Image 4

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

November 26, 2002

TXR#: 0051365

SUBJECT: **ATRAZINE** - NRDC Comments on Preliminary Risk Assessment

TO: Catherine Eiden
Reregistration Branch III, HED (7507C)

FROM: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D.
Branch Senior Scientist, Reregistration Branch I
Health Effects Division (7509C)

Submitter:	Syngenta Crop Protection, Inc.
Chemical:	2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine
Synonym:	atrazine, ATZ
Caswell No.:	063
CAS No.	1912-24-9
PC Code:	080803
DP Barcode:	D284707
Submission:	S619582
Action Requested:	Respond to comments.

INTRODUCTION: Natural Resources Defense Council [NRDC] has submitted comments on EPA's revised human health assessment for atrazine [Docket # OPP-34237C]. The comments, dated July 9, 2002, "supplement" their prior filing, dated June 3, 2002 and their prior comments on the preliminary human health and ecologicalrisk assessments. NRDC "reiterates its previous comments urging EPA to cancel, and to revoke all tolerances for, atrazine."

1

The following responses address various issues and concerns presented in the NRDC comments dated June 9, 2002. In general, there are no new issues that have not been addressed previously, and NRDC has not provided any new data for consideration.

1. CANCER ISSUES

NRDC believes that carcinogenicity to humans must be reconsidered and "new data" supporting an alternative mode of action and "new data" on carcinogenicity must be included in the weight of evidence determination.

HED COMMENT: It is to be noted that no new data were submitted by NRDC. Additionally, the alternative modes of action discussed by NRDC were considered and discussed previously, both by the Agency and by the FIFRA Scientific Advisory Panel [SAP].

A) mammary tumors - estrogen levels

NRDC argues that the failure to demonstrate an increase in serum estradiol levels after atrazine exposure refutes the hypothesis that a hyper-estrogenic state develops in SD rats, thereby leading to mammary tumors.

HED COMMENT: The postulated mode of action is that atrazine exacerbates and accelerates reproductive aging in the SD female rat, causing an earlier onset and higher incidence of mammary gland tumors. The key events in this process include suppressing the pituitary LH surge, thereby prolonging estrous and attendant peak estrogen levels which leads to mammary gland tumors. This postulated mode of action is support by the LH data and data that show that atrazine treated SD rats maintain constant estrous. Thus, it is not hypothesized that there is an increase in estrogen levels *per se* but that the estrogen-responsive tissues are exposed for an extended period of time to estrogen because of prolonged estrous. In chronic bioassays on natural and synthetic estrogens, it has been established that prolong stimulation of the mammary gland with estrogen leads to development of adenocarcinomas. NRDC cites 3 studies which they state demonstrate decreases in measured estradiol levels following atrazine exposure to SD female rats. However, in the Eldridge *et al.*, study (1993), which was not cited by NRDC, estradiol levels were elevated at 3 months in the atrazine-treated Sprague-Dawley female rats compared to the control rats at three months. Although the SAP noted that there is a lack of robust data on hormones in the atrazine database, the SAP stated that the database on atrazine strongly supports the hypothesis that prolonged exposure to estrogen produced by the ovary is requisite for development of the mammary tumors observed.

B) mammary tumors - lengthening of estrous

NRDC argues that EPA fails to explain the discordant finding within its proposed paradigm; *i.e.*, Fischer rat shows lengthening of estrus but no mammary tumors [Simic (1994) - J Appl Toxicol 14(6): 401-404]. Since lengthening of estrus is part of the mode of action, this finding demonstrates, according to NRDC, that the hypothesized MOA is not the only [or even the most important] MOA.

HED COMMENT: In the paper cited by NRDC, the prolonged estrous cycle in the Fischer 344 rat was characterized by extended diestrous, in contrast to prolonged estrous in SD rats. In another paper not cited by NRDC [MRID 43598613], the effects of atrazine exposure [100 and 300 mg/kg/day for 2 weeks] on estrous were compared in SD and Fischer 344 rats. The SD female rats exhibited a treatment-related

2

lengthening of the estrous cycle and an increased number of days characterized by cornified epithelial cells. This resulted in a greater percent of the cycle days spent in estrous and reduction in the percent of the cycle days spent in diestrus. Fischer rats also exhibited a significant trend toward cycle lengthening, but this was due to reduction in the percent of cycle spent in estrus and a concomitant increase in diestrual days. According to the authors, these findings suggest that treatment with atrazine at the dose levels used may result in prolonged exposure to endogenous estrogen in the SD but not the Fischer 344 rat.

In comparable studies in the Sprague-Dawley and Fischer 344 rats, atrazine exposure up to 400 ppm had no effect on the percent days in **estrus** in the Fischer rat or mammary gland tumor incidence [[Thakur, A. K. (1991). MRID 42146101] whereas the SD rats [Thakur, A. K. (1991), MRID 42085001] displayed an earlier appearance [after 9 months of treatment] of altered estrous cycles characterized by an increase in the percent days in estrus. Disrupted estrus cycles in SD female rats precede the appearance of mammary gland tumors in both control and treated groups, the effect of atrazine is to make both of these events occur earlier. The estrus and mammary gland tumor results reinforce the view that female SD rats display an earlier disruption of the estrus cycle than do control (untreated) animals and develop mammary gland tumors earlier. Unlike SD rats, control Fischer-344 female rats do not display abnormal estrous cycles until late in life and do not develop a high incidence of mammary gland tumors. The mode of action for SD female rats is further confirmed by data showing that Fischer -344 rats, which have a different reproductive senescence, do not form atrazine-related mammary gland tumors.

The SAP stated that tumorigenesis in female F-344 rats may have been obscured by the decrease in body weight and that a proper statistical test should be employed. EPA performed such analyses [TXR No. 0051364] of the mammary tumors in the F-344rats. The body-weight adjusted analysis of the data indicates that there is no statistically-significant relationship between body weight and tumor incidence.

C) **mammary tumors - male mammary tumors in F-344 rats**

NRDC argues that although the mammary tumors observed in male F344 rats appeared late in the study [Pinter (1990) - Neoplasms 37: 533-544], they add to the weight-of-evidence.

HED COMMENT: As discussed previously by the SAP, a proper age-adjusted analysis of the tumor data results in the conclusion that the tumors appear to be due to increased survival and not to atrazine exposure.

D) **mammary tumors - ovarian cycle disruption in pigs**

NRDC believes these data [Gojermac (1996) - Toxicol Lett 85(1):9-15] indicate that atrazine disrupts ovarian cycling, inhibits estrus at low doses, and causes multiple ovarian cysts in the pig, and these data should not be ignored.

HED COMMENT: These data were not ignored and were considered previously. It was determined that they are of limited value with respect to the mode of action assessment. These data suggest that atrazine may affect reproduction in pigs. However, there are several aspects of the study that hinder interpretation of the findings. Although the authors conclude that atrazine prolonged the estrus cycle, this endpoint [delayed estrus] was not adequately examined. There is no information regarding the frequency of the checks for estrus each day or whether the checks were preformed daily. The pigs may have had an undetected estrus before the expected

estrus; i.e., short cycles. The paper states that the duration of estrus and the length of the estrus cycle were monitored for two cycles for each pig prior to study start, but no data were provided. It is not apparent whether any control pigs were also monitored prior to the start. In the Methods section of the paper, it states that after the last blood sampling [day 24] and checking the next expected estrus, the animals were sacrificed 7 days after the last blood sampling. It is unclear whether the pigs were monitored for the next expected estrus prior to day 24. According to The Merck Veterinary Manual, the estrous cycle is **18**-24 days [average 21] in sows and gilts.

No hormone data were provided for the dosing period [days 1-19]. Blood samples [for hormone assessment] were collected three times in 6 hours on the first five days post dose [estrus cycle days 20-24]. It is noted that Figure B [control] of the paper shows estrus day 22 to be estrus day 0 also. It is not clear whether this is the day when the control displayed estrus or what was considered to be the next expected estrus.

The estradiol values in the treated pigs did not fluctuate much over the 5-day monitoring period, and they do not appear to be elevated compared to the control values. The progesterone levels were low [atrazine pigs] in the first sample, which might be expected if treatment had induced early luteolysis or the pigs were experiencing short normal cycles. The progesterone levels thereafter increased daily, indicative of new luteal function, not persistence of corpora lutea. Although the profiles of these hormones appear to be different between the treated and control pigs [Figures A and B], there may only be a shift in time. Also, this may be a similar situation as observed in the Fischer 344 rat [prolonged cycle not associated with increased estrogen exposure]. Interpretation of the findings in the pig is further hindered since there are no LH data to determine whether the pigs failed to have an ovulatory surge of LH.

Other limitations with this study include the small sample size [4 animals] and the lack of dose-response data [only one dose evaluated].

E) **mammary tumors - non-estrogenic mechanism/increased susceptibility/tumor promotion**

NRDC believes there is new evidence [references Fenton, 2002] that demands a complete reevaluation of cancer risk. Fenton study found that Long-Evans [LE] rats prenatally-exposed to atrazine and subsequently challenged [PND 45] with known mammary carcinogen [DMBA] were more likely to develop mammary gland tumors/more tumors/larger tumors than LE rats exposed to atrazine or DMBA alone. The paper also reported that atrazine exposure alters pubertal mammary gland development *via* a non-estrogenic mechanism, thereby supporting a mechanism by which pubertal alterations may predispose individuals to tumor development.

HED COMMENT: It should be noted that the Fenton *et al.*, data are preliminary and have not been fully peer reviewed. The preliminary work reported in an abstract by Fenton and Davis (2002), an investigator in NHEERL's Reproductive Toxicology Division, suggests that gestational exposure to atrazine may affect the developing mammary gland. This preliminary work indicates that atrazine causes a developmental delay in mammary gland maturation, which lengthens the window of susceptibility to the carcinogen DMBA. In another abstract, Greiner, Youngblood, and Fenton (2002) suggest that atrazine decreases puberty-induced mammary gland development by altering normal pituitary functions. The Fenton *et al.*, data do not provide evidence of a direct cancer mode of action that may be operative in humans given these studies involve co-treatment with a well known mutagenic carcinogen. This work is, however, consistent with the other reported

findings from Dr. Ralph Cooper's laboratory (e.g.,Laws et al., 2001, Stoker et al., 2001) that atrazine affects/causes developmental effects or delays (e.g., delayed puberty) by altering hypothalamic-pituitary function. EPA agrees that atrazine's ability to affect pituitary function and result in developmental effects should be assumed to be relevant to humans. The NOAELs and safety factors used in the atrazine assessment addresses this preliminary study.

F) **Wilms tumor - data on tadpoles**

Reference [Tavera Mendoza Abstract presented at SETAC 22nd Annual Meeting, 11/13/01] is made to a recent study that found that tadpoles exposed to atrazine during gonadal differentiation developed renal embryonic adenosarcoma [Wilms' tumors]. It is stated that disruption of the WT-1 gene can occur in both the frog and human, and that this alteration has been linked to Wilm's tumor in both species [referencing the above]. NRDC believes that this report merits careful consideration.

Additionally, NRDC associates studies reporting association of pesticide exposure of the parents [Sharpe, *et al.*, Epidemiology study in Brazil] and an increase in this tumor in children. NRDC wants this tumor finding as part of the weight of evidence in the cancer assessment.

HED COMMENT: The potential of atrazine to alter the WT-1 gene is not based on data and is speculation at this time. There are similarities and differences in development and regulation by hormones among vertebrates, and the effects of atrazine on amphibians and how they may relate to humans is under review. EPA is planning to convene an independent scientific peer review [the FIFRA Science Advisory Panel (SAP)] of information related to potential effects of atrazine on amphibians sometime in mid-2003.

With respect to the other cited studies, the associations described in humans [Sharpe, *et al.*] were with pesticides in general (insecticides and herbicides] used in farm work. Information on specific pesticides used [atrazine] was not obtained. Although the results reported in some studies suggest parental exposure to pesticides may be related to the subsequent development of cancer in the offspring, other explanations cannot be excluded. Additionally, there are numerous other studies not cited by NRDC that indicate it unlikely that environmental exposures play a major role in the etiology of Wilms tumor.

In a case-control study conducted with histologically confirmed neuroblastoma cases among New York State residents [Kerr, M. A., *et al.* Cancer Causes Control (2000), Aug. 11 (7): 635-643], the odds ratios were significantly elevated for maternal and paternal occupational exposure to various substances, including *insecticides*; herbicides [atrazine] were not mentioned. However, the authors concluded that due to the uncertainty of the biologic plausibility of these associations and the possibility of alternative explanations, the results should be interpreted cautiously.

In a related issue, but one not involving cancer *per se*, there is a growing concern that exposures to a wide-range of endocrine disrupting chemicals (EDCs) are associated with feminization of birds, fish, alligators, and other animals in the environment. The concern has been raised that EDC related feminization of males observed in the ecosystem is also occurring in humans. This is an emerging area of concern, and the scientific community and other interested parties are engaging in discussions. As mentioned above, EPA is planning to convene an independent scientific peer review [the FIFRA Science Advisory Panel (SAP)] of information related to potential effects of atrazine on amphibians sometime in mid-2003.

G) **lymphoma - several studies**

NRDC states that Non-Hodgkin's lymphoma [NHL] has been on the rise in recent decades, and several studies are cited and discussed.

HED COMMENT: As stated by the SAP previously, "To summarize, there are a few epidemiologic studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies showing an association and internal inconsistencies in the studies available indicates that the human studies by themselves do not make a strong case for an association." Refer also to Jerry Blondell's assessment.

H) **Human cancer - prostate cancer, ovarian cancer, testicular cancer, breast cancer, leukemias/lymphomas**

NRDC discusses Syngenta's St. Gafriel facility data and other epidemiology data and states that these data should not be ignored. NRDC believes the epidemiology results are "not likely to be due to chance", and are "almost certainly related to herbicide exposure".

HED COMMENT: As stated above, the SAP considered the available epidemiology data and concluded that there are a few epidemiologic studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies showing an association and internal inconsistencies in the studies available indicates that the human studies by themselves do not make a strong case for an association. Refer also to Jerry Blondell's assessment.

I) **other modes of action** [page 24]

NRDC wants EPA to fully explore other modes of action and relevance to humans, and the organization believes that EPA has failed to adequately consider other modes of action. Although NRDC states that it is clear that atrazine acts as an endocrine disruptor and that one of the modes of action involves the hypothalamic-pituitary-gonadal axis, they are not convinced that this is the only mode of action. NRDC believes there is evidence of at least three other modes of action.

(1) **aromatase activity** -Aromatase is a cytochrome p450 enzyme that converts steroids or androgens to estrogens, thus increasing estrogen levels in the body. It is found in different species (both mammalian and nonmammalian) and in various tissues (mammary gland, ovary, bone, brain, etc). Data available are primarily from studies in frogs, fish and alligators, in addition to data on human adrenocortical cells [*in vitro*]. NRDC says EPA has not explained why it is ignoring this critical information on an alternative mode of action.

HED COMMENT: It is to be pointed out that no new data are provided by NRDC on any of the other modes of action. Furthermore, the SAP was asked to comment on whether alternative modes of action have been sufficiently discussed and ruled out by the Agency. The SAP stated "There are no data that would suggest other plausible modes of action. The increased level of hormones and the increased level of hormones alone, can account for the increased incidence of mammary tumors in Sprague Dawley female rats. The proposed mode of action is plausible and each step in the pathway has been shown to be affected in atrazine treated rats. None of the effects are based on speculation."

Previously, OPP concluded that it is plausible that **enhanced** aromatase activity may have some influence on the development of mammary tumors in SD female rats. However, whether or not enhanced aromatase activity is a significant contribution to the carcinogenicity, or other effects, of atrazine remains to be determined. EPA acknowledged the fact that an increase in aromatase activity would be consistent with dose-response increases in estradiol and estrone and decreases in testicular testosterone noted in a study that examined the effects of atrazine on pubertal development. The doses that resulted in effects on these hormones were well above doses that led to reproductive/developmental effects. Additionally, it was acknowledged that it is plausible that enhanced aromatase activity may have some influence on the development of mammary tumors in SD female rats; however, there are no data to date on whether enhanced aromatase activity significantly contributes to the carcinogenicity observed. The effect of the chlorotriazines on aromatase remains an active research issue, in general. The EPA's National Health and Environmental Research Laboratory (Dr. Ralph Cooper's laboratory) have recently evaluated the effects of atrazine and diaminochlorotriazine [DACT] on aromatase activity in the rat. Preliminary results show that DACT does not effect aromatase activity and atrazine actually causes a **decrease** in aromatase, but only at high doses. Based on the weight of evidence, enhancing aromatase activity does not appear to be a mode of carcinogenic action, particularly given the recent findings of Ralph Cooper. Further, if this were a primary mode of action, a more consistent finding of tumors at estrogen sensitive sites would be anticipated in the rodent carcinogenicity studies. Lastly, the June 2000 FIFRA Scientific Advisory Panel was specifically asked about OPP's assessment of other possible modes of carcinogenic action, and the SAP stated there is insufficient basis to link effects on aromatase to the mammary gland tumor response in female Sprague Dawley rats.

With regard to research data relating to the effects of atrazine on amphibians, EPA has not yet reached conclusions on these data, and therefore does not have any specific comment on these research efforts. EPA is planning to convene an independent scientific peer review [the FIFRA Science Advisory Panel (SAP)] of information related to potential effects of atrazine on amphibians sometime in mid- 2003.

2) **16-alpha-hydroxyestrone** - NRDC states that there is some evidence that atrazine may affect estrogen metabolism, resulting in a greater production of a mutagenic metabolite.

HED COMMENT: In 1993, it was postulated by Davis et al. that the 16α-hydroxyestrone is a type of estrogen which results in the formation of breast cancer in women. But, this hypothesis is in contrast to the work of Aldercreutz et al., 1994 which showed through epidemiologic studies that involvement of estrogen metabolites as a risk factor for breast cancer, is at best circumstantial. Furthermore, more recent work by Ursin et al., 1999 indicates that 16α-/2-hydroxyestrone ratios are not predictive of breast cancer risk in patients. In 1994, Bradlow et al., reported using MCF-7 cells that atrazine might increase the production of 16α-hydroxyestrone by altering the intracellular metabolism of estrogens. However, more recent studies by Safe and coworkers indicate that decreases or increases in 16α-/2-hydroxyestrone ratios do not predict mammary gland cancer potential. McDougal et al., 1997 evaluated the effects of atrazine and the effects of a variety of chemicals known to inhibit or induce mammary gland tumors in rats on the estradiol-2-hydroxylase activity in the MCF-7 model (McDougal et al, 1997). Atrazine reduced estradiol-2-hydroxylase activity, and no correlation between cancer (or anticancer) potential and estradiol-2-hydroxylase activity could be demonstrated. McDougal and Safe, 1998 studied the effects of several pesticides, mammary gland carcinogens and anti-estrogens on estradiol, 16α- and 2-hydroxylase activities and 16α-/2-hydroxylase ratios in MCF-7 cells. These results also indicated that in MCF-7 cells treated with different chemicals both increases and decreases in 16α-/2-metabolite ratios were found and thus 16α-/2-metabolite ratios were not

7

predictive mammary gland carcinogens.

This issue was addressed previously [in Part B of the May 2000 EPA atrazine document], and NRDC has not provided any new data.

3) **metabolite N-nitrosoatrazine** - NRDC points out that this metabolite is mutagenic, and a mutagenic MOA on the part of a metabolite would imply a cancer risk in humans without a threshold. NRDC states that the overall scientific evidence indicates that atrazine may be acting both as an initiator and as a promoter of cancers in hormonally-sensitive organs.

HED COMMENT: Again, it is pointed out that the SAP addressed this issue in 2000. With respect to the potential role nitrosoatrazine may play in cancer development in humans, it is questionable. Although the mutagenic compound *N*-Nitrosoatrazine (NNAT) can be formed *in vitro* when atrazine and nitrite are mixed at an acid pH., and because nitrites and atrazine can be found together in drinking water, concern has been raised about this mutagenic chemical. Although the hypothesis has been advanced that NNAT can be formed in the acid pH found in the stomach, the formation of NNAT in the stomach *in vivo* has yet to be demonstrated. If indeed the mutagenic compound NNAT could act as an initiator of the cancer process, one would expect NNAT to be carcinogenic. However, the cancer bioassays in female Swiss mice and female Wistar rats failed to show a carcinogenic response following NNAT exposure. Since the June 2000 SAP, there have been no new data on NNAT, and NRDC also has not provide any new data to the Agency.

2. FQPA SAFETY FACTOR

A. **2-hydroxyatrazine** - NRDC considers the lack of a FQPA safety factor for 2-hydroxyatrazine to be a mistake since it shows similar toxicity [adverse reproductive endpoints] as atrazine and DACT. NRDC wants a 10X FQPA safety factor on this metabolite also.

HED COMMENT: Unlike atrazine, 2-hydroxyatrazine [2-OH atrazine] did not cause a delay in vaginal opening but did cause a minimal delay in preputial separation (Laws *et al.*, 2002). Furthermore, there was no increase above control levels in the incidence of mammary gland tumors or tumors of any type in a two-year chronic/carcinogenicity study on 2-hydroxyatrazine (Chow and Hart, 1995). In a recent registrant sponsored study [Eldridge, J. C., Minnema, D., Breckenridge, C. B. , et al.; SOT, March 2001], 2-hydoxyatrazine did not suppress the LH surge. However, Dr. Ralph Cooper at the EPA's NHEERL is currently evaluating whether this metabolite alters the LH surge. However, based on available data, it can not be concluded that 2-hydroxylatrazine shares the same neuroendocrine mode of action with atrazine. Thus, the data do not raise the same issues regarding the potential susceptibility of the young due to its neuroendocrine mode of action. Furthermore, no increase in sensitivity was observed following exposure of rats during gestation days 6-15. Data available on 2-OH atrazine include a subchronic oral toxicity study and a chronic oral toxicity study in rats, a rat developmental toxicity study, and mutagenicity studies. Reproductive organ toxicity was not observed in any of these studies. In a recent study [NHEERL], pregnancy loss was observed at 300 and 500 mg/kg/day, but not at 100 mg/kg/day, following 2-OH atrazine exposure of LE dams on gestation days 6-10..

B. **magnitude of safety factor** - NRDC wants a larger safety factor applied, since they consider the FQPA safety factor of 10X, which accounts for both exposure and risk uncertainty, as unlikely to sufficiently capture the magnitude of uncertainty within this assessment.

8

HED COMMENT: The NOAELs determined for delays in preputial separation and delayed sexual maturation are 6.25 mg/kg/day [males]/25 mg/kg/day [females], respectively. These values, which are indicators of pubertal hypothalamic-pituitary-gonadal related effects, show NOAELs that are 3.5X and 14X, respectively, greater than the adult NOAEL for LH effects [1.8 mg/kg/day]. Therefore, by using the adult NOAEL, an additional >3X is actually included in the safety factor.

The HIARC concluded that due to residual concerns [concern of the potential neuroendocrine effects of atrazine exposure throughout all critical developmental periods, which have not been adequately characterized], the **hazard-based** special FQPA safety factor was required. However, the HIARC concluded that it could be reduced to 3X. This was based on a comparison of the lowest NOAEL available in the young animal [6.25 mg/kg/day; 31-day pubertal development study] with the lowest NOAEL in the adult animal [1.8 mg/kg/day ; 6-month LH surge study]. This comparison suggests that the young would not be expected to be an order of magnitude more sensitive than adults. A similar comparison using studies of comparable duration also indicates that the young would not be expected to be an order of magnitude more sensitive than the adult animal. For example, the NOAEL determined for delayed sexual maturation [20 days exposure] in the female rat is 25 mg/kg/day compared to the NOAEL of 5 mg/kg/day in the 28-day exposure study in adult females [LH surge attenuation and estrous cycle alterations].

C. **underestimate of risk** - NRDC considers the lowest dose tested in the 6-month LH surge study to be an **effect** dose [see discussion of this aspect elsewhere]. Although NRDC supports the EPA conclusions that the neuroendocrine effects associated with atrazine exposure are of extreme concern, are relevant to all populations, and are of greatest concern to fetuses, infants, and children, NRDC states that the demonstrated ability of atrazine and its metabolites to disrupt normal neuroendocrine function will impact growth, development, reproduction, immune, and metabolic functions. NRDC continues by pointing out that "human exposures to abnormal levels of LH during early life may permanently imprint on the hypothalamic-pituitary-gonadal pathway, thereby determining the ability to respond normally to testosterone and other gonadal hormones later in life. The response of the central nervous system to the gonadal hormones during childhood and puberty is tightly regulated by neurotransmitters in the brain, mainly glutamate and GABA (gamma-aminobutyric acid). Without the normal hormonal levels during development of the fetal and infant nervous system, the ability to elicit normal responses to gonadal hormones later in life may be compromised. It is likely that exposure of the infant and toddler to levels of atrazine during early life, at levels which interfere with LH activity, may have adverse effects on pubertal development, and on later reproductive function, negatively impacting on the life-long health of an exposed person."

HED COMMENTS: NRDC's comments are essentially what was stated in the risk assessment. Atrazine is one of the best studied pesticides, and there is an extensive toxicology database on its mechanism of toxicity. The perturbation of the hypothalamic-pituitary-gonadal axis is the primary and only established mode of action of atrazine. Based on the nature of the effect of concern [neuroendocrine disruption] and uncertainties with respect to possible effects from exposure throughout development, which have not been thoroughly examined, a potential for noncancer effects due to atrazine's ability to disrupt hypothalamic-pituitary function could not be discounted. The endpoint selected for the risk assessments [LH surge attenuation and estrous cycle alterations] serves as a **surrogate** for the effect of atrazine on the hypothalamic-pituitary axis/function, and the NOAEL selected is the lowest NOAEL in the database for the endpoint of concern and is considered protective for all population subgroups.

EPA disagrees with NRDC that 1.8 mg/kg/d is a LOAEL in the 6 month LH surge study by Syngenta. EPA believes it is justified in using 3.6 mg/kg/d as a LOAEL for this endpoint. The rationale for the selection of 3.6 mg/kg/d as a LOAEL and 1.8 mg/kg/d as a NOAEL for suppression of the LH surge is based on a weight of evidence argument. There is a dose response trend for suppression of the LH surge, albeit, the 3.6 mg/kg/d dose does not represent a statistically significant decrease in the amount of LH. Importantly, this dose response trend is supported by the statistically significant difference in vaginal cycling at 3.6 mg/kg/d. Vaginal cycling data tend to be less variable than LH data. Thus, EPA acknowledges that selection of 1.8 mg/kg/d as a NOAEL for LH suppression is conservative, but errs on the side of health protection. Although there is one statistically significant response for suppression of the LH surge in the 1.8 mg/kg/d dose group for one time point, this is not sufficient evidence to designate 1.8 mg/kg as a LOAEL, particularly in light of the fact there were no statistically significant differences found for vaginal cycling at this dose.

Aldercreutz H, Gorbach SL, Goldin BR, Woods MN, Dwyer JT, and Harnalaninen E Estrogen metabolism and excretion in oriental and Caucasian women. J. Nat. Cancer Inst. 86:1076-1082. 1994

Bradlow, H.l., Davis, D.L., Lin, g., Sepkovic, D. and Tiwari, R. 1995. Effects of pesticides on the ratio of 16α/2-hydroxy estrone: a biologic marker of breast cancer risk. *Environ.. Health Persp.*: Vol 103, Supplement 7: 147-150.

Davis DL, Bradlow LH, Wolff M, Woodruff T, Hoel DG, and Anton-Culver H. 1993. Medical Hypothesis: Xenoestrogens as preventable causes of breast cancer. Environ. Health Perspect. 101:372-277.

Laws, S.C., Ferrell, J.M., Stoker, T.E., Cooper, R.L. 2002. Pubertal Development in Female Wistar Rats Following Exposure to Propazine and Atrazine Metabolites, Diamino-S-Chlorotriazine and Hydroxyatrazine. Abstract. Society of Toxicology 2002.

McDougal A, Wilson C, and Safe S. Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cell by pesticides and carcinogens. Environ. Toxicol. Pharmacol. 3: 195-199. 1997

McDougal A, and Safe S Induction of 16alpha-/2-hydroxy estrone metabolite ratios in MCF-7 cells by pesticides , carcinogens and antiestrogens does not predict mammary carcinogens Environ Health Perspect. 106:203-206. 1998

Ursin G, London S Stanczyk FG, Gentzschein E, Pagannini-Hill A, Ross RK and Pike MC 1999 Urinary 2-hydroxy estrone ratio and risk of breast cancer in postmenopausal women. J. Nat. Cancer Institute 91(12) 1067-1072.

11