Atrazine

by David M. DeMarini PhD and Shelia Hoar Zahm PhD

Citation for most recent IARC review

IARC Monographs 73, 1999

Current evaluation

Conclusions from the previous Monograph:
Atrazine is not classifiable as to its carcinogenicity to humans (Group 3). There is *inadequate evidence* in humans for the carcinogenicity of atrazine. There is *sufficient evidence* in experimental animals for the carcinogenicity of atrazine. The Working Group concluded that the animal mammary tumors associated with exposure to atrazine involve a non-DNA-reactive, hormonally mediated mechanism that is not relevant to humans.

Exposure and biomonitoring

Atrazine (6-Chloro-N-ethyl-N'-(1-methylethyhl)-1,3,5-triazine-2,4-diamine) is a triazine herbicide used widely on a variety of crops, especially maize, sorghum, and sugar-cane, for the pre- and post-emergent control of broad-leaved weeds. Occupational exposure may occur through both inhalation and dermal adsorption during the manufacture of atrazine, its formulation, and its application. It is found widely, together with its dealkylated degradation products, in rivers, lakes, estuaries, groundwater, and reservoirs. In drinking-water, the levels rarely exceed 1 μg/L. Surveys of various foods and feeds have generally found no detectable atrazine residue.

In water systems, atrazine is generally dealkylated to form desethylatrazine (DEA), desisopropylatrazine (DIA), and diaminochlorotriazine (DACT). As many as 8-12 metabolites of atrazine have been identified or postulated to occur in animals and humans, with some studies showing DACT as the primary metabolites and others showing atrazine mercapturate (AM) as the primary one (Barr et al., 2007). Typically, the level of AM in the urine is the only metabolite of atrazine that is measured in most biomonitoring studies, and this approach has generally found atrazine exposure in <5% of subjects at a detection level of <0.8 ng/ml (CDC, 2005).

A recent study evaluated the urinary levels of 9 atrazine metabolites in humans and concluded that DACT was the primary metabolite regardless of exposure scenario, and that exposure assessment based on measuring only AM or any single atrazine metabolite resulted in an underestimate of atrazine exposure (Barr et al., 2007). This study found that AM accounted for only 2-12% of the detected metabolites, that most of the subjects had detectable atrazine exposure, and that future biomonitoring studies would likely need to include analysis of at least AM, DACT, DIA, and DEA in order to characterize the extent of atrazine exposure in a population. The study also found that the proportion of these urinary metabolites varied considerably depending on whether the subjects had high or low acute exposures or whether the subjects had general environmental exposures (i.e., not agricultural workers).

Based on the observations above, Panuwet et al. (2008) have developed an analytical method that measures the 7 primary metabolites of atrazine in urine using an on-line solid phase extraction-high-performance liquid chromatography-tandem mass spectrometry (SPE-HPLC-MS/MS) and isotope dilution quantification method. Applying this method to maize farmers, Bakke et al. (2008) concluded that the amount of atrazine applied to the fields by an agricultural worker is likely to provide a valid surrogate of atrazine exposure in epidemiologic studies.

A study of pesticide urinary metabolite levels of farm worker children aged 1-6 found detectable levels of the metabolite atrazine mercapturate (Arcury et al., 2007). The authors noted that the children were most likely exposed to pesticide drift or contaminated water supplies.

Cancer in humans

(*inadequate*, Vol. 73, 1999)

At the time of the previous IARC evaluation of atrazine (Vol. 73, 1999), the most relevant epidemiologic studies consisted of two cohort studies of manufacturing workers, three population-based case-control studies of lymphatic and hematopoietic malignancies in agricultural areas of the U.S., and a population-based case-control study of ovarian cancer in a rice-growing area of Italy. Notable findings included a non-significant excess, based on small numbers, of non-Hodgkin lymphoma (NHL) among manufacturing workers, a significant association with NHL among exposed U.S. farmers, and a two- to threefold increase of borderline significance in the risk for ovarian cancer among women in Italy. The risk of NHL among U.S. farmers was attenuated when multiple exposures were considered.

Of note since the last evaluation, there have been further investigations among one of the manufacturing cohorts and the U.S. case-control studies of NHL, a new case-control study of ovarian cancer, analyses from the prospective U.S. Agricultural Cohort Study, and several ecological studies of environmental exposure.

Manufacturing

Approximately 2,000 workers manufacturing atrazine and other triazine herbicides at a plant in Louisiana were studied for cancer incidence during the time period 1985-1997 (MacLennan et al., 2002) and mortality during 1970-1997 (MacLennan et al., 2003). A nonsignificant excess of prostate cancer incidence was observed based on 11 cases (standardized incidence ratio = 175, 95 % confidence interval = 87-312). The plant had a prostate cancer screening program, which the authors and Hessel et al. (2004) suggest may be responsible for the apparent excess. The mortality study revealed a nonsignificant excess of deaths due to NHL (4 observed/1.1 expected, standardized mortality ratio = 372, 95 confidence interval = 101-952).

Agriculture

To evaluate the possible role of pesticides in the etiology of NHL, De Roos et al. (2003) pooled data from three population-based case-control studies in the Midwestern U.S. The resultant large sample size allowed for analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides. Atrazine was significantly associated with risk of NHL in both logistic and hierarchical regression analyses. Furthermore, there was an indication of a superadditive effect of atrazine in combination with carbofuran, diazinon, or alachlor. Using archival biopsies from the Iowa/Minnesota case-control study, Schroeder et al. (2001) found atrazine to be associated with risk of NHL among t(14;18) cases only.
The role of atrazine and cancer was investigated in the Agricultural Health Study, a large, prospective cohort study of licensed pesticide applicators and spouses from Iowa and North Carolina. Rusiecki et al. (2004), reported on cancer incidence among 36,513 applicators who ever used atrazine, based on follow-up through 2001. There were suggestive, nonsignificant excess risks for lung cancer, bladder cancer, NHL, and multiple myeloma. Rate ratios increase with lifetime days and intensity-weighted lifetime days of atrazine exposure, however confidence intervals were wide, and tests for trend were not significant. There were no excess risks for other cancers, including prostate, which was consistent with an earlier negative report on prostate cancer from the same study based on follow-up through 1999 (Alavanja et al., 2003).

A population-based case-control study conducted in Italy reported a nonsignificant association of triazines and the incidence of leukemia (odds ratio=1.7, 95 % confidence interval = 0.6 -4.7, six exposed cases) for men and women combined (Miligi et al., 2006). A population-based case-control study conducted in an agricultural area of central California evaluated the risk of ovarian cancer in relation to occupational and residential exposures to triazines (Young et al., 2005). Exposure indices were created using work and residential histories obtained by interviews along with the state Pesticide Usage Database. Ever having occupational exposure to triazines was associated with a nonsignificant excess risk (odds ratio = 1.3, 95% confidence interval = 0.8, 2.3). Exposure to atrazine specifically was not

associated with excess risk, based on two exposed cases. Residential exposure to atrazine also showed no increased risk (OR=0.9), based on eight exposed cases.

Environment

Since the last evaluation, the effects of environmental exposure to atrazine were evaluated in several ecological studies. Hopenhayn-Rich et al. (2002) investigated the relationship between atrazine exposure in drinking water to ovarian and breast cancer incidence rates for 1993-1997 in Kentucky, a slightly longer time period than a similar earlier report by Kettles et al. (1997). District exposure indices were based on public drinking water atrazine levels, acreage of corn planted, and atrazine sales. Atrazine levels were not associated with breast cancer and were inversely associated with ovarian cancer. Muir et al. (2004) observed an inconsistent but significant association between breast cancer incidence rates and kilograms of atrazine active ingredient applied in the rural areas of one agricultural county in England using spatial autocorrelation techniques; however, the association was not observed in a second county in the same study. Mills et al. (2006) found no increased risk of breast cancer incidence among California Latinas during 1988-1999 and county levels of atrazine use during 1970-1988. Van Leeuwen et al. (1999) reported that atrazine levels in drinking water in Ontario were positively associated with stomach cancer incidence and negatively associated with colon cancer incidence. The ecological approach is a relatively weak methodology because it uses county-level exposure information to impute "possible" exposure status for individuals instead of individual-level exposure data. In addition, some ecologic studies are cross-sectional whereby current exposure status is linked to measures of current cancer status, without any consideration of latency for tumor development.

Cancer in experimental animals

(*sufficient*, Vol 73, 1999)

Atrazine was tested for carcinogenicity in one study in CD-1 mice by oral administration in the diet, and no increase in tumor incidence was observed. Atrazine was also tested by oral administration in two studies in Fisher rats and in five studies in Sprague-Dawley rats, including a comparison of intact and ovariectomized females of the latter strain. No increase in tumor incidence was observed in the one adequate study in Fisher rats. In contrast, the incidence of mammary tumors was increased in intact Sprague-Dawley females in four studies, but no increase was seen in ovariectomized Sprague-Dawley females. Atrazine increased the incidence of lymphomas in one study in CD-1 mice tested by intraperitoneal injection.

Fukamachi et al. (2004) found that atrazine enhanced 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors in a transgenic strain of Sprague-Dawley female rat containing copies of the human c-Ha-*ras* proto-oncogene. In the comparable strain of male rat, atrazine decreased DMBA-induced skin tumor development (Fukamachi et al., 2004). The authors noted that the doses (5-50 ppm in the diet) were 10,000 times higher than those expected via environmental exposure in humans. The relevance to humans of the co-carcinogenicity study in transgenic animals is unclear. As summarized by Kandori et al. (2005), the observed suppression of prostate cancer in atrazine-treated transgenic rats expressing the probasin/SV40 T antigen was most likely caused by a decrease in calorie intake rather than by atrazine-related endocrine disruption.

Use of atrazine in the presence of nitrogen fertilizers has raised the possibility of *N*-nitrosation in soil. There may also be endogenous formation of *N*-nitrosoatrazine from precursors ingested in the diet and drinking water. A carcinogenicity study of *N*-nitrosoatrazine administered to female Swiss mice and female Wistar rats was cut short due to excessive toxicity, with insufficient duration of exposure for an adequate study (Weisenburger et al., 1990).

Mechanisms of carcinogenicity

IARC (1999), two reviews (Stevens et al., 1999; Cooper et al., 2007), a risk assessment b the California EPA (Gammon et al., 2005), and a U.S. EPA Scientific Advisory Panel (Schoeny et al., 2006) have reached similar conclusions regarding the mechanisms by which atrazine causes mammary tumors in female Sprague-Dawley rats. The mechanism involves an effect by atrazine on the hypothalamus, leading to decreased secretion of hypothalamic norepinenphrine. This results in decreased release of gonadotropin-releasing hormone (GnRH) and decreased levels of GnRH, which affects the pituitary gland (atrazine does not directly affect the pituitary gland). The pituitary gland then attenuates the release of luteinizing hormone (LH) (atrazine alters LH cycling), and disruption of LH cycling results in increased exposure to both endogenous estrogen and prolactin, affecting the function of the ovaries. These altered exposures to reproductive hormones enhance the growth of mammary tumors.

The prolonged atrazine exposure in the female Sprague-Dawley rats seems to accelerate aging within the brain-pituitary-ovarian axis, and this premature reproductive senescence (i.e., constant estrus) establishes the hormonal environment conducive to the development of mammary gland tumors. Because the causative factors associated with reproductive aging in the rat (i.e., impaired hypothalamic function) and human (depletion of primary follicles) are characteristically different, it seems unlikely that a similar process occurs in women.

Nonetheless, the hypothalamic regulation of LH and prolactin secretion is similar in the rat and human; thus, atrazine could possibly influence the secretion of these pituitary hormones in humans. Although the doses used in the rodent studies are extremely high, current estimates of exposure in humans may be too low because of the failure to analyze for the presence of various atrazine metabolites (Barr et al., 2007). Other chlorotriazine herbicides produce metabolites similar to those of atrazine; thus, the combined exposure to such agents may produce an exposure level higher than is currently appreciated or from atrazine alone.

Microarray analysis of 1185 cancer-related genes from RNA extracted from bone marrow of CD-1 mice exposed for 4 months to atrazine in drinking water (1 mg/L) found that atrazine did not alter expression of any of the genes (Cimino-Reale et al., 2008). Proteomic analysis of pituitary removed 5 days after exposure of ovariectomized female Wistar rats to a single dose of atrazine by gavage (200 mg/kg) detected the induction of several proteins that contained active-site or solvent-exposed cysteine residues, making them viable targets for covalent modification by diaminochlorotriazine (DACT), an electrophilic metabolite of atrazine (Dooley et al., 2008).

Atrazine is not mutagenic (IARC, 1999), and two studies published since the last review have confirmed this both in vitro (Kligerman et al., 2000a) and in vivo (Kligerman et al., 2000b).

Reports suggest that atrazine is an immune disruptor in frogs (Brodkin et al., 2007), suppresses immune function in male but not female Sprague-Dawley rats (Rooney et al., 2003), and alters expression of the *rag1* gene in zebrafish, which is involved in acquired immune system disruption ((Liedtke et al., 2008). However, this literature is inconsistent, and a critical review by Solomon et al. (2008) concluded that environmentally relevant concentrations of atrazine do not affect reproduction and/or reproductive development in fish, amphibians, or reptiles. Although some studies have argued that atrazine induces aromatase (Fan et al., 2007), which converts testosterone to estradiol, the review by Solomon et al. (2008) concluded that the literature does not support such a role for atrazine. The authors also concluded that the literature does not permit definitive conclusions regarding the ability of atrazine to affect immune function, stress endocrinology, parasitism, or population-level effects among fish, amphibians, or reptiles. The relevance of this literature to humans remains unclear.

Research needs and future recommendations

The finding that atrazine is carcinogenic at a single organ, sex, strain, and species of rodent makes it unlikely that atrazine is a human carcinogen, considering that most IARC Group 1 carcinogens are trans-species carcinogens. Nonetheless, clear mechanistic data are lacking to show that atrazine does or does not alter the secretion of luteinizing hormone (LH) and prolactin in humans. Thus, further studies are needed to characterize the ability of atrazine to interfere with the hypothalamic-pituitary-ovarian axis in women. Clarification of this issue would help to show whether atrazine is a mammary carcinogen in women.

The U.S. Agricultural Health Study team has conducted an intensive biomarker study among 30 male corn farmers and 10 agricultural extension agents exposed to atrazine and other pesticides (Vermuelen et al., 2005; Bakke et al., 2008), collecting blood and urine at six times during the year coinciding with critical periods in the growing season (e.g., prior, during, and after planting; prior and after harvest; off-season). Although the main focus is on immunological effects, the biospecimens may be suitable for studies of hormonal effects of exposure, which could pertain to prostate cancer risk. If a sufficient number of women who apply atrazine could be identified within the Agricultural Health Study or other populations, a similar biomarker study could shed light on atrazine's hormonal effects among women and its possible role in breast and ovarian cancer.

The epidemiologic report by Rusiecki et al. (2004) on 36,513 atrazine-exposed pesticide applicators within the Agricultural Health Study cohort found suggestions of trends for risk of lung cancer, bladder cancer, NHL, and multiple myeloma, based on follow-up through December 31, 2001. Follow-up of the Agricultural Health Study cohort has recently been extended through 2006. The final numbers are not available yet, but it appears that the number of incident cancer cases has increased by over 65% since 2001. Analyses incorporating these additional data may clarify some of the previously reported nonsignificant findings that were based on small numbers of events. Larger numbers may also allow better control for multiple exposures. In addition, it may be useful to include the spouse members of

the cohort (the majority of whom are female), who were not represented in the Rusiecki et al. (2004) report, to examine risk of breast and ovarian cancers.

The 1990 carcinogenicity study of *N*-nitrosoatrazine was terminated early due to excessive toxicity (Weisenburger et al., 1990). Conducting a study at lower doses might clarify its carcinogenicity.

More extensive microarray and proteomic studies in rodents and humans would also help to characterize the pathways disrupted by atrazine. It is important to verify all the steps in the putative pathway that might explain the unique effects in Sprague-Dawley rats, and the absence in other strains, species, and humans. In addition, investigation of possible effects of *in utero* exposure among humans and animals is needed. .

Although atrazine has been studied extensively for its ability to alter immune function and aromatase in amphibians, reptiles, and fish, only a limited literature has addressed this issue in mammals or humans. Thus, there is a need to explore atrazine's ability to alter these functions in species relevant to humans as well as in human molecular epidemiology studies.

Current biomonitoring methods that have evaluated just one metabolite of atrazine in the urine have probably underestimated exposure. Thus, exposure assessment is needed of a wide array of subjects by the method of Barr et al. (2007), which detects the urinary levels of seven metabolites of atrazine.

Selected relevant publications since IARC review

Alavanja MCR, Samanic C, Dosemeci M. et al. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study Cohort. *Am J Epidemiol* 2003; 157: 800-814.

Arcury TA, Grzywacz JG, Barr DB et al. Pesticide urinary metabolite levels of children in Eastern North Carolina farmworker households. *Environ Health Perspect* 2007: 115, 1254-1260.

Bakke B, De Roos AJ, Barr DB et al. Exposure to atrazine and selected non-persistent pesticides among corn farmers during a growing season. *J Expo Sci Environ Epidemiol* 2009; 19: 544-554.

Barr DB, Panuwet P, Nguyen JV, Udunka S, Needham LL. Assessing exposure to atrazine and its metabolites using biomonitoring. *Environ Health Perspect* 2007; 115: 1474-1478.

Brodkin MA, Madhoun H, Rameswaran M, Vatnick I. Atrazine is an immune disruptor in northern leopard frogs (*Rana pipiens*). *Environ Toxicol Chem* 2007; 26: 80-84.

Cimino-Reale G, Ferrario D, Casati B, et al. Combined in utero and juvenile exposure of mice to arsenate and atrazine in drinking water modulates gene expression and clonogenicity of myeloid progenitors. *Toxicol Lett* 2008; 180: 59-66.

Cooper RL, Laws SC, Das PC et al. Atrazine and reproductive function: mode and mechanism of action studies. *Birth Defects Res B Dev Reprod Toxicol* 2007; 80: 98-112.

De Roos AJ, Zahm SH, Cantor KP et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med* 2003; 60 : e11.

Dooley GP, Reardon KF, Prenni JE et al. Proteomic analysis of diaminochlorotriazine adducts in Wistar rat pituitary glands and Lbeta T2 rat pituitary cells. *Chem Res Toxicol* 2008; 21: 844-851.

Fan,WQ, Yanase T, Morinaga H et al. Atrazine-induced aromatase expression is SF-1 dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans. *Environ Health Perspect* 2007; 115: 720-727.

Fukamachi K, Han BS, Kim CK et al. Possible enhancing effects of atrazine and nonylphenol on 7,12-dimethylbenz[*a*]anthracene-induced mammary tumor development in human c-Ha-*ras* proto-oncogene transenic rats. *Cancer Sci* 2004; 95: 404-410.

Gammon DW, Aldous CN, Carr Jr. WC, Sanborn JR, Pfeifer KF. A risk assessment of atrazine use in California: human health and ecological aspects. *Pest Manag Sci* 2005; 61: 331-355.

Hessel PA, Kalmes R, Smith TJ et al. A nested case-control study of prostate cancer and atrazine exposure. J. *Occup Environ Med* 2004; 46, 379-385.

Hopenhayn-Rich C, Stump ML, Browning SR. Regional assessment of atrazine exposure and incidence of breast and ovarian cancers in Kentucky. *Arch Environ Contam Toxicol* 2002; 42: 127-136.

Kandori H, Suzuki S, Asamoto M, et al. Influence of atrazine administration and reduction of calorie intake on prostate carcinogenesis in probasin/SV40 T antigen transgenic rats. *Cancer Sci* 2005; 96: 221-226.

Kligerman AD, Doerr CL, Tennant AH, Zucker RM. Cytogenetic studies of three triazine herbicides. I. In vitro studies. *Mutat Res* 2000a; 465: 53-59.

Kligerman AD, Doerr CL, Tennant AH, Peng B. Cytogenetic studies of three triazine herbicides. II. In vivo micronucleus studies in mouse bone marrow. *Mutat Res* 2000b; 471: 107-112.

Liedtke A, Muncke J, Rűfenacht K, Eggen RI. Molecular multi-effect screening of environmental pollutants using the MolDarT. *Environ Toxicol* 2008; 23: 59-67.

MacLennan PA, Delzell E, Sathiakumar N, Myers SL. Mortality among triazine herbicide manufacturing workers. *J Toxicology Environ Health Part A* 2003; 66: 501-507.

MacLennan PA, Delzell E, Sathiakumar N, et al. Cancer incidence among triazine herbicide manufacturing workers. *J Occup Med* 2002; 44: 1048-1058.

Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. *Ann NY Acad Sci* 2006; 1076: 366-377.

Mills PK, Yang R. Regression analysis of pesticide use and breast cancer incidence in California Latinas. *J Environ Health* 2006; 68: 15-22.

Muir K, Rattanamongkolgul S, Smallman-Raynor M, et al. Breast cancer incidence and its possible spatial association with pesticide application in two counties of England. *Public Health* 2004; 118: 513-520.

Panuwet P, Nguyen JV, Kuklenyik P, et al. Quantification of atrazine and its metabolites in urine by on-line solid-phase extraction-high-performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 2008; 391: 1931-1939.

Rooney AA, Matulka RA, Luebke RW. Developmental atrazine exposure suppresses immune function in male, but not female Sprague-Dawley rats. *Toxicol Sci* 2003; 76: 366-375.

Rusiecki JA, De Roos A, Lee WJ, et al. Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. *J Natl Cancer Inst* 2004; 96: 1375-1382.

Schoeny R, Haber L, Dourson M. Data considerations for regulation of water contaminants. *Toxicology* 2006; 221: 217-224.

Schroeder JC, Olshan AF, Baric R, et al. Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma. *Epidemiology* 2001; 12: 701-709.

Solomon KR, Carr JA, Du Preez LH, et al. Effects of atrazine on fish, amphibitans, and aquatic reptiles: a critical review. *Crit Rev Toxicol* 2008; 38: 721-772.

Stevens JT, Breckenridge CB, Wetzel L. A risk characterization for atrazine: oncogenicity profile. *J Toxicol Environ Health A* 1999; 56: 69-109.

Van Leeuwen JA, Waltner-Toews D, Abernathy T, Smit B, Shoukri M. Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987-1991. *Int J Epidemiol* 1999; 28: 836-840.

Vermuelen R, De Roos AJ, Bakke B, Blair A, Hildesheim, et al. A study on immunological responses to exposures encountered in corn farming. *J Biochem Molec Toxicol* 2005; 19: 172.

Weisenburger D, Hickman T, Patil K, Lawston T, Mirvish S. Carcinogenesis tests of atrazine and *N*-nitrosoatrazine. *Proc Am Assoc Canc Res* 1990; 31:102 (abstract 603).

Young HA, Mills PK, Riordan DG, Cress RD. Triazine herbicides and epithelial ovarian cancer risk in Central California. *J Occup Environ Med* 2005; 47: 1148-1156.