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November 14, 2000

MEMORANDUM

SUBJECT: *ATRAZINE* - Re-evaluation by the FQPA Safety Factor Committee.

FROM: Brenda Tarplee, Executive Secretary
FQPA Safety Factor Committee
Health Effects Division (7509C)

THROUGH: Ed Zager, Chair
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PC Code: 080803

The Health Effects Division (HED) FQPA Safety Factor Committee (FQPA SFC) met on October 23, 2000 and November 8, 2000 to evaluate the hazard and exposure data for atrazine (including its chloro- and hydroxy-metabolites) in order to recommend the FQPA safety factor to be used when assessing the risks posed by the use of this chemical. The SFC concluded that the FQPA safety factor should be retained at 10x when assessing parent atrazine and its chloro-metabolites (represented by DACT) but could be removed (1x) when assessing the hydroxy-metabolites.

I. Background

The HED FQPA Safety Factor Committee (FQPA SFC) met on **July 19, 1999 and September 27, 1999** to evaluate the hazard and exposure databases for atrazine, simazine, and propazine in order to make the FQPA safety factor recommendation to be used in risk assessment. A final recommendation report did not result from this meeting since the Committee had concerns for evidence of developmental toxicity in the absence of maternal toxicity in the prenatal developmental toxicity study conducted with the terminal mammalian metabolite of atrazine (simazine and propazine) diaminochlorotriazine (DACT). Since additional data, including a 28-day luteinizing hormone surge study using atrazine, simazine and DACT, were expected to be submitted to the Agency, the final recommendation report was held pending review of these data. A draft interim report of this study was received (MRID 45058701) in mid-March, 2000 and a HIARC meeting was scheduled shortly thereafter.

On **May 4, 2000**, the Hazard Identification Assessment Review Committee (HIARC) reevaluated Atrazine including additional data generated by the Agency's National Health Effects Environmental Research Laboratories (NHEERL) pertaining to the potential effects to infants and children. DACT and hydroxyatrazine were also evaluated at this meeting including toxicological dose /endpoint selection and FQPA assessment. (For the conclusions of the HIARC, refer to HED Doc. Nos. 014308, 014311, and 014312).

On **June 27-29, 2000**, the toxicological and mechanistic information available on Atrazine, including whether or not its CNS neuroendocrine mode of action is relevant to children, was presented to the **FIFRA Scientific Advisory Panel**. The final report of this meeting has not been released (Refer to summary document prepared by V. Dellarco; final version dated November 8, 2000; Atrazine: Considerations Relating to the Potential Susceptibility of the Developing Fetus and Young).

The **HED FQPA SFC** met on **October 23, 2000** and again on **November 8, 2000** to evaluate the hazard and exposure data for atrazine (including its chloro- and hydroxy-metabolites). The Committee's conclusions follow.

II. HAZARD ASSESSMENT

(Correspondence: R. Hawks to B. Tarplee received Oct. 17, 2000 and V. Dellarco received Nov. 6, 2000)

1. Adequacy of Toxicology Database

Although the toxicology database for atrazine was considered adequate by the HIARC for consideration of potential adverse health consequences to infants and children under FQPA (see HIARC Report dated August 28, 2000; HED Document No 014308), the FQPA SFC determined that uncertainties remain. While atrazine has been evaluated for potential reproductive effects under the Subdivision F 83-4 two-generation protocol in rats, and for prenatal developmental toxicity in rabbits and rats, these studies did not assess important endocrine endpoints, which are included in the 1998 OPPTS guidelines.

However, several non-guideline studies have been conducted which assessed endocrine-related toxicity. These include the following:

Stoker *et al.*, 1999 demonstrated that exposure of a lactating dam to atrazine during the days shortly after parturition may result in increased incidence and severity of prostate inflammation in male offspring.

More recently, atrazine was evaluated under a study protocol designed for endocrine disruptors. Stoker *et al.*, 2000 and Laws *et al.*, 2000 conducted pubertal assays in the rat where positive findings were observed for both males and females.

It should be noted that NHEERL has also generated data on the chloro-metabolites of atrazine that demonstrate effects on male puberty similar to atrazine (Unpublished SOT Abstract - *The Effects of Atrazine Metabolites on Puberty in the Male Wistar Rat*. D L Guidici, R L Cooper and T E Stoker. Endocrinology Branch, NHEERL, U.S. Environmental Protection Agency, RTP, NC.).

Additionally, the HIARC recommended that studies be conducted to further assess the neurotoxic potential of atrazine, and that a two-generation reproduction study in rats (OPPTS 870.3400) be conducted with DACT. The FQPA SFC determined that these considerations demonstrated uncertainties regarding potential toxicity to infants and children, for both atrazine and DACT.

2. Determination of Susceptibility

HIARC concluded that there is no evidence of increased susceptibility (quantitative or qualitative) seen in the standard developmental or two generation reproduction studies conducted using pre-1998 Subdivision F guidelines.

There is, however, evidence of increased susceptibility given the prostate inflammation and delayed puberty (in males and females with atrazine and in males with DACT) found in rat studies consistent with the atrazine CNS mode of action. These endpoints were not assessed in the guideline studies that had been conducted on atrazine and DACT.

DACT, the terminal metabolite, causes effects similar to atrazine (delayed puberty). Additionally, quantitative increased susceptibility was demonstrated in a prenatal developmental toxicity study with DACT in rats: developmental effects were seen in the absence of maternal toxicity. The maternal NOAEL was 25 mg/kg/day based on statistically significant decrease in body weight gain at 75 mg/kg/day (LOAEL). The developmental NOAEL was 2.5 mg/kg/day based on increase incidences of incompletely ossified parietals, interparietals and unossified hyoids at 25 mg/kg/day (LOAEL).

3. Mode of Action

Recent research studies at EPA's National Health and Environmental Effects Laboratory have provided evidence that atrazine alters the CNS (hypothalamic) control of pituitary-

ovarian function (Cooper *et. al.*, 2000; Stoker *et.al.*, 2000; Laws *et al.*, 2000; also see OPP Atrazine Health Assessment Document May 22, 2000). Atrazine has been shown by NHEERL to disrupt critical reproductive processes including puberty, ovarian cyclicality, pregnancy and lactation (milk quality/production) in treated rats. All of these effects are consistent with a CNS-hypothalamic mode of action. This CNS mode of action is operative in both adults and the young, and results in altered pituitary hormone function, especially luteinizing hormone (LH) and prolactin (PRL) secretions. Atrazine has been shown to decrease the neurotransmitter, norepinephrine, which impairs the pulsatile release of gonadotropin releasing hormone (GnRH), thus leading to a suppression of the pituitary LH release. Atrazine also increases the neurotransmitter dopamine, which in turn leads to a decrease in pituitary prolactin release. The HIARC determined that there is a cause for concern for infants and children, given the available information on the mode of action (specifically, neuroendocrine alterations at the hypothalamus).

4. Requirement of a Developmental Neurotoxicity Study

HIARC concluded that a standard guideline (OPPTS 870.6300) developmental neurotoxicity study (DNT) is not required at this time. However, due to concern for atrazine's CNS mode of action affecting neuroendocrine function, the HIARC did recommend that studies be performed which examine the specific CNS alterations described in the studies conducted by the registrant and the Agency's NHEERL labs. It was noted that any additional testing on atrazine should consider incorporation of hypothalamic neurotransmitter, hormone, and reproductive/developmental measures following developmental exposures (gestation through lactation, pre-weaning, up to day 60), as well as sensitive functional/behavioral neurological evaluations.

5. Summary of June 27-29, 2000 FIFRA Scientific Advisory Panel Comments (Summary document prepared by V. Dellarco; final version dated November 8, 2000)

On June 27-29, 2000, the toxicological and mechanistic information available on Atrazine, including whether or not its CNS neuroendocrine mode of action is relevant to children, was presented to the FIFRA Scientific Advisory Panel (SAP). **The final report has not been released** however, a summary document has been prepared by HED Senior Scientist, V. Dellarco, which includes a brief section on the discussion at that meeting (Atrazine: Considerations Relating to the Potential Susceptibility of the Developing Fetus and Young; See Attachment 1).

In general, the SAP agreed that the rat findings on atrazine and its CNS mode of action in the rat raised a concern for children since:

- ▶ delayed puberty and prostatitis effects consistent with perturbation of hypothalamic-pituitary-gonadal axis were observed;
- ▶ neurotransmitter effects could potentially translate into severe effects in children that may not be manifested until later in life;

- ▶ there are gaps in the available data resulting in uncertainties (e.g., there is no information regarding the potential for atrazine to alter estrogen receptors; additionally there is a lack of information on the pre- and peri-natal periods and on the potential of atrazine to result in neurodevelopmental effects).

III. EXPOSURE ASSESSMENTS

1. Dietary (Food) Exposure Considerations

(Correspondence: C. Eiden to B. Tarplee dated October 17, 2000)

Although there are some post-emergence uses, the triazines in general are primarily used in early spring preplant and pre-emergence soil applications. Uses for atrazine exist on corn, sorghum, sugarcane and wheat, guava, and macadamia nuts.

Atrazine is 6-chloro-N²-isopropyl, N⁴-ethyl, 1,3,5 triazine-2,4-diamine. In a generalized scheme of metabolism the chloro-atom can be replaced either by a hydroxy- group or by a glutathione conjugate. This leads to three families of metabolites: the chloro metabolites, the hydroxy metabolites, and the glutathione metabolites.

Residues of atrazine that are soil applied and are absorbed through the root system are systemic, and are translocated throughout the plant. Therefore, residues cannot be removed by simple washing or peeling. Foliar applied residues are apparently not translocated to other parts of the plant but do penetrate into the local tissues and also cannot be removed by simple washing or peeling.

Should cancer dietary risk assessment be needed, the residues of concern for estimating human exposure would be parent atrazine and the chloro-metabolites. (Memo, 9/29/95, J. Abbotts). Pending concurrence from the metabolism committee, exposure assessment for chronic non-cancer dietary risk should be performed on two different sets of residues. One assessment should be based on combined parent and chloro-metabolites, using the RfD for parent atrazine. The second evaluation should be based on anticipated residues of combined free hydroxy-metabolites, using an RfD assigned for hydroxy-atrazine. The tolerance expressions will include parent and the chloro-metabolites.

Monitoring data is available for parent atrazine for many foods. The Pesticide Data Program (PDP), Food and Drug Administration (FDA), and Food Safety and Inspection Service (FSIS) have all monitored for atrazine. In general, these monitoring data suggest that exposure to the parent atrazine through food is small. However, because the parent atrazine is also not found in the fruiting parts of plants in the metabolism studies, it is difficult to estimate the total chloro- or hydroxy- metabolites in fruits, nuts or grains based upon testing for parent atrazine, alone. Therefore, for dietary food exposure assessments, residues of atrazine, its chloro- and hydroxy metabolites were estimated from field trial and metabolism study data where possible as these studies included analyses for the chloro- and hydroxy metabolites, as well as the parent compound, in the main crops

on which atrazine is used. Except for sugar cane, all human foods treated with atrazine are fruits, nuts or grains. Sugar cane, of course, is highly processed before it is consumed by humans.

In addition, BEAD has recently updated percent crop treated information for atrazine. Data is good for the major crops. Uses of atrazine are 76% average to 100% maximum on sugarcane, approximately 50% to 60% max on sweet corn, 82% to 97% max on other corn, 60 to 74% max on sorghum and less than 1% on wheat. BEAD has information that use on macadamia nuts may be around 57%. Novartis has suggested that there is not more than 10% use on guava and BEAD has affirmed that this estimate should be conservative.

HED conducts dietary risk assessments using the Dietary Exposure Evaluation Model (DEEM™), which incorporates consumption data generated in USDA's Continuing Surveys of Food Intakes by Individuals (CSFII), 1989-1992. Refinements to the analyses included the use of anticipated residues from field trial data, available percent crop treated data and processing data where applicable. Because the human food portions of crops treated with atrazine were generally free of residues, estimated exposure was very predominantly through milk and meat from animals fed the feed and forage portions of crops that did contain residues of atrazine. These estimates in animals foods, however, are based upon conservative assumptions that may overestimate the residues in these foods.

2. Dietary (Drinking Water) Exposure Considerations

(Correspondences: J. Lin to B. Tarplee dated October 18, 2000 and M. Frankenberry to B. Tarplee dated November 8, 2000)

The environmental fate database is complete enough to characterize drinking water exposure resulting from the use of atrazine. The half-life of atrazine in loamy soils ranges from 60 to 150 days. However, when conditions in soils are changed from aerobic to anaerobic, the rate of degradation slows considerably. In addition, atrazine degrades very slowly once it enters the water column. Half-life in reservoirs may be 1 to 2 years. Atrazine has both a low vapor pressure and low Henry's law constant, and thus volatilization from soil surfaces and water is negligible. The moderate water solubility and small K_d and K_{oc} favor movement of atrazine in the dissolved state from treated soil in surface or subsurface waters during irrigation or rain events.

Known metabolites of atrazine are deethylatrazine (DEA), deisopropylatrazine (DIA), diaminochlorotriazine (DACT), hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA). DEA would be expected to be more mobile in the aquatic environment than parent or other metabolites because of its lesser K_d and K_{oc} values. According to the measured K_d and K_{oc} values, HA is expected to be least mobile in soil/water systems. Soil half-lives for DEA, DIA, DACT, and HA have been reported to be 26, 17, 19, and 121 days, respectively.

The parent and several of its metabolites are considered to be highly mobile and persistent due to the wide detections in the monitoring studies. Based on the information of the environmental fate profiles and the results of the monitoring programs, the drinking water exposure potential is high.

The PRZM/EXAMS modeling with the considerations of Index Reservoir and Percent Crop Area (IR/PCA) were implemented to generate the estimated drinking water concentrations from surface water source for corn use. SCIGROW screening model was used to estimate drinking water concentrations from groundwater source however, due to the abundance of available monitoring data, the drinking water estimations will be based on monitoring data.

Several monitoring databases, including Novartis PLEX (population lined exposure) database (21 States), Novartis voluntary monitoring study (8 States), Acetochlor Registration Partnership (ARP) study (12 corn States), Novartis Rural Well survey (19 States), and various State EPAs' Monitoring Results, will be used to derive the estimations of drinking water for both surface water and groundwater sources. Due to the large coverage area, it would be expected that the vulnerable areas have been included in the monitoring programs.

Uncertainty in the monitoring data exists for the estimation of degradates in both surface and ground water. Surface water degradate estimates have been derived from regression equations calculated from data collected for 17-20 CWSs only in one year of monitoring. In addition to the limited data for surface water, there are no data for degradates in ground water CWSs, a gap which the registrant is seeking to fill. And data collected for ground water estimates from rural wells consist of one sample only per well for the survey. Given the variability over time that is apparent in monitoring from other ground water surveys, one sample per well is not adequate to characterize the concentrations. Finally, all estimates derived for the chloro-atrazine degradates may underestimate total chloro-triazine levels because total levels consist of chlorinated contributions from the other triazines (simazine and cyanazine) which are not assessed here.

3. Residential Exposure Considerations

(Correspondences: G. Bangs to B. Tarplee dated October 10, 2000 and October 17, 2000)

Based on the product labels, meetings with registrants, and usage reports, children and infants would be exposed to atrazine after application of the herbicide to sod, golf course turf, and residential lawns. The formulated product has a non-restricted label. Atrazine is contained in several homeowner-usable "weed and feed" formulations. There is limited information available, but BEAD estimates the average rate on sod to be 2.3 lb ai per acre annually (typically one application at 2 lbs ai per acre per year).

The following chemical-specific studies will be used for residential post-application exposure scenarios: Dissipation of turf transferable residues after application of (1) granular and (2) dry flowable formulations. For some scenarios, such as soil ingestion,

the Residential SOPs will be used. In addition, golfing exposure is assessed using guidelines developed by the Exposure SAC. BEAD and/or marketing data that determine frequency, seasonality, and geographic distribution of use are also available.

It is noted that all of the available ORE handler and postapplication exposure studies use dosimeters and measure for the parent atrazine only. The hydroxy- and chlorometabolites are not measured (or at least not quantified using that method).

IV. SAFETY FACTOR RECOMMENDATION AND RATIONALE

1. FQPA Safety Factor Recommendation

The Committee recommended that the FQPA safety factor be retained at 10x when assessing parent atrazine and its chloro-metabolites (represented by DACT) but could be removed (1x) when assessing the hydroxy-metabolites.

2. Rationale for Retaining the FQPA Safety Factor for Atrazine and Chloro-metabolites

The FQPA SFC concluded that the FQPA safety factor be **retained** at 10x when assessing parent atrazine and its chloro-metabolites (represented by DACT) because:

- ▶ There is qualitative evidence of increased susceptibility, given the prostate inflammation and delay in puberty in rat studies (in males and females with atrazine and in males with DACT), which is consistent with the atrazine mode of action;
- ▶ There is cause for concern for infants and children given the evidence from special studies describing the central nervous system (CNS) toxicity (specifically, neuroendocrine alterations at the hypothalamus);
- ▶ Quantitative increased susceptibility was demonstrated in a prenatal developmental toxicity study with DACT in rats (developmental effects were seen in the absence of maternal toxicity);
- ▶ There is uncertainty in the toxicology data base since HIARC recommended that studies examining the specific CNS alterations be performed. Additionally, the HIARC required that a two-generation study be conducted with DACT employing the OPPTS Series 870 guidelines; and
- ▶ There is some uncertainty in the water monitoring data for the estimation of degradates in surface water and, to a greater extent, in ground water (See Section III.2. above).

3. Application of the Safety Factor - Population Subgroups / Risk Assessment Scenarios

The FQPA safety factor for atrazine and its chloro-metabolites is applicable to **All Population Subgroups for dietary and non-dietary exposure assessments of All Durations** since there are concerns and uncertainties in the toxicology and exposure data bases which could impact all population subgroups during all durations of exposure.

4. Rationale for Removing the FQPA Safety Factor for Hydroxy-metabolites

The FQPA SFC determined that it is appropriate to consider hydroxyatrazine separately given its toxicological profile and expected exposure pattern and concluded that the FQPA safety factor could be **removed** (1x) when assessing the hydroxy-metabolites since:

- ▶ There was no evidence of increased susceptibility in the prenatal developmental toxicity study in rats with hydroxyatrazine;
- ▶ There is no evidence of neurotoxicity from the submitted toxicity studies;
- ▶ The neuroendocrine effects described for atrazine are postulated to be part of a cancer mode of action for atrazine. Because hydroxyatrazine is non-carcinogenic, the current belief is that the neuroendocrine effects described for atrazine are not occurring following hydroxyatrazine exposure;
- ▶ The drinking water exposure concerns expressed for atrazine and the chloro-metabolites (Section III.2.) do not apply to hydroxyatrazine, given its dissimilar toxicological profile and environmental fate properties (measured Kd and Koc values indicate that hydroxyatrazine is less mobile in soil/water systems); and
- ▶ The dietary and non-dietary exposure assessments will not underestimate the potential exposures for infants and children.



November 8, 2000
Prepared by V. Dellarco

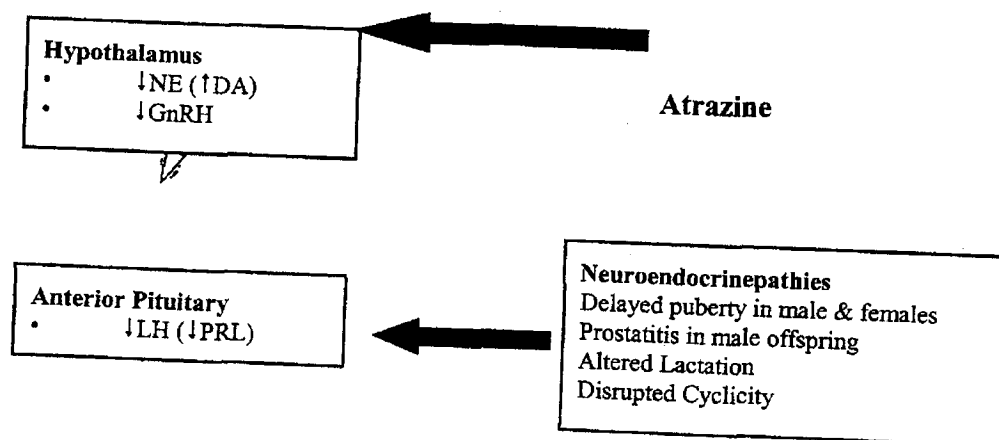
Atrazine: Considerations Relating to the Potential Susceptibility of the Developing Fetus and Young

THIS IS A DISCUSSION PIECE TO ASSIST THE FQPA SAFETY FACTOR COMMITTEE IN THEIR DELIBERATIONS. PERTINENT INFORMATION IS SUMMARIZED REGARDING THE IMPLICATIONS OF ATRAZINE'S MODE OF ACTION ON CHILDREN'S HEALTH, AND ON RESIDUAL UNCERTAINTIES IN THE AVAILABLE EVIDENCE.

For noncancer effects relevant to children's health, Atrazine's neuroendocrine mode of action is an important consideration in the determination of the FQPA safety factor.

Mode of Action:

Recent research studies at EPA's National Health and Environmental Effects Laboratory have provided evidence that atrazine alters the CNS (hypothalamic) control of pituitary-ovarian function (Cooper *et. al.*, 2000; Stoker *et.al.*, 2000; Laws *et al.*, 2000; also see OPP Atrazine Health Assessment Document May 22, 2000). Atrazine has been shown by NHEERL to disrupt critical reproductive processes including puberty, ovarian cyclicity, pregnancy and lactation (milk quality/production) in treated rats. All of these effects are consistent with a CNS-hypothalamic mode of action. This CNS mode of action is operative in both adults and the young, and results in altered pituitary hormone function, especially luteinizing hormone (LH) and prolactin (PRL) secretions. Atrazine has been shown to decrease the neurotransmitter, norepinephrine, which impairs the pulsatile release of gonadotropin releasing hormone (GnRH), thus leading to a suppression of the pituitary LH release. Atrazine also increases the neurotransmitter dopamine, which in turn leads to a decrease in pituitary prolactin release.



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Although the mode of action has been reasonably established for atrazine, the exact mechanism by which it changes neurotransmitters and neuropeptides within the CNS is not understood. Although atrazine alters hypothalamic norepinephrine and dopamine, these effects do not necessarily represent its primary site of action. These CNS alterations may be a signal of potential upstream effects on other neurotransmitters.

Implications of the Mode of Action on the Young & Relevance to Humans:

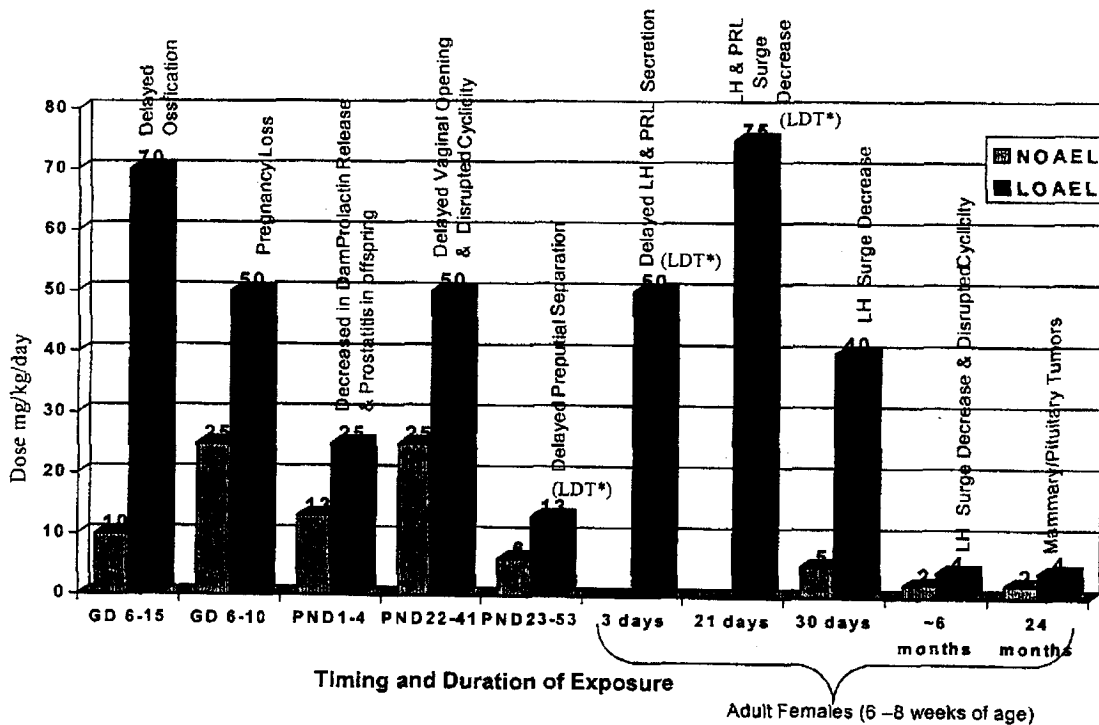
Gonadal development and reproductive growth are dependent on the GnRH regulation of pituitary LH and prolactin. Thus, it is not surprising that administration of atrazine during critical periods of development resulted in delayed puberty in both female and male rats, and in a decrease in suckling -induced prolactin release in lactating dams that lead to prostatitis in adult male offspring. The pubertal and prostatitis effects are viewed as evidence consistent with atrazine's CNS mode of action. The health consequences in children of these hypothalamic changes are not known. Nevertheless, atrazine's CNS effect on the rat hypothalamic-pituitary-gonadal axis should be of concern. There is evidence in the literature that hypothalamic neurotransmitters and neuropeptides are involved in the modulation of GnRH during reproductive and pubertal development in primates. The primate GnRH pulse generator can be modulated by hypothalamic neuronal inputs like in the rat. For example, treatment of GnRH antagonists such as methyl aspartate can prevent the re-awakening of the pulsatile LH release in primates (Wu et al., 1996; Gay and Plant, 1987). Furthermore, neurotransmitters such as NE and dopamine play an important role in brain development. Thus, the rodent findings raise a concern for children if exposed to atrazine.

Completeness of the Toxicity Database:

The toxicology database for atrazine is considered adequate by the HIARC (see August 28, 2000 report) for consideration of potential health to infants and children under FQPA. Prenatal developmental toxicity studies in rabbits and rats are available. Although atrazine has been evaluated for potential reproductive effects, this was done under the old (*i.e.*, pre-1998) two- generation protocol in rats. Therefore, the lack of observed susceptibility in the atrazine guideline reproductive study may be misleading because these pre-1998 guidelines did not include sensitive measures of endocrine disruption that are now included (e.g., estrous cyclicity, sperm measures, sexual maturation, expanded postmortem observations).

More recently, atrazine was evaluated under a study protocol designed for endocrine disruptors. It was evaluated by NHEERL in the rat pubertal assays where positive findings were observed for both males and females (Stoker *et al.*, 2000 and Laws *et al.*, 2000).

It should be noted that although atrazine has a CNS mode of action, it and its metabolites have not been evaluated in any standard guideline neurotoxicity assays. Below is a summary of the NOAELs and LOAELs for the developmental and reproductive effects of atrazine.



Key neuroendocrine related effects following atrazine treatment of rats. [NOTE: It is unlikely that the delayed ossification found in developmental studies is related to Atrazine's neuroendocrine mode of action.]

*LDT = lowest dose tested

Data Base Issues:

Although information has been developed on atrazine's mode of action and resulting neuroendocrinopathies (delayed puberty, prostatitis, pregnancy loss, altered lactation), there are some issues and uncertainties that arise from the available data.

- The focus of testing with atrazine in young rats has been limited to short term dosing of a specific developmental period (postnatal days ~20 - 50 in the rat pubertal assays).** This raises two issues: (1) the uncertainty associated with the apparent sensitivity during earlier developmental periods, and (2) the uncertainty of the consequence of a longer duration of dosing throughout development. From a review of the literature on endocrine disruptors (EPA 1997 Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis by Crisp et al., and the 1999 NAS Report on *Hormonally Active Agents in the Environment*), an increased sensitivity can be found with exposures to early developmental periods with other endocrine disruptors. Therefore, it is important for any chemical that is anti-estrogenic or anti-androgenic to evaluate critical periods throughout development. Although atrazine does not bind to estrogen or androgen receptors directly, it behaves like an anti-estrogenic or

androgenic chemical for studies done by NHEERL (Stoker et al., and Laws et al., papers). Furthermore, it has been demonstrated in rats and mice that suppression of prolactin in the lactating dam during postnatal days 1-10 will result in the disruption of neuronal development within the tuberoinfundibular dopaminergic neurons (TIDA). In the rat, this in turn will lead to the development of hyperprolactinemia prior to puberty (at ~PND 25-30) (References are in the Stoker et al 2000 paper). Evidence indicative of a loss of a specific population of hypothalamic neurons that play a key role in the regulation of prolactin has been demonstrated for atrazine. Therefore, it is important to consider evaluation of earlier developmental periods for atrazine.

Data on atrazine suggest that the longer the duration of exposure to young animals, the lower the dose that is needed to produce effects. For example, a lower NOAEL is found in the male pubertal assay with a longer duration of exposure (30 days) compared to the female pubertal assay (20 days).

In summary, there is a reason basis to believe that longer term dosing to atrazine that covers all critical developmental periods, gestation through puberty in both male and female rats might lead to lower NOAELs than those identified.

- **Studies on the effects of NE, Dopamine, and GnRH have been acute (3 day) treatments at high doses.** Atrazine's effects on these neurotransmitters/peptides at longer exposures and longer doses is not known.
- **The focus of testing has been on cancer and its endocrine reproductive effects:** Atrazine's endocrine effects on reproduction are secondary to its CNS effects on hypothalamic neurotransmitters and neuropeptides. No evaluation of neurotoxicity has been conducted on atrazine or its metabolites. It is not known whether atrazine's CNS mode of action would lead to behavioral effects in the young or at what dose compared to its reproductive developmental effects. Thus, in addition to functional neurological evaluations, more sensitive CNS measures relevant to atrazine's mode of action should be discussed and considered, such as endpoints indicative of CNS toxicity or measures of sexual differentiation in the brain.

[NOTE: Data on the neurological effects of hormonally active environmental contaminants are very limited. Unfortunately, little is known about the neurological effects of endocrine disruptors. The EPA 1997 *Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis* (Crisp et al.), and the 1999 NAS Report on *Hormonally Active Agents in the Environment* point out the major role played by the CNS in integrating hormonal and behavioral activity and disturbances in these finely coordinated mechanisms can impair normal adaptive behavior and reproduction.]

Testing Recommendations:

Although HIARC (August 28, 2000 report) determined that a standard DNT is not required because atrazine's CNS mode of action affected pituitary endocrine function, HIARC did recommend that studies examining the specific CNS alterations described in the studies conducted by the registrant and the Agency's NHEERL labs be performed.

NOTE: Based on the issues presented above for atrazine, the June 2000 SAP comments (see below), and more recent studies from the NHEERL, any additional testing on atrazine should consider incorporation of hypothalamic neurotransmitter, hormone, and reproductive/developmental measures following developmental exposures (gestation through lactation, pre-weaning, up to day 60), as well as sensitive neurological evaluations.

Determination of Susceptibility

HIARC (August 28, 2000) concluded that there was evidence of increased susceptibility given the delayed puberty found in rat studies consistent with atrazine's CNS mode of action.

Metabolites

DACT is a key metabolite that occurs in drinking water and thus will be included in the aggregate exposure assessment. Given that it causes similar effects as atrazine, it is important to determine its potential susceptibility in the young. Some toxicological data are also available on hydroxyatrazine, and it is also addressed below.

- In a prenatal developmental toxicity study with DACT in rats developmental effects were seen in the absence of maternal toxicity. The maternal NOAEL was 25 mg/kg/day based on statistically significant decrease in body weight gain at 75 mg/kg/day (LOAEL). The developmental NOAEL was 2.5 mg/kg/day based on increase incidence of incompletely ossified parietals, interparietals and unossified hyoids at 25 mg/kg/day (LOAEL). (See HIARC Report dated 8/28/0).

It should be noted that very recently, NHEERL have generated data on the chloro metabolites of atrazine that demonstrates effects on male puberty similar to atrazine (Unpublished SOT Abstract - *The Effects of Atrazine Metabolites on Puberty in the Male Wistar Rat*. D L Guidici, R L Cooper and T E Stoker. Endocrinology Branch, NHEERL, U.S. Environmental Protection Agency, RTP, NC.). In the male pubertal assay, significant delay was found at the isomolar equivalents of 25 mg of atrazine/kg/day for all three metabolites, that is deisopropyl chlorotriazine, deethyl chlorotriazine and diaminochlorotriazine.

- There was no evidence of increased susceptibility in the prenatal developmental toxicity study in rats, however there is more limited information to judge susceptibility compared to atrazine and the chloro metabolites. The data with OH-hydroxy in the pubertal assays by NHEERL is incomplete at this time.

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Placental Transfer and Lactational Exposure

Both atrazine and its metabolites (DACT) are found in the milk. However, the % of administered dose is very small.

- In goats, 2% of the atrazine dose is transferred to milk. Qualitatively, the major metabolite in goats milk is DACT and it accounts for 45% of the residue found.
 - In cows, 69% of the residue found in milk is DACT. Quantitatively, 2.7% of the administered dose comes out in the milk.
 - R. Cooper's laboratory has completed a study (unpublished) with tritiated atrazine using 2 and 4 mg/kg doses to the dam and allowed the dam to nurse her pups for 30 minutes. One percent of those two doses were found in the stomach of the pups. Negligible concentrations were found in the pup brain
- No studies have been identified that directly examine transplacental transfer of atrazine or its metabolites. It is reasonable, however, to assume that atrazine and its metabolites would cross the placenta barrier.

June 27-29 SAP Comments (the below summary is based on notes taken at the June meeting and not on the final written report):

In general, the SAP agreed that the rat findings on atrazine and its CNS mode of action raised a concern for children:

- delayed puberty and prostatitis effects are consistent with perturbation of hypothalamic-pituitary-gonadal axis.
- neurotransmitter effects (i.e., changes in NE and DA) were considered to be a concern, and could potentially translate into effects in children that may not be manifested until later in life.
- there are holes in the available data: there is a lack of information on peri-natal period of treatment and on the potential of atrazine to result in neurodevelopmental effects.

Selection of Endpoints for RfD Derivation:

The acute RfD of 0.1 mg/kg is based on delayed or absence of ossification found in a rat developmental study (NOAEL 10 mg/kg; LOAEL 70 mg/kg) and an uncertainty factor of 100. It should be noted that this developmental effect is not likely to be associated with atrazine's CNS mode of action. Three other studies were considered to support this RfD: another rat-developmental study (delayed ossification -NOAEL 25 mg/kg/day, LOAEL 100 mg/kg/day), a rabbit developmental study (delayed ossification -NOAEL 5 mg/kg/day, LOAEL 75 mg/kg/day), and a study by R. Cooper on hyperprolactinemia prior to puberty leading to lateral prostate inflammation in young adult male rats (NOAEL 12.5 mg/kg/day, LOAEL 25 mg/kg/day).

NOTE: Since the May 4, 2000 HIARC meeting, a recent published study by R. Cooper showed delayed pubertal effects in female rat pups (NOAEL 25 mg/kg/day; LOAEL 50 mg/kg/day) and identified a NOAEL of 6.25 mg/kg/day (LOAEL 12.5 mg/kg/day) for delayed puberty in male rats. These recent results are consistent with the HIARC report.

The chronic RfD of 0.02 mg/kg is based on a 6 month study for suppression of the preovulatory LH surge in adult female rats (NOAEL 1.8 mg/kg; LOAEL ~4 mg/kg) and an uncertainty factor of 100.

Therefore, the acute RfD is based on an endpoint in the young animal, while the chronic RfD is based on adult effects.

Key References Relevant to Atrazine's CNS Mode of Action and Effects on Reproductive Development

Cooper, R.L., Robinette CL, and Stoker T.E., 1999. Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. *Tox. Sci.* 1999 Nov; 52(1):68-79.

Cooper, R.L., Stoker, T.E., Tyrey, L., Goldman, J.M., and McElroy, W.K. 2000. Atrazine disrupts the hypothalamic control of pituitary-ovarian function. *Tox. Sci.* 53: 297-307

Laws, S.C., Ferrell, J.M., Stoker, T.E., Schmid, J. and Cooper, R.L. 2000. The Effects of Atrazine on Female Wistar Rats: An Evaluation of the Protocol for Assessing Pubertal Development and Thyroid Function. *Toxicological Sciences*, in press.

Stoker, T.E., Robbinette, C.L., Cooper, R.L. 2000b. Perinatal exposure to estrogenic compounds and the subsequent effects on the prostate of the adult rat: Evaluation of inflammation in the ventral and lateral lobes. *In press. Repro. Tox.*

Stoker, T.E., Laws, S.C., Guidici, D, and Cooper, R.L. (2000) The Effects of Atrazine on Puberty and Thyroid Function in the Male Wistar Rat: An Evaluation in the Male Pubertal Protocol. *Toxicological Sciences*, in press

Example References Pertinent in Evaluating Issue of Human Relevance of Atrazine's CNS Mode of Action in Children:

Pubertal studies

Wu, F.C., Butler, GE., Kelnar, C.J., Huhtaniemi, I., and Veldhuis, JD. (1996). Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through

puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J. Clin Endocrinol. Metab.* 81:798-805

"We conclude that the onset of puberty in man is heralded by the reawakening of a partially quiescent GnRH pulse generator. This predominantly involves an amplification of a pre-existing pattern of hypothalamic GnRH secretion leading to major augmentation of total quantity of LH molecules released per burst. (Midchildhood, 2 yrs prior to puberty).

Whether the endogenous excitatory amino acids such as glutamate and aspartate which stimulate GnRH pulse amplitude in particular, the GABA system or neuropeptide Y system are involved in the modulation of GnRH release are possibilities that must be examined experimentally. Nevertheless, any constraining mechanisms must act via inhibition of GnRH release rather than synthesis because NMDA can acutely release GnRH from the prepubertal hypothalamus (Gay and Plant, 1987) and GnRH antagonist abolish pulsatile LH secretion.

Gay, VL, and Plant, TM (1987) N-methyl-D, L-aspartate (NMDA) Elicits hypothalamic gonadotropin releasing hormone release in prepubertal male rhesus monkeys (*Macaca mulatta*). *Endocrinology* 120:2289-2296.

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