- Atrazine transformation products, including deethylatrazine, deisopropylatrazine, and diaminoatrazine, should be tested for their oncogenicity and other health effects.
- Studies are needed to further monitor the impact of atrazine use on levels in precipitation and patterns of deposition in high-use states.
- Since atrazine does have the capacity to affect endocrine pathways, assessment of reproductive effects from pre-natal and juvenile exposures to atrazine should be conducted. Reproductive toxicology studies in laboratory animals should include determining the effects on levels of endocrine hormones (e.g. estrogen, progesterone, prolactin, 5-alpha reductase, DHT), patterns of estrous cyclicity, effects on reproduction and fertility, and pathological changes in estrogen-responsive tissues, including the ovary, uterus and breast (S. Snedeker has nominated atrazine to the NIEHS Juvenile Pesticide-Exposure Reproductive-Toxicology Study, personal communication with R. Chapin).

IX. Summary of Studies Currently Being Conducted:

The following studies were abstracted from the Computer Retrieval of Information on Scientific Projects (CRISP) database, which lists studies funded by federal agencies (i.e. NIH, EPA, USDA), or were obtained through personal communications with the principal investigator (PI).

Agricultural Health Study; joint intramural research, NCI and NIEHS
Dr. Michael Alavanja, Project Officer, NCI (personal communication with Dr. Alavanja)
This 10-year prospective study, which is in its fourth year, will follow 90,000 farmers, commercial pesticide applicators, and spouses of farmers and applicators in Iowa and North Carolina. The survey will document pesticide usage by questionnaire, and in a sub-set of the population, actual pesticide exposures will be measured in the urine and blood using validated biomarkers. Information will also be gathered on home use of pesticides, as well as agricultural uses of pesticides. This study is unique, since it will include one of the largest cohorts of female pesticide applicators ever followed, as well as including the female spouses of farmers and pesticide applicators. Approximately 58,000 men and 32,000 women are enrolled in this study. Case-control breast cancer and ovarian cancer studies as well as other case-control studies of cancer are planned.

Interventions to Reduce Cancer Risk Among Farm Families
Dr. Melissa Perry, P.I., Medical College of Wisconsin
This study proposes to translate prior epidemiologic, laboratory, clinical, and behavior information on cancer risks into a primary prevention program to reduce cancer among farmers and their families. The preventive interventions will target pesticide applicators, most of whom are farmers, and their families through community-based educational programs designed to increase cancer prevention knowledge, risk perception, and self-efficacy in order to create behavior change to reduce cancer risks. Because the majority of applicators are male and because other family members are likely to be exposed to pesticides by virtue of living in the farm setting, wives and adult daughters of the applicators will also receive an educational intervention. This intervention will be designed to increase knowledge of pesticides risks and increase cancer preventive behaviors including breast self exam and mammography screening among women of recommended age. To be conducted in Vermont.

Reducing Pesticide Exposure in Minority Families
Dr. Linda Mc Cauley, P.I., Oregon Health Sciences University
The specific aims of the study are to: (1) compare the levels of pesticides in homes as a function of the type of agricultural crop the parents work with, the types of pesticides commonly used on the crops, proximity of housing to the field and characteristics of the home; (2) evaluate specific health outcomes associated with pesticide over-exposure in both workers and their children and to evaluate specific biomarkers; and (3) assess the effectiveness of the Migrant Headstart program as a mechanism for delivering culturally appropriate environmental health prevention strategies.

Studies of Manufacturing Workers Exposed to Triazines
Ciba-Giegy Corp (now Norvartis)
(personal communication; letter from Mr. Kerry Miller, Regional State Gov. Relations Manager for Ciba, dated October 21, 1996).

Epidemiological studies are ongoing to monitor workers exposed to atrazine at production facilities. However, since entrance of women into these facilities is relatively recent, it is possible that this small female cohort may not have been monitored for a sufficient period of time to date to warrant valid conclusions.

X. Bibliography

Adams, N. H., Levi, P., and Hodgson, E. (1990). *In vitro* studies of the metabolism of atrazine, simazine, and terbutryn in several vertebrate species. J. Agric. Food Chem. *38*, 1411-1417.

Ahrens, W. H. (1994). Atrazine. In Herbicide Handbook, W. H. Ahrens, ed. (Champaign, IL: Weed Science Society of America), pp. 20-23.

Alavanja, M. C. R., Sandler, D. P., McMaster, S. B., Zahm, S. H., McDonnell, C. J., Lynch, C. F., Pennybacker, M., Rothman, N., Dosemeci, M., Bond, A. E., and Blair, A. (1996). The Agricultural Health Study. Environ. Health Perspect. *104*, 362-369.

Aspelin, A. L. (1997). Pesticides Industry Sales and Usage, 733-R-97-002, USEPA, ed. (Washington, D.C.: Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides and Toxic Substances, US Environmental Protection Agency).

Babic-Gojmerac, T., Kniewald, Z., and Kniewald, J. (1989). Testosterone metabolism in neuroendocrine organs in male rats under atrazine and deethylatrazine influence. J. Steroid Biochem. *33*, 141-146.

Baker, D. B. (1983). Herbicide Contamination in Municipal Water Supplies of Northwestern Ohio [draft final report] (Tiffin, Ohio: Heidelberg College).

Bakke, J., Larson, J. D., and Price, C. E. (1972). Metabolism of atrazine and 2-hydroxyatrazine by the rat. J. Agric. Food Chem. *20*, 602-607.

Balaguer, P., Joyeux, A., Denison, M. S., Vincent, R., Gillesby, B. E., and Zacharewski, T. (1996). Assessing the estrogenic and dioxin-like activities of chemicals and complex mixtures using *in vitro* recombinant receptor-reporter gene assays. Can. J. Physiol. Pharmacol. *74*, 216-222.

Balu, K., Holden, P. W., Johnson, L. C., and Cheung, M. W. (1998). Chapter 19, Summary of Ciba Crop Protection groundwater monitoring study for atrazine and its degradation products in the United States. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 227-238.

Bartowiak, D., Newhart, K., Pepple, M., Troiano, J., and Weaver, D. (1995). Sampling for Pesticide Residues in California Well Water; 1995 Update of the Well Inventory Data Base (Sacremento, CA: California Environmental Protection Agency, Dept. of Pesticide Regulation).

Biradar, D. P., and Rayburn, A. L. (1995). Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. J. Environ. Qual. *24*, 1222-1225.

Blair, A., Dosemeci, M., and Heineman, E. (1993). Cancer and other causes of death among male and female farmers from twenty-three states. Am. J. Ind. Med. *23*, 729-742.

BNA (1997). Drinking Water; Changes predicted in final regulation to protect ground water from herbicides. Environ. Rep. *28*, 317-318.

Bontoyan, W. R., Law, M. W., and Wright, D. P., Jr. (1979). Nitrosamines in agricultural and home-use pesticides. J. Agric. Food Chem. *27*, 631-635.

Bradlow, H. L., Davis, D. L., G., L., Sepkovic, D., and Tiwari, R. (1995). Effects of pesticides on the ratio of 16α/2-Hydroxyestrone: A biologic marker of breast cancer risk. Environ. Health Perspect. *103*, 147-150.

Bradway, D. E., and Moseman, R. F. (1982). Determination of urinary residue levels of the *N*-dealkyl metabolites of triazine herbicides. J. Agric. Food Chem. *30*, 244-247.

Brown, L. M., Blair, A., Gibson, R., Everett, G. D., Cantor, K. P., Schumann, L. M., Burmeister, L. F., Van Lier, S. F., and Dick, F. (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res. *50*, 6585-6591.

Brown, L. M., Burnmeister, L. F., Everett, G. D., and Blair, A. (1993). Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes Control *4*, 153-156.

Brusick, D. J. (1994). An assessment of the genetic toxicity of atrazine: Relevance to human health and environmental effects. Mutat. Res. *317*, 133-144.

Buchanan, G. A., and Hiltbold, A. E. (1973). Performance and persistence of atrazine. Weed Sci. *21*, 413-416.

Buhler, D. D., Randall, G. W., Koskinen, W. C., and Wyse, D. L. (1993). Atrazine and alachlor losses from subsurface tile drainage of a clay loam soil. J. Environ. Qual. *22*, 583-588.

Burmeister, L. F. (1990). Cancer mortality in Iowa farmers: Recent results. Am. J. Ind. Med. *18*, 295-301.

Cantor, K. P., Blair, A., Everett, G., Gibson, R., Burmeister, L. F., Brown, L. M., Schumann, L., and Dick, F. R. (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res. *52*, 2447-2455.

Capel, P. D., Ma, L., Schroyer, B. R., Larson, S. J., and Gilchrist, T. A. (1995). Analysis and detection of the new corn herbicide acetochlor in river water and rain. Environ. Sci. Technol. *29*, 1702-1705.

Catenacci, G., Maroni, M., Cottica, D., and Pozzoli, L. (1990). Assessment of human exposure to atrazine through the

determination of free atrazine in urine. Bull. Environ. Contam. Toxicol. *44*, 1-7.

Chapin, R. E., Stevens, J. T., Hughes, C. L., Kelce, W. R., Hess, R. A., and Daston, G. P. (1996). Symposium overview: Endocrine modulation of reproduction, paper presented by: Eldridge, J. C., Stevens, J. T., Wetzel, L. T., Tisdel, M. O., Brechenridge, C. B., McConnell, R. F., and Simpkins, J. W. , Atrazine: mechanisms of hormonal imbalance in female SD rats. Fundam. Appl. Toxicol. *29*, 1-17.

Clarkson, J. R., Golden, K. A., Tierney, D. P., and Christensen, B. R. (1996). Human exposure to atrazine and simazine via ground and surface drinking water: Update I, January 25, 1996, no. 2852.0480 (Greensboro, N.C.: Ciba Crop Protection and Ciba-Geigy Corp).

Clements, C., Ralph, S., and Petras, M. (1997). Genotoxicity of selected herbicides in *Rana catesbeiana* tadpoles using the alkakine singe-cell gel DNA electrophoresis (Comet) assay. Environ. Mol. Mutagen. *29*, 277-288.

Connor, K., Howell, J., Safe, S., Chen, I., Liu, H., Berhane, K., Sciarretta, C., and Zacharewski, T. (1998). Chapter 33, Failure of chloro-s-triazine-derived compounds to induce estrogenic responses *in vivo* and *in vitro*. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 424-431.

Cooper, R. L., Conn, P. M., and Walker, R. F. (1980). Characterization of the LH surge in middle-aged female rats. Biol. Reprod. *23*, 611-615.

Cooper, R. L., Goldman, J. M., and Rehnberg, G. L. (1986). Neuroendocrine control of reproductive function in the aging female rodent. J. Am. Geriat. Soc. *34*, 735-751.

Cooper, R. L., Stoker, T. E., Goldman, J. M., Parrish, M. B., and Tyrey, L. (1996). Effect of atrazine on ovarian function in the rat. Reprod. Toxicol. *10*, 257-264.

Cooper, R. L., Stoker, T. E., McElroy, W. K., and Hein, J. (1998). Atrazine (ATR) disrupts hypothalamic catecholamines and pituitary function (abstract no. 789). Proceedings of the Society of Toxicology 1998 Annual Meeting, pp. 160.

Cooper, R. L., and Walker, R. F. (1979). Potential therapeutic consequences of age-dependent changes in brain physiology. Interdiscipl. Topics Geront. *15*, 54-76.

Crosignani, P., and Berrino, F. (1994). Re: "Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma" [letter]. Scand. J. Work Environ. Health *20*, 223-225.

Danzo, B. J. (1997). Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environmental Health Perspectives *105*, 294-301.

Dauterman, W. C., and Muecke, W. (1974). *In vitro* metabolism of atrazine by rat liver. *4*, 212-219.

Dieter, M. P., and Garnett, J. (1993). Proceedings of the American Association for Cancer Research [abstract 1034]: Use of a F344 rat leukemia model to test the farm chemical pesticides, parathion, chlorpyrifos and atrazine for potential tumorigenicity. Carcinogenesis *34*, 173.

Donna, A., Betta, P.-G., Robutti, F., and Bellingeri, D. (1986). Carcinogenicity testing of atrazine: preliminary report on a 13-month study on male Swiss albino mice treated by intraperitoneal administration. Giornale Italiano di Medicina del Lavoro *8*, 119-121.

Donna, A., Betta, P.-G., Robutti, F., Crosignani, P., Berrino, F., and Bellingeri, D. (1984). Ovarian mesothelial tumors and herbicides: A case-control study. Carcinogenesis *5*, 941-942.

Donna, A., Betta, P.-G., Gagliardi, F., Ghiazza, G. F., Gallareto, M., and Gabutto, V. (1981). Preliminary experimental contribution to the study of possible carcinogenic activity of two herbicides containing atrazine-simazine and trifuralin as active principles. Pathologica *73*, 707-721.

Donna, A., Crosignani, P., Robutti, F., Betta, P. G., Bocca, R., Mariani, N., Ferrario, F., Fissi, R., and Berrino, F. (1989). Triazine herbicides and ovarian epithelial neoplasms. Scand. J. Work Environ. Health *15*, 47-53.

Dunkelberg, H., Fuchs, J., Hengstler, J. G., Klein, E., Oesch, F., and Strüder, K. (1994). Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline, and simazine in mammalian cells. Bull. Environ. Contam. Toxicol. *52*, 498-504.

Dunnick, J. K., Elwell, M. R., Huff, J., and Barrett, J. C. (1995). Chemically induced mammary gland cancer in the National Toxicology Program's carcinogenesis bioassay. Carcinogenesis *16*, 173-179.

Eisenbeis, S. J., Lynch, D. L., and Hampel, A. E. (1981). The Ames mutagen assay tested against herbicides and herbicide combinations. Soil Sci. *131*, 44-47.

Eldridge, J. C., Fleenor-Heyser, D. G., Extrom, P. C., Wetzel, L. T., Breckenridge, C. B., Gillis, J. H., Luempert III, L. G., and Stevens, J. T. (1994 a). Short-term effects of chlorotriazines on estrus in female Sprague-Dawley and Fischer 344 rats. J. Toxicol. Environ. Health *43*, 155-167.

Eldridge, J. C., Tennant, M. K., Wetzel, L. T., Breckenridge, C. B., and Stevens, J. T. (1994 b). Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: Hormonal properties, dosage, and animal strain. Environ. Health Perspect. *102 (Suppl 11)*, 29-36.

Eldridge, J. C., McConnnell, R. F., Wetzel, L. T., and Tisdel, M. O. (1998). Appearance of mammary tumors in atrazine-treated rats: probable mode of action involving strain-related control of ovulation and estrous cycling. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 414-423.

Erickson, M. D., Frank, C. W., and Morgan, D. P. (1979). Determination of *s*-triazine herbicide residues in urine: Studies of excretion and metabolism in swine as a model to human metabolism. J. Agric. Food Chem. *27*, 743-746.

FDA. (1998). Food and Drug Administration Pesticide Program, Residue Monitoring 1996 (http://vm.cfsan.fda.gov/~dms/pest96rep.html: Food and Drug Administration).

Finch, C. E., Felicio, L. S., Mobbs, C. V., and Nelson, J. F. (1984). Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. Endocrine Rev. *5*, 467-497.

Flury, M. (1996). Experimental evidence of transport of pesticides through field soils- a review. J. Environ. Qual. *25*, 25-45.

Frank, R., Logan, L., and Clegg, B. S. (1991). Pesticide and polychlorinated biphenyl residues in waters at the mouth of the Grand, Saugeen, and Thames Rivers, Ontario, Canada, 1986-1990. Arch. Environ. Contam. Toxicol. *21*, 585-595.

Funari, E., Brambilla, A. L., Camoni, I., Canuti, A., Cavallaro, A., Chierici, S., Cialella, G., Donati, G., Jaforte, A., Prandi, L., Salamana, V., Silano, V., and Zapponi, G. A. (1988). Extensive atrazine pollution of drinking water in the Lombardia region and related public health aspects. Biomed. Environ. Sci. *1*, 350-355.

Gebel, T., Kevekorder, S., Pav, K., Edenharder, R., and Dunkelberg, H. (1997). *In vivo* genotoxicity of selected herbicides in the mouse bone-marrow micronucleus test. Arch. Toxicol. *71*, 193-197.

Gianessi, L. P., and Anderson, J. E. (1995 a). Pesticide Use in New York Crop Production (Washington, D.C.: National Center for Food and Agricultural Policy).

Gianessi, L. P., and Anderson, J. E. (1995 b). Pesticide Use in US Crop Production (Washington, D.C.: National Center for Food and Agricultural Policy).

Gruessner, B., and Watzin, M. C. (1995). Patterns of herbicide contamination in selected Vermont streams detected by enzyme immunoassay and gas chromatography/mass spectrometry. Environ. Sci. Technol. *29*, 2806-2813.

Guddewar, M. B., and Dauterman, W. C. (1979). Studies on a glutathione S-transferase preparation from mouse liver which conjugates chloro-*s*-triazine herbicides. Pestic. Biochem. Physiol. *12*, 1-9.

Harris, C. I. (1967). Fate of 2-chloro-s-triazine herbicides in soil. J. Agric. Food Chem. *15*, 157-162.

Hazelette, J. R., and Green, J. D. (1988). Atrazine Technical: 91-Week Oral Carcinogenicity Study in Mice, MRID No. 40431302, Study No. 842120, October 30, 1987 (Testing Facility: Division of Toxicology/Pathology, Ciba-Geigy Corp.).

Heindel, J. J., Chapin, R. E., Gulati, D. K., George, J. D., Price, C. J., Marr, M. C., Myers, C. B., Barnes, L. H., Fail, P. A., Grizzle, T. B., Schwetz, B. A., and Yang, R. S. H. (1994). Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. Fundam. Appl. Toxicol. *22*, 605-621.

Hill, R. H., Jr., Barr, J., Driskell, W. J., Patterson, D. G., Needham, L. L., and Bond, A. E. (1996). Biologic monitoring for pesticide residues among farm families: Atrazine exposure (abstract no. 135) (New Orleans, LA: American Chemical Society).

Hoar, S. K., Blair, A., Holmes, F. F., Boysen, C. D., Robel, R. J., Hoover, R., and Fraumeni, J. F., Jr. (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. J. Am. Med. Assoc. *256*, 1141-1147.

HSDB. (1996). Atrazine; Hazardous Substances Database (TOXNET: National Library of Medicine).

Huang, H. H., and Meites, J. (1975). Reproductive capacity of aging female rats. Neuroendocrinology *17*, 289-295.

Huang, H. H., Steger, R. W., Bruni, J. F., and Meites, J. (1978). Patterns of sex steroid and gonadotropin secretion in aging female rats. Endocrinology *103*, 1855-1859.

Hurle, K., and Kibler, E. (1976). The effect of changing moisture conditions on the degradation of atrazine in soil. In 1976 British Weed Control Conference (13th British Weed Control Conference) (Hotel Metropole, Brighton, England: British Crop Protection Council), pp. 627-633.

IARC. (1991). Occupational Exposures in Insecticide Application, and Some Pesticides; Atrazine. In IARC monographs on the evaluation of carcinogenic risks to humans (Lyon: IARC, World Health Organization), pp. 441-466.

Ikonen, R., Kangas, J., and Savolainen, H. (1988). Urinary atrazine metabolites as indicators for rat and human exposure to atrazine. Toxicol. Lett. *44*, 109-112.

Infurna, R., Levy, B., Meng, E., Yau, E., and Traina, V. (1988). Teratologial evaluations of atrazine technical, atrazine herbicide, in rats and rabbits. J. of Toxicol. and Environ. Health *24*, 307-319.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., and Peters, J. (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl. Cancer Inst. *42*, 1101-1114.

IRIS. (1998). Atrazine, CASRN 1912-24-9 (http://www.epa.gov/ngispgm3/iris/subst/02909.htm: EPA Integrated Risk Information Service).

Janzowski, C., Klein, R., and Preussmann, R. (1980). Formations of *N*-nitroso compounds of the pesticides atrazine, simazine, and carbaryl with nitrogen oxides. In *N*-Nitroso Compounds: Analysis, Formation and Occurrence (IARC Scientific Publications No. 31), E. A. Walker, L. Griciute, M. Castegnaro and M. Borzsonyi, eds. (Lyon: IARC), pp. 329-339.

Jayachandran, K., Steinheimer, T. R., Somasundaram, L., Moorman, T. B., Kanwar, R. S., and Coats, J. R. (1994). Occurrence of atrazine and degradates as contaminants of subsurface drainage and shallow groundwater. J. Environ. Qual. *23*, 311-319.

Kappas, A. (1988). On the mutagenic and recombinogenic activity of certain herbicides in *Salmonella typhimurium* and in *Aspergillus nidulans*. Mutat. Res. *204*, 615-621.

Kearney, P. C., Oliver, J. E., Helling, C. S., Isensee, A. R., and Kontson, A. (1977). Distribution, movement, persistence, and metabolism of *N*-nitrosoatrazine in soils and a model aquatic ecosystem. J. Agric. Food Chem. *25*, 1177-1181.

Kello, D. (1989). WHO drinking water quality guidelines for selected herbicides. Food Addit. Contam. *6 Supplement 1*, S79-S85.

Kettles, M., Browning, S. R., Prince, T. S., and Horstman, S. W. (1997). Triazine herbicide exposure and breast cancer incidence: An ecologic study of Kentucky counties. Environ. Health Perspect. *105*, 1222-1227.

Khan, S. U. (1978). Kinetics of hydrolysis of atrazine in aqueous fulvic acid solution. Pestic. Sci. *9*, 39-43.

Kniewald, J., Peruzovic, M., Gojmerac, T., Karmela, M., and Kniewald, Z. (1987). Indirect influence of *s*-triazines on rat gonadotrophic mechanism at early postnatal period. J. Steroid Biochem. *27*, 1095-1100.

Kogevinas, M., Kauppinen, T., Winkelmann, R., Becher, H., Bertazzi, P. A., Bueno-de-Mesquita, H. B., Coggon, D., Green, L., Johnson, E., Littorin, M., Lynge, E., Marlow, D. A., Mathews, J. D., Neuberger, M., Benn, T., Pannett, B., Pearce, N., and Saracci, R. (1995). Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. Epidemiology *6*, 396-402.

Kolpin, D. W., Barbash, J. E., and Gillion, R. J. (1998). Occurrence of pesticides in shallow groundwater of the United States: Initial results from the National Water-Quality Assessment Program. Environ. Sci. Technol. *32*, 558-566.

Kolpin, D. W., Kalkhoff, S. J., Goolsby, D. A., Sneck-Fahrer, D. A., and Thurman, E. M. (1997 a). Occurrence of selected herbicides and herbicide degradation products in Iowa's ground water. Ground Water *35*, 679-688.

Kolpin, D. W., Sneck-Fahrer, D., Hallberg, G. R., and Libra, R. D. (1997 b). Temporal trends of selected agricultural chemicals in Iowa's groundwater, 1982-1995: Are things getting better? J. Environ. Qual. *26*, 1007-1017.

Kolpin, D. W., Thurman, E. M., and Goolsby, D. A. (1996). Occurrence of selected pesticides and their metabolites in near-surface aquifers of the Midwestern United States. Environ. Sci. Technol. *30*, 335-340.

Koskinen, W. C., and Clay, S. A. (1997). Factors affecting atrazine fate in north central US soils. Rev. Environ. Contam. Toxicol. *151*, 117-165.

Lamoureux, G. L., Simoneaux, B., and Larson, J. (1998). The metabolism of atrazine and related 2-chloro-4,6-bis(alkylamino)-*s*-triazines in plants. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hacket, eds. (Washington, D.C.: American Chemical Society).

LeFevre, J., and McClintock, M. K. (1988). Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. Biol. Reprod. *38*, 780-789.

Levy, J., and Chesters, G. (1995). Simulation of atrazine and metabolite transport and fate in a sandy-till aquifer. J. Contam. Hydro. *20*, 67-88.

Lu, K. H., Hopper, B. R., Vargo, T. M., and Yen, S. S. C. (1979). Chronological changes in sex steroid, gonadotropin and prolactin secretion in aging female rats displaying different reproductive states. Biol. Reprod. *21*, 193-203.

Lunchick, C., and Selman, F. (1998). Chapter 14, The assessment of worker exposure to atrazine and simazine: A tiered approach. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 141-155.

Lusby, A. F., Simmons, Z., and McGuire, P. M. (1979). Variation in mutagenicity of *s*-triazine compounds tested on four *Salmonella* strains. Environ. Mutagen. *1*, 287-290.

Lynge, E. (1985). A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. Br. J. Cancer *52*, 259-270.

Ma, L., and Selim, H. M. (1996). Atrazine retention and transport in soils. Rev. Environ. Contam. Toxicol. *145*, 129-173.

Mayhew, D. A., Taylor, G. D., Smith, S. H., and Banas, D. A. (1986). Twenty-four Month Combined Oral Toxicity and Oncogenicity Study in Rats Utilizing Atrazine Technical, Study No. 410-1102, Accession No. 262714-2226, April 29, 1986 (Conducted by American Biogenics Corp. for Ciba-Geigy Corp.).

McDougal, A., and Safe, S. (1998). Induction of 16α-/2-hydroxyestrone metabolite ratios in MCF-7 cells by pesticides, carcinogens, and antiestrogens does not predict mammary carcinogens. Environmental Health Perspectives *106*, 203-206.

Meisner, L. F., Roloff, B. D., and Belluck, D. A. (1993). *In vitro* effects of N-nitrosoatrazine on chromosome breakage. Arch. Environ. Contam. Toxicol. *24*, 108-112.

Meister, R. T. (1998). Pesticide Dictionary; Atrazine. In Farm Chemicals Handbook '98, R. T. Meister, ed. (Willoughby, OH: Meister Publishing Company), pp. C34-35.

Meites, J. (1972). Relation of prolactin and estrogen to mammary tumorigenesis in the rat. J. Natl. Cancer Inst. *48*, 1217-1224.

Miles, C. J., and Pfeuffer, R. J. (1997). Pesticides in canals of south Florida. Arch. Environ. Contam. Toxicol. *32*, 337-345.

Muir, D., and Baker, E. B. (1978). The disappearance and movement of three triazine herbicides and several of their degradation products in soil under field conditions. Weed Res. *18*, 111-120.

Muir, D. C., and Baker, B. E. (1976). Detection of triazine herbicides and their degradation products in tile-drain water from fields under intensive corn (maize) production. J. Agric. Food Chem. *24*, 122-125.

Nations, B. K., and Hallberg, G. R. (1992). Pesticides in Iowa precipitation. J. Environ. Qual. *21*, 486-492.

Nelson, J. F., Felicio, L. S., Randall, P. K., Sims, C., and Finch, C. E. (1982). A longitudinal study of estrous cyclicity in aging C57BL/6J Mice: I. Cycle frequency, length and vaginal cytology. Biol. Reprod. *27*, 327-339.

Nequin, L. G., Alvarez, J., and Schwartz, N. B. (1979). Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat. Biol. Reprod. *20*, 659-670.

Norvartis. (1998). AAtrex Nine-0® label: Norvartis/Ciba-Geigy).

NTP. (1996). Tox-36, Toxicity studies of pesticide/fertilizer mixtures administered in drinking water to F344/N Rats and B6C3F Mice (URL = <http://ntp-server.niehs.nih.gov/htdocs/ST-studies/TOX036.html>: National Toxicology Program).

Pantone, D. J., Potter, K. N., Torbert, H. A., and Morrison, J., J.E. (1996). Atrazine loss in runoff from no-tillage and chisel-tillage systems on a Houston black clay soil. J. Environ. Qual. *25*, 572-577.

Peters, J. W., and Cook, R. M. (1973). Effects of atrazine on reproduction in rats. Bull. Environ. Contam. Toxicol. *9*, 301-304.

Phillips, P. J., Wall, G. R., Eckhardt, D. A., Freehafer, D. A., and Rosenmann, L. (1998). Pesticide Concentrations in Surface Waters of New York State in Relation to Land Use-1997, USGS Water Resources Investigation Report 98-4101 (http:ny.usgs.gov/projects/nypesticides/reports/WRIR4101.html: US Geological Survey), pp. 1-5.

Pinter, A., Torok, G., Borzsonyi, M., Surjan, A., Csik, M., Kelecsenyi, Z., and Kocsis, Z. (1990). Long-term carcinogenicity bioassay of the herbicide atrazine in F344 rats. Neoplasma *37*, 533-544.

Pionke, H. B., Glotfelty, D. E., Lucas, A. D., and Urban, J. B. (1988). Pesticide contamination of groundwaters in the Mahantango Creek Watershed. J. Environ. Qual. *17*, 76-84.

Plewa, M. J., Wagner, E. D., Gentile, G. J., and Gentile, J. M. (1984). An evaluation of the genotoxic properties of herbicides following plant and animal activation. Mutat. Res. *136*, 233-245.

Pylypiw, H. M., Jr., Bugbee, G. J., and Frink, C. R. (1993). Uptake of pre-emergent herbicides by corn: Distribution in plants and soil. Bull. Environ. Contam. Toxicol. *50*, 474-478.

Redondo, M. J. (1997). Dissipation and distribution of atrazine, simazine, chlorpyrifos, and tetradifon residues in citrus orchard soil. Arch. Environ. Contam. Toxicol. *32*, 346-352.

Ribas, G., Frenzilli, G., Barale, R., and Marcos, R. (1995). Herbicide-induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay. Mutat. Res. *344*, 41-54.

Ribaudo, M. O., and Bouzaher, A. (1994). Atrazine: environmental characteristics and economics of management (Washington, D.C.: United States Department of Agriculture - Economic Research Service).

Ritter, W. F. (1990). Pesticide contamination of ground water in the United States - a review. J. Environ. Sci. Health *25*, 1-29.

Rodriguez, C. J., and Harkin, J. M. (1997). Degradation of atrazine in subsoils, and groundwater mixed with aquifer sediments. Bull. Environ. Contam. Toxicol. *59*, 728-735.

Ruiz, M. J., and Marzin, D. (1997). Genotoxicity of six pesticides by *Salmonella* mutagenicity test and SOS chromotest. Mutat. Res. *390*, 245-255.

Sathiakumar, N., Delzell, E., Austin, H., and Cole, P. (1992). A follow-up study of agricultural chemical production workers. Am. J. Ind. Med. *21*, 321-330.

Sathiakumar, N., Delzell, E., and Cole, P. (1996). Mortality among workers at two triazine herbicide manufacturing plants. Am. J. Ind. Med. *29*, 143-151.

Simoneaux, B. J., Hackett, D. S., Bray, L. D., and Thalaker, F. (1998). Chapter 10, Magnitude and nature of (*s*)-triazine residues in foodstuffs as predicted from radiolabeled studies on selected animals and plants. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 104-114.

Simpkins, J. W., Eldridge, J. C., and Wetzel, L. T. (1998). Chapter 31, Role of strain-specific reproductive patterns in the appearance of mammary tumors in atrazine-treated rats. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 399-413.

Solomon, K. R., Baker, D. B., Richards, R. P., Dixon, K. R., Klaine, S. J., La Point, T. W., Kendall, R. J., Weisskopf, C. P., Giddings, J. M., Giesy, J. P., Hall, L. W., Jr., and Williams, W. M. (1996). Ecological risk assessment of atrazine in North American surface waters. Environ. Toxicol. Chem. *15*, 31-76.

Sorenson, B. A., Wyse, D. L., Koskinen, W. C., Buhler, D. D., W.E., L., and Jorgenson, M. D. (1993). Formation and movement of ^{14}C-atrazine degradation products in a sandy loam soil under field conditions. Weed Sci. *41*, 239-245.

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., and Serrano, F. O. (1995). The E-Screen Assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. Environ. Health Perspect. *103*, 113-122.

Stevens, J. T., Breckenridge, C. B., Wetzel, L. T., Gillis, J. H., Luempert III, L. G., and Eldridge, J. C. (1994). Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides. J. Toxicol. Environ. Health *43*, 139-153.

Stevens, J. T., and Sumner, D. D. (1991). Chapter 20, Herbicides; 20.17.1 Atrazine. In Handbook of Pesticide Toxicology, W. J. Hayes, Jr. and E. R. Laws, Jr., eds. (San Diego: Academic Press, Inc.), pp. 1381-1383.

Tennant, M. K., Hill, D. S., and Eldridge, J. C. (1994). Possible antiestrogenic properties of chloro-*s*-triazines in rat uterus. J. Toxicol. Environ. Health *43*, 183-196.

Tezak, Z., Simic, B., and Kniewald, J. (1992). Effect of pesticides on oestradiol-receptor complex formation in rat uterus cytosol. Food Chem. Toxicol. *30*, 879-885.

Thakur, A. K., Wetzel, L. T., Tisdel, M. O., and Stevens, J. T. (1992). Comparison of the potential effects of atrazine on the development of mammary, pituitary, uterine, and ovarian tumors in Sprague-Dawley and Fischer 344 female rats [abstract no. 1490]. Toxicologist *12*, 380.

Thakur, A. K., Wetzel, L. T., Voelker, R. W., and Wakefield, A. E. (1998). Chapter 30, Results of a two-year oncogenicity study in Fischer 344 Rats with atrazine. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 384-398.

Thurman, E. M., Kolpin, D. W., Goolsby, D. A., and Meyer, M. T. (1998). Chapter 17, Source and transport of desethylatrazine and desisopropylatrazine to groundwater of the midwestern United States. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 189-207.

Tierney, D. P., Clarkson, J. R., Christensen, B. R., Golden, K. A., and Hines, N. A. (1998). Chapter 21, Exposure to the herbicide atrazine and simazine in drinking water. In Triazine Herbicides: Risk Assessment, L. G. Ballantine, J. E. McFarland and D. S. Hackett, eds. (Washington, D.C.: American Chemical Society), pp. 252-265.

Tran, D. Q., Kow, K. Y., McLachlan, J. A., and Arnold, S. F. (1996). The inhibition of estrogen receptor-mediated responses by chloro-*s*-triazine-derived compounds is dependent on estradiol concentration in yeast. Biochem. Biophys. Res. Commun. *227*, 140-146.

USDHHS. (1998). Report on Carcinogens, Eighth Edition Summary, 1998, I. L. Systems, ed. (Rockville, MD: US Dept. of Health and Human Services, and the National Toxicology Program).

USEPA. (1989). Atrazine. In Drinking Water Health Advisory: Pesticides (Lewis Publishers), pp. 43-67.

USEPA (1994). Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review. Fed. Reg. *59*, 60412-60443.

USEPA. (1998). Atrazine, Tolerances and Exemptions from Tolerances for Pesticide Chemicals in or on Raw Agricultural Commodities, 40 CFR 180.220. In Code of Federal Regulations, pp. 348-349.

USEPA. (1996). Drinking Water Regulations and Health Advisories, EPA 822-B-96-002 (Washington, D.C.: Office of Water, US Environmental Protection Agency).

USEPA. (1990). National Pesticide Survey: Summary Results of EPA's National Survey of Pesticides in Drinking Water Wells (Washington, D.C.: United States Environmental Protection Agency, Office of Water, Office of Pesticides and Toxic Substances).

USEPA (1991). Notice of MCL for Atrazine (56 FR 3526). January 10, 1991. Fed. Reg.

Vineis, P., Terracini, B., Ciccone, G., Cignetti, A., Colombo, E., Donna, A., Maffi, L., Pisa, R., Ricci, P., Zanini, E., and Comba, P. (1986). Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. A population based case-referent study. Scand. J. Work Environ. Health *13*, 9-17.

Wall, G. R., and Phillips, P. J. (1997 a). Pesticides in surface waters of the Hudson River basin, New York and Adjacent states (US Geological Survey Fact Sheet no. FS 238-96).

Wall, G. R., and Phillips, P. J. (1997 b). Pesticides in surface waters of the Hudson River basin-Mohawk River subbasin (US Geological Survey Fact Sheet no. F2-237-96).

Wall, G. R., Riva-Murrary, K., and Phillips, P. J. (1998). Water Quality in the Hudson River Basin, New York and Adjacent States, 1992-95, USGS Circular no. 1165 (Denver, CO: US Geological Survey Information Services).

Weisenburger, D. D. (1990 a). Environmental epidemiology of non-Hodgkin's lymphoma in eastern Nebraska. Am. J. Ind. Med. *18*, 303-305.

Weisenburger, D. D., Hickman, T. J., Patil, K. D., Lawson, T. A., and Mirvish, S. S. (1990 b). Carcinogenesis tests of atrazine and N-nitrosoatrazine-compounds of special interest to the Midwest, Proceedings of the American Association for Cancer Research, Vol. 31 [abstract 603]. Carcinogenesis, 102.

Wetzel, L. T., Luempert III, L. G., Breckenridge, C. B., Tisdel, M. O., Stevens, J. T., Thakur, A. K., Extrom, P. J., and Eldridge, J. C. (1994). Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. J. Toxicol. Environ. Health *43*, 169-182.

WHO. (1987). Drinking-Water Quality: Guidelines for Selected Herbicides (triazines) (Copenhagen: World Health Organization, Regional Office for Europe), pp. 4, 8-11.

Wiklund, K., and Dich, J. (1994). Cancer risks among female farmers in Sweden. Cancer Causes Control *5*, 449-457.

Wilson, M. P., Savage, E. P., Adrian, D. D., Aaronson, M. J., Keefe, T. J., Hamar, D. H., and Tessari, J. T. (1987). Groundwater transport of the herbicide, atrazine, Weld county, Colorado. Bull. Environ. Contam. Toxicol. *39*, 807-814.

Winkelmann, D. A., and Klaine, S. J. (1991). Degradation and bound residue formation of four atrazine metabolites, deethylatrazine, deisopropylatrazine, dealkyatrazine and hydroxyatrazine, in a western Tennessee soil. Environ. Toxicol. Chem. *10*, 347-354.

Wise, P. (1984). Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. Endocrinology *115*, 801-809.

Wise, P. M. (1987). The role of the hypothalamus in aging of the female reproductive system. J. Steroid Biochem. *27*, 713-719.

Wise, P. M., Scarbrough, K., Larson, G. H., Lloyd, J. M., Weiland, N. G., and Chiu, S. (1991). Neuroendocrine influences on aging of the female reproductive system. Frontiers in Neuroendocrinology *12*, 323-356.

WRI. (1989). Assessment of Pesticides in Upstate New York Groundwater: Water Resources Institute, Center for Environmental Research, Cornell University).

WSSA. (1994). Atrazine. In Herbicide Handbook, 7th ed., W. H. Ahrens, ed. (Champaign, IL: Weed Science Society of America), pp. 20-23.

Wu, T. L. (1981). Atrazine residues in estuarine water and the aerial deposition of atrazine into Rhode River, Maryland. Water Air Soil Pollut. *15*, 173-184.

Yoder, J., Watson, M., and Benson, W. W. (1973). Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat. Res. *21*, 335-340.

Zahm, S. H., and Blair, A. (1994). Author's reply, Re: "Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma" [letter]. Scand. J. Work Environ. Health *20*, 225-226.

Zahm, S. H., Weisenburger, D. D., Babbitt, P. A., Saal, R. C., Cantor, K. P., and Blair, A. (1988). A case-control study of non-Hodgkin's lymphoma and agricultural factors in eastern Nebraska [abstract]. Am. J. Epidemiol. *128*, 901.

Zahm, S. H., Weisenburger, D. D., Babbitt, P. A., Saal, R. C., Vaught, J. B., Cantor, K. P., and Blair, A. (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology *1*, 349-356.

Zahm, S. H., Weisenburger, D. D., Cantor, K. P., Holmes, F. F., and Blair, A. (1993 a). Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma. Scand. J. Work Environ. Health *19*, 108-114.

Zahm, S, Weisenburger, D. D., Saal, R. C., Vaught, J. B., Babbitt, P. A., and Blair, A. (1993 b). The role of agricultural pesticide use in the development of Non-Hodgkin's lymphoma in women. Arch. Environ. Health *48*, 353-358.

XI. Appendix A. Common Abbreviations, Acronyms and Symbols

ADI	Acceptable Daily Intake, set by the World Health Organization
AI	Active Ingredient
BCERF	Cornell Program on Breast Cancer and Environmental Risk Factors in New York State
bd wt	body weight
C	carbon
°C	degrees Centigrade
CAS	Chemical Abstract Service
CE	constant estrus
CfE	Cornell University Center for the Environment
CHO	Chinese hamster ovary cells
CI	Confidence Interval
Cl	chlorine
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra- and extra mural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
CWS	Community Water Supply
DAR	deethylatrazine to atrazine ratio; index of atrazine transport to groundwater
DEN	diethylnitrosamine; a liver carcinogen
DHT	dehydrotestosterone
DMBA	7,12-dimethylbenz[*a*]anthracene; known mammary carcinogen
DNA	deoxyribonucleic acid
EPA	United States Environmental Protection Agency
ER	estrogen receptor
E-SCREEN	screening assay for estrogenicity that measures proliferative response in estrogen-dependent breast tumor cells
F344	Fischer 344, rat strain
FDA	Food and Drug Administration
GnRH	gonadotropin releasing hormone
GSH	glutathione
ha	hectacre (area equal to 2.471 acres)
HA	The health advisories are non-enforceable limits of the concentration of the chemical in the drinking water that is not expected to cause any adverse noncarcinogenic health effects when consumed for no more than the time period specified, with a margin of safety
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Cornell Institute for Comparative and Environmental Toxicology
i.p.	interperitoneal
IRIS	Integrated Risk Information System; Database maintained by the EPA
IUGR	Intrauterine growth retardation
kg	kilogram
L	liter
LH	luteinizing hormone
lbs	pound(s)
mg	microgram
mg	milligram
MCF-7	Michigan Cancer Foundation; cells derived from human breast tumor
MCL	Maximum Contaminant Level; enforceable limit set by the EPA which sets the maximum level of a contaminate in a public drinking water supply
mmol	millimole(s)
MTD	Maximum Tolerated Dose
n	number of subjects/animals in the group
ng	nanogram
NHL	non-Hodgkin's lymphoma
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health and Safety
NIH	National Institutes of Health
NMU	*N*-nitroso-*N*-methyurea; mammary carcinogen
NOEL	No observed effect level
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OP	organophosphate pesticides
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
pg	picogram
PHED	Pesticide Handlers Exposure Database
pM	picomole(s)
ppm	parts per million
ppb	parts per billion
ppt	parts per trillion
RACB	Reproductive Assessment by Continuous Breeding; National Toxicology Program protocol
RR	Relative Risk
RRWS	Rathburn Rural Water System
RfD	Reference Dose
RUP	Restricted Use Pesticide
SCGE	single-cell gel electrophoresis

SD	Sprague Dawley rat
SHE	Syrian hamster embryo
SMR	Standardized Mortality Ratio; SMR= the ratio of "observed" to "expected" deaths; in some studies, this ratio is multiplied by 100
TMA	Time-weighted average
US	United States
USGS	United States Geological Survey
USC	University of Southern California
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
2-OHE1	2-hydroxyestrone
16-OHE1	16-alpha hydroxyestrone

Symbols:

α	alpha
β	beta
γ	gamma
μ	micro
<	less than
>	greater than
≥	greater than or equal to
%	percent
p	p value
±	plus or minus
=	equal
®	registered trademark

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Cancer Risk Classification Scheme (revised 10/98 sms).

The Process:

Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity
IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
ATDSR (**A**gency for **T**oxic **D**isease **S**ubstance **R**egistry)

Conduct **Literature Searches** using databases to obtain historical and the most recent information; i.e. Toxline, Medline, Biosis, Cancerlit
-Peer-reviewed scientific literature-available through Cornell libraries and interlibrary loans.
-Technical Reports-NTIS-National Technical Information Service
-TOXNET databases—USEPA's IRIS database source of oncogenicity and regulatory status information
-Gray literature—Studies submitted to US Environmental Protection Agency (EPA) that are not published—i.e. industry generated oncogenicity studies
-Some abstracts of cancer bioassays are on line (IRIS database)
-Request reports from industry
-Request reports from EPA through Freedom of Information Act

The Critical Evaluation includes some general background information, including: chemical name, chemical formula, Chemical Abstract Subject Registry no. (CAS #), chemical structure, trade name(s), trade names of mixtures, metabolites/degradation products, history of use, and current regulatory status.

Evidence of cancer in other (non-breast) organ systems is provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and US Federal Agencies (NTP, USEPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See attached sheets for a more detailed explanation of the BCERF Cancer Risk classification scheme)

The **emphasis of the document** is a critical evaluation of the evidence for breast cancer risk, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section is devoted to brief summaries of new research studies that are in progress. A bibliography with all cited literature is included in each critical evaluation. Major international, federal and state agencies will be provided with copies of our report.

General Outline of BCERF Critical Evaluations-revised 10/98 sms

I Chemical Information
- A. Common Name
- B. Chemical Name(s)
- C. Chemical Formula(s)
- D. CAS # (Chemical Abstract Service Number)
- E. Chemical Structure
- F. Trade Name(s)
- G. Trade Names of Mixtures
- H. Major Metabolite(s)/Breakdown Products

II. History of Use, Usage
- A. History of Usage and Uses
- B. Current Usage (when applicable)

III. Current Regulatory Status
- A. Current Regulatory Status, EPA
- B. Drinking Water Standards and Health Advisories
- C. Food Residue Tolerances and Action Levels (when applicable)
- D. Workplace Regulations (when applicable)

IV. Summary of Evidence of Overall Carcinogenicity (non-breast sites)
- A. Human Studies
- B. Experimental Animal Studies
- C. Current Classification of Carcinogenicity by other Agencies
    1. IARC (International Agency for Research on Cancer)
    2. NTP (National Toxicology Program)
    3. USEPA (Environmental Protection Agency)

V. Critical Evaluation of the Scientific Evidence for Breast Cancer Risk
- A. Humans Studies
    1. Case-Studies
    2. Human Epidemiological Cohort Studies
    3. Human Epidemiological Case-Control Studies
    4. When available will summarize information on detection/accumulation in human tissues / and validation of biomarkers
- B. Experimental Animal Studies
- C. Other Relevant Information, including mechanisms by which exposure may affect breast cancer risk (examples: co-carcinogenicity, tumor promotion estrogenicity, endocrine disruption, reproductive toxicology, mutagenicity, cell proliferation, oncogene/tumor suppressor gene expression, immune function, etc.)

VI. Other Relevant Information
- A. Specific for the pesticide; (i.e. may include information on environmental fate, potential for human exposure)

VII. Summary, Conclusions, Recommendation for Breast Cancer Risk Classification

VIII. Identification of Research Gaps, and Other Recommendations

IX. Brief Summaries of New Human Studies Currently Being Conducted

X. Bibliography

XI. Appendix A. Common Abbreviations, Acronyms and Symbols

XII. Appendix B. BCERF Critical Evaluations of Breast Cancer Risk

BCERF Breast Cancer Risk Classification Scheme

(adapted from the IARC Preamble by S.M. Snedeker; **revised 12/97, 10/98 sms**)

Group 1: **Human breast carcinogen;** *sufficient evidence* of carcinogenicity to humans is necessary. *Sufficient evidence* is considered to be evidence that a **causal** relationship has been established between exposure to the agent and human breast cancer.

Group 2A: **Probable breast carcinogen**; this category generally includes agents for which there is *1) limited evidence* of breast carcinogenicity in humans and *sufficient evidence* of mammary carcinogenicity in experimental animals. The classification may also be used when there is *2) limited evidence* of breast carcinogenicity in humans and strong supporting evidence from other relevant data, or when there is 3) *sufficient evidence* of mammary carcinogenicity in experimental animals and strong supporting evidence from other relevant data.

Group 2B: **Possible breast carcinogen;** this category generally includes agents for which there is 1) *limited evidence* in humans in the absence of *sufficient evidence* in experimental animals; 2) *inadequate evidence* of carcinogenicity in humans or when human data is nonexistent but there is *sufficient evidence* of carcinogenicity in experimental animals, 3) *inadequate evidence* or no data in humans but with *limited evidence* of carcinogenicity in experimental animals together with strong supporting evidence from other relevant data.

Group 2C: **Potential to affect breast cancer risk**; this category includes agents for which there is *inadequate or nonexistent human and animal data*, but there *is supporting evidence from other relevant data* that identifies a mechanism by which the agent may affect breast cancer risk. Examples are, but are not limited to: evidence of agent's estrogenicity, disruption of estrogen metabolism resulting in potential to affect exposure to estrogen; evidence of breast tumor promotion, progression or co-carcinogenicity; increased expression of proto-oncogenes or oncogenes; evidence of inactivation of tumor suppressor gene associated with breast cancer; evidence of adverse effect on immune function; or evidence of a structural similarity to a known breast carcinogen (structure-activity relationship).

Group 3: **Not classifiable** as to its breast carcinogenicity to humans. Agents are placed in this category when they do not fall into any other group.

Group 4: **Probably not a breast carcinogen in humans**: This category is used for agents for which there is evidence suggesting a lack of breast carcinogenicity in human studies and in animal studies, together with a lack of related evidence which may predict breast cancer risk. The absence of studies does **not** constitute evidence for a lack of breast carcinogenicity.

Brief Definitions of Sufficient, Limited, and Inadequate Evidence:
(adapted from the IARC Preamble by S.M. Snedeker)

Human Studies

Sufficient evidence of carcinogenicity in humans: Must have established evidence between exposure to the agent and human breast cancer. Case-reports are given the least weight in considering carcinogenicity data in humans—they are suggestive of a relationship, but by themselves cannot demonstrate causality. Consistent, case-control studies which have controlled for confounding factors and have found high relative risks of developing breast cancer in relation to an identified exposure are given the most weight in determining a causal relationship.

Limited evidence of breast carcinogenicity in humans: A positive association has been observed between exposure to the agent and breast cancer, but chance, bias or confounding factors could not be ruled out.

Inadequate evidence of breast carcinogenicity in humans: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Experimental Animal Studies:

Sufficient evidence of breast carcinogenicity in animals: Evidence of malignant tumors or combination of benign and malignant tumors in (a) two or more species of animals, (b) or two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Limited evidence of breast carcinogenicity in animals: The studies suggest a carcinogenic effect, but are limited for making a definitive evaluation because: (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence of only benign neoplasms of lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains of animals.

Inadequate evidence of breast carcinogenicity in animals: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.