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TOXIC SUBSTANCES

TXR NO. 0050592

DATE: April 5, 2002

MEMORANDUM

SUBJECT: ATRAZINE/DACT - Fourth Report of the Hazard Identification Assessment Review Committee.

FROM: Linda L. Taylor 
Reregistration Branch
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair 
and
Elizabeth Doyle, Co-Chair 
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Catherine Eiden, Risk Assessor
Reregistration Branch III
Health Effects Division (7509C)

PC Code: 080803

On March 19, 2002, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) re-evaluated atrazine and diaminochlorotriazine [DACT], a chloro-metabolite of atrazine, with regard to the potential for increased susceptibility of infants and children from exposure to atrazine/DACT as required by the Food Quality Protection Act (FQPA) of 1996. The toxicological endpoints used for the acute and chronic Reference Doses (RfDs) and occupational/residential exposure risk assessments were re-evaluated. The re-evaluation was due to a change in the assessment of the rat developmental toxicity study on DACT, upon which was based the determination of increased quantitative susceptibility in the previous atrazine [TXR # 014421] and DACT [TXR # 014311] assessments. The HIARC was requested to (1) determine whether

maternal toxicity was demonstrated at the 25 mg/kg/day dose level [previously considered the NOAEL]; (2) determine whether a 2-generation reproduction study for DACT was required; (3) reevaluate the toxicological dose/endpoint selection for DACT, in light of the fact that the current acute RfD and the short-term incidental oral exposure scenarios are based on the 25 mg/kg/day NOAEL; and (4) reevaluate the FQPA assessment of DACT. The conclusions drawn at the March 19, 2002 meeting are presented in this report. This report supersedes all previous HIARC reports on atrazine, as well as the previous HIARC report on DACT.

Committee Members in Attendance

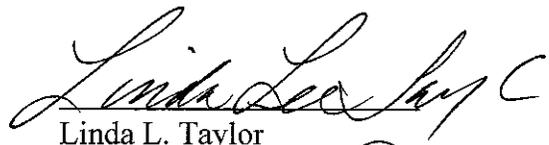
Members present were: Ayaad Assaad, William Burnam, Jonathan Chen, Elizabeth Doyle, Virginia Fornillo, Pamela Hurley, Elizabeth Mendez, David Nixon, Jess Rowland, and Brenda Tarplee.

Member(s) in absentia:

Data evaluation prepared by: Linda L. Taylor

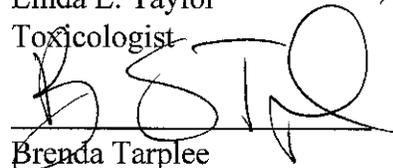
Also in attendance were: Artensie Flowers, Susan Makris, Catherine Eiden, Steve Knizner, Karl Baetcke, Michael Metzger, Whang, Phang

Data Evaluation / Report Presentation



Linda L. Taylor
Toxicologist

Report Concurrence



Brenda Tarplee
Branch Senior Scientist
Science Info. Management Branch

INTRODUCTION

On March 19, 2002, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) re-evaluated both atrazine and DACT with regard to the potential for increased susceptibility of infants and children from exposure to atrazine/DACT as required by the Food Quality Protection Act (FQPA) of 1996. The toxicological endpoints used for the acute and chronic Reference Doses (RfDs) and occupational/residential exposure risk assessments were re-evaluated. The re-evaluation was due to a change in the assessment of the rat developmental toxicity study on the chloro-metabolite DACT, upon which was based the determination of increased quantitative susceptibility in the previous assessment [TXR # 014311]. At the March 19, 2002 meeting, the HIARC concluded that the same studies and endpoints/dose levels selected for atrazine were applicable to DACT also. Therefore, a separate assessment for DACT was not performed. The conclusions drawn at the 3-19-02 meeting are presented in this report.

I. FQPA HAZARD CONSIDERATIONS

1. Adequacy of the Toxicity Data Base

The HIARC concluded that the toxicology database for atrazine is complete, and it is considered adequate for evaluation of potential adverse health consequences to infants and children under FQPA. One rabbit and two rat developmental toxicity studies and a 2-generation reproduction study are available on atrazine, and all are considered acceptable. There are several non-guideline studies on atrazine that assess endocrine-related toxicity of atrazine [see below under Additional Information from Literature Sources]. The HIARC also noted that there is no information on atrazine concerning exposure throughout all critical developmental periods (i.e., gestation through puberty in both sexes), in particular, early in development and, therefore, atrazine's CNS mode of action (effects on neurotransmitters/peptides) has not been fully characterized in the young.

HIARC determined that because of the structural similarities between atrazine and DACT, information from the atrazine toxicology database may be used to gain a better understanding of potential DACT toxicities. At the March, 2002 meeting, the HIARC concluded that the same studies and endpoints/dose levels selected for atrazine were applicable to DACT also.

2. Evidence of Neurotoxicity

The HIARC concluded that there is a concern for neurotoxicity resulting from exposure to atrazine/DACT. Acute and subchronic neurotoxicity studies are not available for either compound. Special studies submitted by the registrant provide evidence of atrazine-associated neurotoxicity. The neurotoxicity seen in these studies is a central nervous system (CNS) toxicity (specifically, neurotransmitter and neuropeptide alterations at the level of the hypothalamus).

3. Developmental Toxicity Study Conclusions

Executive Summary: Developmental toxicity study in Charles River CD rats (MRID 40566302). Atrazine (96.7%) was administered to 27 rats/dose by gastric intubation at 0, 10, 70, or 700 mg/kg/day from days 6 through 15 of gestation.

Mortality was very high for the 700 mg/kg/day animals in this study. All but 6 of the 27 females in this group died during the gestation period. Other statistically significant findings in this group included: salivation; oral and nasal discharge; ptosis; swollen abdomens; blood on the vulva; enlarged stomachs and adrenal; and discolored lungs. Body weight gains and food consumption were statistically significantly reduced throughout most of the gestation period. Pregnancy rates (85.2% for controls vs 96.3% high dose) and the numbers of live fetuses at c-section (mean of 12.7 per litter for controls vs 13.4 for the high dose) for the high dose group were comparable to controls. There were few findings in either the low or mid dose animals.

The maternal LOAEL is 70 mg/kg/day, based on reduced body weight gain. The maternal NOAEL is 10 mg/kg/day.

Fetal weights were statistically significantly reduced in the high dose group. Skeletal examinations were not conducted in the high dose group due to the extremely low fetal weights. Visceral and external examinations were conducted. No group, including the high dose group, displayed any findings significantly different from control values. Skeletal anomalies were observed in the mid dose group.

The developmental LOAEL was found to be 70 mg/kg/day, based on delayed or no ossification at several sites. The developmental NOAEL is 10 mg/kg/day.

Executive Summary: In a developmental toxicity study (MRID 41065201) atrazine (97.6%) was administered by gavage to 104 mated female Sprague-Dawley rats, 26/dose, at dose levels of 0, 5, 25, 100 mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity findings were almost exclusively confined to the high dose group. Compared to controls high dose dams displayed: reduced food consumption (decreased 13%, $p \leq 0.5$); reduced total body weight gain (reduced 18% during dosing period, $p \leq 0.5$); reduced corrected (minus uterine weight) weight gain (reduced 20% for entire gestation, $p \leq 0.5$); and increased alopecia 1/26 controls vs 5/26 high dose). One high dose animal died on gestation day 20 and salivation was noted as an observation in 18/26 high dose animals. The only observations seen outside the high dose group were: an abortion from one of the mid-dose animals on gestation day 19; a fluid-filled hollow right kidney in a mid-dose animal; and hollow discolored kidneys in a low dose animal.

The maternal LOAEL is 100 mg/kg/day based on reduced body weight gain and food consumption. The maternal NOAEL is 25 mg/kg/day.

The few malformations seen upon external examination of the fetuses were seen only in the control groups and clearly could not be compound related. Likewise, there was no increased incidence of visceral malformation in dosed groups vs control groups. There were no skeletal malformations observed but there was an increased incidence of incomplete ossification of various bones in the high dose. Hyoids (control fetal incidence of 11% vs 21.7% high dose), occipitals (7.7% vs 21.1%) and parietals (2.2% vs 8.4%) showed incomplete ossification. There was also an increased incidence ($p \leq 0.05$) of incomplete ossification of the interparietals in all dose groups compared to controls.

Fetal body weight, number of resorptions and implantations, and live fetuses/litter were not significantly affected by atrazine treatment. Exposure of gravid Sprague Dawley rats to atrazine under the conditions described in this study seemed to have few embryo/fetotoxic effects.

The developmental LOAEL is 100 mg/kg/day, based on increased incidence of delayed ossification of skull bones. The developmental NOAEL is 25 mg/kg/day.

Executive Summary: In a developmental toxicity study (Acc. No. 254979; MRIDs 00143006, 40566301) atrazine (96.3%) was administered by gavage to 76 mated female New Zealand White rabbits, 19/dose, at dose levels of 0, 1, 5, or 75 mg/kg/day, from days 7 through 19 of gestation.

Clinical signs seen in 75 mg/kg/day animals that were considered to be related to compound treatment were stool changes (none, little or soft stool; 9/19 controls vs 19/19 high dose), and the appearance of blood in the cage or on the vulva (0/19 controls vs 4/19 high dose). Body weight gain was reduced in high dose dams and, at many time points, body weight was below day zero values. At gestation days 14, 19, 21 and 25, mean maternal body weights were 12%, 19%, 18%, and 10% below control values ($p \leq 0.01$ for all four of these time points).

High dose animals displayed significantly reduced food consumption during treatment. During gestation days 12 to 17 the high dose average feed consumption was only 1-6 g of feed per animal per day compared to 175-182 g for the controls. The mid and low dose groups had no alterations that could be attributed to atrazine exposure.

The maternal toxicity LOAEL is 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs. The maternal toxicity NOAEL is 5 mg/kg/day.

Increased resorptions - mean of 1.3/dam in controls vs 4.8/dam in high dose - ($p \leq 0.01$), reduced live fetuses per litter - mean of 8.8/dam in controls vs 5.9/dam in high dose -

($p \leq 0.05$), and increased delayed ossification of appendicular elements were observed in the high dose group. The low and intermediate groups had no fetal findings that could be attributed to compound exposure. The findings in the high dose group were determined to be secondary to maternal toxicity and thus the LOAEL and NOAEL for embryo/fetotoxicity match the maternal LOAEL and NOAEL.

The developmental toxicity LOAEL is 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification. The developmental toxicity NOAEL is 5 mg/kg/day.

Executive Summary: In a developmental toxicity study (MRID 41392402), diaminochlorotriazine [DACT] (98.2% a.i., batch # FL 871423) was administered to 26 female Sprague-Dawley rats/dose *via* gavage at dose levels of 0, 2.5, 25, 75, and 150 mg/kg bw/day from days 6 through 16 of gestation.

There were no treatment-related effects on mortality or clinical signs. Maternal body weight-gain was reduced significantly [33.2%] during the 20-day gestation interval at the high-dose level, and there was a decrease in body-weight gain during the dosing interval [days 6-16] at the two-highest dose levels. During the initial dosing interval [days 6-8], dams at the 25 mg/kg/day dose level displayed a 32% decrease in body-weight gain, and the dams at the 75 and 150 mg/kg/day dose levels displayed negative body-weight gains. Decreased food consumption was observed at the highest dose level [days 6-16; 66% of control value] during the dosing period. Only a slight decrease in food consumption was observed at the 25 and 75 mg/kg/day dose levels during the dosing period.

The maternal LOAEL is 25 mg/kg bw/day, based on decreased body-weight gain during the initial three days of the dosing period.. The maternal NOAEL is 2.5mg/kg bw/day.

Pregnancy rate was not adversely affected by treatment, and there were no abortions. Comparable numbers of corpora lutea/dam and implantations/dam were observed among the groups, and there was no dose-related effect on pre-implantation loss. At the high-dose level [150 mg/kg/day], there was an increase in resorptions [both early and late], and post-implantation loss was significantly increased [18.9% vs 5.6% in the control] at this dose level. There were fewer live fetuses at 150 mg/kg/day [86% of control]. There were no dead fetuses, and the sex ratio was comparable among the groups. Gravid uterine weight was decreased significantly compared to the control [77% of control] at the 150 mg/kg/day dose level, and fetal body weights were lower at 75 mg/kg/day [91%-92% of control] and 150 mg/kg/day [81% of controls] in both sexes. There was an increased incidence of absent renal papilla [22% vs 3.3%] and pitted kidneys [4.8% vs 0%] at the high-dose level compared to the control. There was a dose-related increase in incomplete ossification of several bones at the 75 and 150 mg/kg/day dose levels and in three bones at 25 mg/kg/day [interparietal, parietals, and hyoids (unossified)].

The developmental toxicity LOAEL is 25 mg/kg bw/day, based on increases in the incidences of incompletely ossified parietals, interparietals, and unossified hyoids. The developmental toxicity NOAEL is 2.5 mg/kg bw/day.

4. Reproductive Toxicity Study Conclusions

Executive Summary: In a 2-generation reproduction study (MRID 40431303) atrazine, (purity not specified but said to be technical grade) was administered to 240 Charles River (CRCD, VAF/PLUS) rats 30/sex/dose in the diet at dose levels of 0, 10, 50, and 500 ppm.

There was very little variation in test article consumption between generations; the F₀ and F₁ males had similar test article consumption during the 70-day pre-mating period as did the F₀ and F₁ females. The average values for the two generations are 0, 0.75, 3.78, 39.0 mg/kg/day for males and 0, 0.86, 3.70, 42.8 mg/kg/day for females. Test article consumption for the F₀ and F₁ generation females during their gestation period did not vary greatly between generation. Mean compound consumption for both generations were 0, 0.66, 3.33 and 35.43 mg/kg/day. Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 ppm dose in both sexes and both generations throughout the study. Compared to controls, body weights for F₀ high dose males and females at 70 days into the study were decreased by 12% and 15%, respectively while F₁ body weight for the same time period was decreased by 15% and 13% for males and females, respectively. The only other parental effect which may have been treatment related was a slight, but statistically significant, increase in relative testes weight which occurred in both generations of the high dose.

The LOAEL is 500 ppm (39 mg/kg/day in males, 42.8 mg/kg/day in females) based on decreased body weights, body weight gains and food consumption. The NOAEL is 50 ppm (3.78 mg/kg/day in males, 3.7 mg/kg/day in females).

There did not appear to be any reproductive effects from compound exposure. Measured reproductive parameters from both generations did not appear to be altered in a dose-related manner.

The developmental toxicity LOAEL is 39 mg/kg/day in males, 42.8 mg/kg/day in females. The developmental toxicity NOAEL is 3.78 mg/kg/day in males and 3.7 mg/kg/day in females.

5. Additional Information from Literature Sources

In a study protocol designed for endocrine disruptors [pubertal assays in rats], atrazine was positive in both sexes [delayed preputial separation and delayed vaginal opening]. Following atrazine exposure to lactating female rats from postnatal day [PND] 1 to 4, suckling-induced

prolactin release was inhibited in the dams, and there was an increase in the incidence and severity of inflammation of the prostate in the male offspring. There is no information on atrazine concerning dosing throughout all critical developmental periods [gestation through puberty in both sexes of rats], in particular, dosing early in development.

Executive Summary: In a study to evaluate the effect of atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 43934406), atrazine, 97.1% a.i., was administered to 450 female Sprague Dawley rats in the diet. Dose levels were 0 (vehicle control), 2.5, 5, 40 and 200 mg/kg/day for 28 days.

Mortality, clinical signs, gross pathology and pituitary weights were not affected in this study. Food consumption and body weights are decreased at the 40 and 200 mg/kg/day doses (body weight decreased 13% and 47% at 40 and 200 mg/kg/day respectively). The number of animals with diestrus blocks were increased at 40 and 200 mg/kg/day. The number of animals with estrus blocks were increased at 200 mg/kg/day. The prolactin surge was attenuated at 200 mg/kg/day. The LH surge was attenuated at 40 and 200 mg/kg/day.

The LOAEL is 40 mg/kg/day, based on decreases in food consumption, body weight, body weight gain, estrous cycle alterations and LH surge attenuation. The NOAEL is 5 mg/kg/day.

NHEERL Publications

Substantial research has been conducted on the toxicologic effects of atrazine exposure at the Reproductive Toxicology Division of EPA's National Health and Environmental Effects Research Laboratories (NHEERL) at Research Triangle Park, N.C. . This research includes studies investigating the neuroendocrine basis of the mode of action for atrazine-associated carcinogenesis as well as studies investigating developmental and reproductive effects associated with atrazine exposure. Much of these data have been published in the open literature. The abstracts from these open literature publications are shown below.

● Cooper, R.L., Stoker, T.E., Goldman, J.M., Parrish, M.B., Tyrey, L. (1996). Effect of Atrazine on Ovarian Function in the Rat. *Reprod. Toxicol.* 1996 Jul-Aug;10(4):257-64.

The effect of the chlorotriazine herbicide, atrazine, on ovarian function was studied in Long-Evans hooded (LE-hooded) and Sprague-Dawley (SD) rats. Atrazine was administered by gavage for 21 d to females displaying regular 4-d estrous cycles. In both strains, 75 mg/kg/day disrupted the 4-d ovarian cycle; however, no distinct alteration (*i.e.*, irregular cycles but not persistent estrus or diestrus) was apparent at this dose. At 150 mg/kg/day, atrazine induced repetitive pseudopregnancies in females of both strains. The highest dose tested (300 mg/kg/day) also induced repetitive pseudopregnancies in the SD females, while the ovaries of the LE-hooded female appeared regressed and the smear cytology was

indicative of the anestrus condition. Although a NOAEL was not established, the doses employed in this experiment were in excess of those used in chronic feeding studies in which an early onset of mammary gland tumors was noted. These data demonstrate that atrazine can disrupt ovarian function and bring about major changes in the endocrine profile of the female.

●Stoker, T.E., Robinette, C.L., Cooper, R.L. (1999) Maternal Exposure to Atrazine During Lactation Suppresses Suckling-Induced Prolactin Release and Results in Prostatitis in the Adult Offspring. *Toxicol Sci* 1999 Nov;52(1):68-79 MRID 45166902

The availability of prolactin to the neonatal brain is known to affect the development of the tuberoinfundibular (TIDA) neurons and, as a consequence, lead to alterations in subsequent prolactin regulation. Without early lactational exposure to prolactin (derived from the dam's milk), TIDA neuronal growth is impaired and elevated prolactin levels are present in the prepubertal male. These observations, combined with the finding that alterations in prolactin secretion (*i.e.*, hyperprolactinemia) in the adult male rat have been implicated in the development of prostatitis, led us to hypothesize that early lactational exposure to agents that suppress suckling-induced prolactin release would lead to a disruption in TIDA development, altered prolactin regulation, and subsequent prostatitis in the male offspring. To test this hypothesis, suckling-induced prolactin release was measured in Wistar dams treated twice daily with the herbicide atrazine (by gavage, on PND 1-4 at 0, 6.25, 12.5, 25, and 50 mg/kg body weight), or twice daily with the dopamine receptor agonist bromocriptine (BROM, sc, at 0.052, 0.104, 0.208, and 0.417 mg/kg); BROM is known to suppress prolactin release. Similarly, atrazine has also been reported to suppress prolactin in adult females. Serum prolactin was measured on postnatal day [PND] 3 using a serial sampling technique and indwelling cardiac catheters. A significant rise in serum prolactin release was noted in all control females within 10 min of the initiation of suckling. Fifty-mg/kg atrazine inhibited suckling-induced prolactin release in all females, whereas 25 and 12.5 mg/kg atrazine inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of atrazine was without effect. BROM, used here as a positive control, also inhibited suckling-induced prolactin release at doses of 0.104 to 0.417 mg/kg, with no effect at 0.052 mg/kg. To examine the effect of postnatal atrazine and BROM on the incidence and severity of inflammation of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of atrazine-treated dams (25 and 50 mg/kg). The 12.5 mg/kg atrazine and the two highest doses of BROM increased the incidence, but not the severity, of prostatitis. Combined treatment of ovine prolactin and 25 or 50 mg/kg atrazine on PND 1-4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in inflammation, seen after atrazine alone, resulted from the suppression of in the dam.

To determine whether or not there is a critical period for these effects, dams were dosed with 25 and 50 mg/kg on PND 6-9 and PND 11-14. Inflammation was increased in those offspring

from dams treated on PND 6-9, but this increase was not significant. Dosing on PND 11-14 was without effect. These data demonstrate that atrazine suppresses suckling-induced prolactin release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND 1-9.

●Cooper, R.L., Stoker, T.E., Tyrey, L., Goldman, J.M., McElroy, W.K. (2000) Atrazine Disrupts the Hypothalamic Control of Pituitary-Ovarian Function. *Toxicol Sci* 2000 Feb;53(2):297-307. MRID 45166901

The chloro-S-triazine herbicides (*i.e.*, atrazine, simazine, cyanazine) constitute the largest group of herbicides sold in the United States. Despite their extensive usage, relatively little is known about the possible human-health effects and mechanism(s) of action of these compounds. Previous studies in our laboratory have shown that the chlorotriazines disrupt the hormonal control of ovarian cycles. Results from these studies led us to hypothesize that these herbicides disrupt endocrine function primarily through their action on the central nervous system. To evaluate this hypothesis, we examined the estrogen-induced surges of luteinizing hormone (LH) and prolactin in ovariectomized Sprague-Dawley (SD) and Long-Evans hooded (LE) rats treated with atrazine (50-300 mg/kg/day, by gavage) for 1, 3, or 21 days. One dose of atrazine (300 mg/kg) suppressed the LH and prolactin surge in ovariectomized LE, but not SD female rats. Atrazine (300 mg/kg) administered to intact LE females on the day of vaginal proestrus was without effect on ovulation but did induce a pseudopregnancy in 7 of 9 females. Three daily doses of atrazine suppressed the estrogen-induced LH and prolactin surges in ovariectomized LE females in a dose-dependent manner, but this same treatment was without effect on serum LH and prolactin in SD females. The estrogen-induced surges of both pituitary hormones were suppressed by atrazine (75-300 mg/kg/day) in a dose-dependent manner in females of both strains evaluated after 21 days of treatment. Three experiments were then performed to determine whether the brain, pituitary, or both organs were the target sites for the chlorotriazines. These included examination of the ability of (1) the pituitary lactotrophs to secrete prolactin, using hypophysectomized females bearing pituitary autotransplants (ectopic pituitaries); (2) the synthetic gonadotropin-releasing hormone (GnRH) to induce LH secretion in females treated with high concentrations of atrazine for 3 days; and (3) atrazine (administered *in vivo* or *in vitro*) to suppress LH and prolactin secretion from pituitaries, using a flow-through perfusion procedure. In conclusion, the results of these studies demonstrate that atrazine alters LH and prolactin serum levels in the LE and SD female rats by altering the hypothalamic control of these hormones. In this regard, the LE female appeared to be more sensitive to the hormone suppressive effects of atrazine, as indicated by the decreases observed on treatment-day 3. These experiments support the hypothesis that the effect of atrazine on LH and prolactin secretion is mediated via a hypothalamic site of action.

●Cummings, A. M., Rhodes, B.E., and Cooper, R.L. (2000). Effect of Atrazine on Implantation and Early Pregnancy in Four Strains of Rats. *Tox. Sci.* Nov. 58: 135-143.

Atrazine is an herbicide that has been shown to have adverse reproductive effects including alterations in levels of pituitary hormones such as prolactin and luteinizing hormone (LH) in female LE rats when administered at doses of 200 mg/kg/day for 1 and 3 days. Since prolactin's action to promote progesterone secretion is essential for the initiation of pregnancy in rats, this study was designed to examine the effect of exposure to atrazine during early pregnancy on implantation and short-term pregnancy maintenance. Rats were divided into two groups representing periods of dosing with atrazine prior to the diurnal or nocturnal surges of prolactin. Within each group, four groups consisting of four strains of rats (Holtzman, HLZ; Sprague Dawley, SD; Long Evans, LE; Fisher 344, F344) were each further subdivided into four atrazine dosages. Rats were dosed by gavage with 0, 50, 100, or 200 mg/kg/day atrazine on days 1-8 of pregnancy (day 0 = sperm +). All animals were necropsied on day 8 or 9 of pregnancy. The 200 mg/kg dose of atrazine reduced body weight gain in all but one group. Two groups of animals dosed at 100 and 200 mg/kg/day in the nocturnal dosing period showed an increase in percent preimplantation loss, and both of these were F344 rats. Holtzman rats were the only strain to show a significant level of postimplantation loss and a decrease in serum progesterone at 200 mg/kg/day both following diurnal and nocturnal dosing. Doses of 100 mg/kg/day also produced postimplantation loss following diurnal and nocturnal dosing, but progesterone levels were only decreased after nocturnal dosing. Alterations in serum LH were seen in several groups. Serum estradiol was significantly increased only in Sprague Dawley rats dosed at the diurnal interval with 200 mg/kg atrazine. We conclude that F344 rats are most susceptible to preimplantation effects of atrazine and that HLZ rats appear most sensitive to the postimplantation effects of the chemical. LE and SD rats were least sensitive to effects of atrazine during very early pregnancy.

●Narotsky M. G., Best, D.S., Guidici, D. L., and Cooper, R.L. (2000). Strain Comparisons of Atrazine-Induced Pregnancy Loss in the Rat. *Repro Toxicol.* 15, 61-69.

Atrazine was administered by gavage, in 1% methylcellulose, to F344 Sprague-Dawley (SD), and Long Evans (LE) rats at 0, 25, 50, 100, or 200 mg/kg/d on gestation days 6 through 10. The dams were allowed to deliver and litters were examined postnatally. The F344 strain was the most sensitive to atrazine's effects on pregnancy, showing full-litter resorption at ≥ 50 mg/kg. In surviving F344 litters, prenatal loss was increased at 200 mg/kg. In SD and LE rats, full-litter resorption occurred only at 200 mg/kg. Delayed parturition was seen at ≥ 100 mg/kg in F344 and SD rats. Regarding maternal toxicity, the SD dams were the most sensitive, with weight loss at ≥ 25 mg/kg. When 200 mg/kg was administered to F344 rats on days 11 through 15 (after the LH-dependent period of pregnancy), no full-litter resorption was seen. These findings suggest that atrazine-induced full-litter resorption is maternally mediated, and consistent with loss of LH support of the corpora lutea.

●Laws, S.C., Ferrell, J.M., Stoker, T.E., Schmid, J., and Cooper, R.L. (2000). The Effect of Atrazine on Puberty in Female Wistar Rats: An Evaluation in the Protocol for the Assessment of Pubertal Development and Thyroid Function. *Tox. Sci.* 58 366-376.

The effects of atrazine, a chlorotriazine herbicide, on the onset of puberty were evaluated in Wistar rats. Female rats were dosed by oral gavage from postnatal day (PND) 22 through PND 41 with 0, 12.5, 25, 50, 100 or 200 mg atrazine/kg. Vaginal opening was significantly delayed 3.4, 4.5 or greater than 6.8 days by 50, 100 and 200 mg/kg, respectively. Vaginal opening did not occur in 4 of 15 females in the 200 mg/kg group by the time of necropsy (PND 41). Body weight at necropsy was reduced in the 200 mg/kg group by 11.6%, but was not different from the control (0) in the 50 and 100 mg/kg groups. To examine the influence of reduced body weight on pubertal development, a group of pair-fed controls was included whose daily food intake was dependent upon the amount consumed by their counterpart in the 200 mg/kg group. Although necropsy body weight was reduced to the same extent as the atrazine females, vaginal opening in the pair-fed controls was not significantly delayed. Adrenal, kidney, pituitary, ovary and uterine weights were reduced by 200 mg/kg atrazine. Serum T3, T4 and TSH were unaltered by atrazine which were consistent with no histopathologic/morphologic changes in the thyroid. Estrous cyclicity was monitored in a second group of females from vaginal opening - PND 149. The number of females displaying regular 4 or 5-day estrous cycles during the first 15-day interval after vaginal opening, was lower in the 100 and 200 mg/kg atrazine and pair-fed controls. Irregular cycles were characterized by extended periods of diestrus. By the end of the second 15-day interval (PND 57-71), no effects on estrous cyclicity were observed. These data show that atrazine can delay the onset of puberty and alter estrous cyclicity in the female Wistar rat (NOAEL of 25 mg/kg). Reduced food consumption and body weight did not account for the delay in vaginal opening because this effect was not observed in the pair-fed controls. In addition, the effect on estrous cyclicity was observed in the 100 mg/kg atrazine group where no significant reduction in body weight was observed.

●Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The Effect of Atrazine on Puberty in Male Wistar Rats: An Evaluation in the Protocol for the Assessment of Pubertal Development and Thyroid Function. *Toxicol. Sci.* Nov. 58: 50-59.

Since atrazine, a chlorotriazine herbicide, has been shown previously to alter the secretion of luteinizing hormone (LH) and prolactin through a direct effect on the central nervous system (CNS), we hypothesized that exposure to atrazine in the EDSTAC male pubertal protocol (juvenile to peripubertal) would alter the development of the male rat reproductive system. We dosed intact male Wistar rats from postnatal day (PND) 23 to 53 and examined several reproductive endpoints. Atrazine (0, 12.5, 25, 50, 100, 150 or 200 mg/kg) was administered by gavage and an additional pair-fed group was added to compare the effects of any decreased food consumption in the high dose group. Preputial separation was significantly delayed in the 12.5, 50, 100, 150 and 200 mg/kg atrazine dose groups. Preputial separation was also delayed in the pair-fed group, although significantly less than in the high dose atrazine group. The males were killed on PND 53 or 54 and pituitary, thyroid, testes, epididymides, seminal vesicles, ventral and lateral prostates were removed. Atrazine (50 to 200 mg/kg) treatment resulted in a significant reduction in ventral prostate weights, as did the pair-fed group. Testes weights were unaffected by atrazine treatment. Seminal vesicle and

epididymal weights were decreased in the high dose atrazine group and the control pair-fed group. However, the difference in epididymal weights was no longer significantly different when body weight was entered as a covariable. Intratesticular testosterone was significantly decreased in the high dose atrazine group on PND 45, but apparent decreases in serum testosterone were not statistically significant on PND 53. There was a trend for a decrease in luteinizing hormone (LH) as the dose of atrazine increased, however, dose group mean LH were not different from controls. Due to the variability of serum prolactin concentrations on PND 53, no significant difference was identified. Although prolactin is involved in the maintenance of LH receptors prior to puberty, we observed no difference in LH receptor number at PND 45 or 53. Serum estrone and estradiol showed dose-related increases that were significant only in the 200 mg/kg atrazine group. No differences were observed in thyroid stimulating hormone (TSH) and thyroxine (T4) between the atrazine groups and the control, however tri-iodothyronine (T3) was elevated in the high dose atrazine group. No differences in hormone levels were observed in the pair-fed animals. These results indicate that atrazine delays puberty in the male rat and its mode of action appears to be altering the secretion of steroids and subsequent effects on the development of the reproductive tract, which appear to be due to atrazine's effects on the CNS. Thus, atrazine tested positive in the pubertal male screen that EDSTAC is considering as an optional screen for endocrine disrupters.

●Cooper, R.L., Stoker, T.E., McElroy, W.K., and Hein, J. F. (1998) Atrazine (ATR) Disrupts Hypothalamic Catecholamines and Pituitary Function. *Toxicologist* 42, 160.

Neurotransmitters and their regulation of pituitary hormone synthesis and secretion are critical for the onset of puberty and the maintenance of reproductive capability in the adult female. In rats, hypothalamic catecholamines, norepinephrine [NE] and dopamine [DA], modulate the secretion of LH and prolactin. NE is critical for the LH surge and pulsatile GnRH release. DA released into the portal blood inhibits prolactin secretion. In both sexes, atrazine has been shown to decrease NE and increase DA. Thus, atrazine-induced changes in DA and NE concentrations are consistent with a CNS site of action of atrazine's effect on pituitary hormone secretion and may provide an explanation for some of the adverse health effects noted in long-term studies.

6. Pre-and/or Postnatal Toxicity

The HIARC concluded that there is a concern for pre- and/or postnatal toxicity resulting from exposure to Atrazine.

A. Determination of Susceptibility

The HIARC concluded that there was no increased quantitative or qualitative susceptibility in any of the guideline studies on atrazine in the rat, and there was no increased quantitative susceptibility in the rabbit study. However, there was increased qualitative susceptibility in the rabbit study [increased resorptions (deaths) at a dose level that resulted in decreased body-weight gain and clinical signs in the maternal animal]. There are other non-guideline studies on atrazine that show evidence of endocrine disruption [prostatitis study, delayed

puberty study, and data on LH surge attenuation, and estrous cycle alterations]. The primary underlying events that lead to mammary and pituitary tumor formation following atrazine exposure of Sprague-Dawley female rats involve disruption of the hypothalamic-pituitary-ovarian axis. Since aspects related to this axis are involved in reproductive and developmental competency, there is a concern for adverse reproductive and developmental effects in maternal animals and their offspring. Several special studies have been performed that show that treatment of pregnant rats with atrazine can lead to reproductive and developmental effects that may be associated with endocrine alterations. Additionally, the neurotoxicity seen in the non-guideline studies with atrazine is a central nervous system (CNS) toxicity - specifically, neurotransmitter and neuropeptide alterations at the level of the hypothalamus.

Studies in the open literature indicate increased qualitative susceptibility. Dosing of dams immediately following parturition [postnatal days 1-4] resulted in prostatitis in male offspring, and dosing of the young following weaning resulted in delayed puberty in both sexes. The mode of action for these two effects (prostate inflammation and delayed puberty) is believed to be similar to the mode of action described for atrazine-associated cancer and involves the CNS neuroendocrine alterations, specifically, neuroendocrine alterations at the hypothalamus.

In the previous HIARC assessment of DACT, it was determined that increased quantitative susceptibility of the young was observed in the rat developmental toxicity study on DACT. A re-examination of the maternal body-weight gain data from that study was performed subsequently, and it was determined that decreased body-weight gain was evident during the initial dosing period [gestation days 6-8] at 25 mg/kg/day, and the magnitude of the decrease [32%] is considered to be evidence of maternal toxicity. Therefore, the NOAEL for maternal toxicity has been changed to 2.5 mg/kg/day, and the LOAEL for maternal toxicity is 25 mg/kg/day. 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). Therefore, developmental toxicity and maternal toxicity occurred at the same dose level [25 mg/kg/day], and there is no apparent increased quantitative susceptibility following DACT exposure in this study. Additionally, it was determined that a 2-generation reproduction study on DACT is not required.

B. Degree of Concern Analysis and Residual Uncertainties

Since there is evidence of increased susceptibility of the young following exposure to Atrazine in the rabbit developmental study and in several special studies conducted to evaluate endocrine effects, HIARC performed a Degree of Concern Analysis to: 1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and 2) identify any residual concerns after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of this chemical. If residual concerns are identified, HIARC examines whether these residual concerns can be addressed

by a special FQPA safety factor and, if so, the size of the factor needed. The results of the HIARC Degree of Concern analyses for Atrazine (and DACT) follow.

A. Prenatal Developmental Study with Atrazine in Rabbits

The HIARC concluded that there is low concern for the qualitative increased susceptibility (increased fetal resorptions at a dose level that resulted in decreased body-weight gain and clinical signs in the maternal animal) because: 1) the NOAELs in the study are well characterized; and 2.) the fetal effects seen occurred at a high dose level (75 mg/kg/day).

The HIARC also concluded that there are no residual concerns for these effects considering that the Acute RfD established for Atrazine/DACT is based on a NOAEL of 10 mg/kg which is protective of the fetal effects observed at 75 mg/kg/day in the developmental rabbit study.

B. Special Studies with Atrazine

A substantial amount of research has been conducted on the toxicologic effects of Atrazine exposure at the Reproductive Toxicology Division of EPA's National Health and Environmental Effects Research Laboratories (NHEERL) at Research Triangle Park, N.C. This research includes studies investigating the neuroendocrine basis of the mode of action for Atrazine-associated carcinogenesis as well as studies investigating developmental and reproductive effects associated with Atrazine exposure. Much of these data have been published in the open literature and the abstracts from these publications are shown below (Section I.C.). Several of these studies indicate increased susceptibility of the young following exposure to Atrazine:

- Stoker, T.E., Robinette, C.L., Cooper, R.L. (1999) demonstrated that Atrazine suppresses suckling-induced prolactin release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND 1-9;
- Laws, S.C., Ferrell, J.M., Stoker, T.E., Schmid, J., and Cooper, R.L. (2000) demonstrated that Atrazine can delay the onset of puberty and alter estrous cyclicity in the female Wistar rat (NOAEL of 25 mg/kg); and
- Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) concluded that Atrazine delays puberty in the male rat and its mode of action appears to be altering the secretion of steroids and subsequent effects on the development of the reproductive tract (apparently due to Atrazine's effects on the CNS).

The mode of action for these effects (prostate inflammation and delayed puberty) is believed to be similar to that described for Atrazine-associated cancer and involves the CNS neuroendocrine alterations at the hypothalamus (Refer to HED CARC document; TXR # 014431).

After considering the effects observed in these studies with Atrazine in the context of establishing toxicity endpoints for risk assessment, the HIARC identified the following residual concerns:

Since the focus of the testing with Atrazine in the young rat has been limited to short periods of dosing to specific developmental periods, uncertainties are raised for susceptibility during earlier developmental periods as well as for consequences of earlier developmental exposure with longer duration of dosing throughout development. The effects of neurotransmitters/peptides (known to be critical for normal development and which could potentially translate into severe effects in children that may not be manifested until later in life) have not been fully characterized. And as the FIFRA Scientific Advisory Panel noted, there are concerns for behavioral effects in the young resulting from Atrazine's CNS mode of action and the dose level at which these effects might occur compared to reproductive/developmental effects¹.

7. Recommendation for a Developmental Neurotoxicity Study

The HIARC concluded that there is a concern for developmental neurotoxicity resulting from exposure to atrazine.

A. Evidence that suggest requiring a Developmental Neurotoxicity study:

Acute and subchronic neurotoxicity studies are not available for atrazine. Special studies submitted by the registrant (MRIDs 44152102 and 43934406) and published in the open literature [MRID 45166902] provide evidence of atrazine-associated neurotoxicity. The neurotoxicity seen in these studies was a central nervous system (CNS) toxicity (specifically, neurotransmitter and neuropeptide alterations at the level of the hypothalamus).

B. Evidence that do not support a need for a Developmental Neurotoxicity study:

Evidence of neurotoxicity was seen following atrazine exposure. The neurotoxicity seen following atrazine exposure is a CNS mode of action supported through a series of registrant submitted studies and studies performed by EPA scientists at NHEERL.

¹SAP Report No. 2000-05; Atrazine: Hazard and Dose Response Assessment and Characterization. "Because of the rapid developmental brain changes...the influence of Atrazine on neurotransmitters in the hypothalamus and on GnRH may well have a differential, permanent effect on children. This phenomenon is the basis of the relatively new field of behavioral teratology. Atrazine could influence the migration of cells and the connectivity of the CNS. The influence of Atrazine on the hypothalamus and on GnRH may have a differential effect on children. This effect could be latent, and emerge later during the challenge of puberty, or during senescence. Behavioral alterations may be the most sensitive outcome. This possibility should be addressed..."

Based on the weight of evidence presented, the HIARC concluded that a developmental neurotoxicity study is not required for atrazine. A standard DNT is not recommended because atrazine's CNS mode of action primarily affects pituitary endocrine function, and the parameters measured in the DNT, i.e., the functional endpoints (motor activity tests, auditory startle tests, and learning and memory tests) may not be sensitive to detect behavioral consequences of this hypothalamic disruption. Certain measures performed in the DNT (such as determination of onset of developmental landmarks and neuropathology) would be useful in examining this CNS neuroendocrine toxicity. However, special studies designed specifically to examine these endpoints would be much more useful in this regard.

8. Hazard-based Special FQPA Safety Factor

Considering the existing data used for toxicity endpoint selection, the HIARC used the following rationale to conclude that an additional Special FQPA Safety Factor of 3X would be adequate to account for these hazard-based residual uncertainties:

The toxicology endpoints selected for risk assessment are all consistent with Atrazine's mode of toxicity using the most sensitive endpoint with the lowest NOAEL (1.8 mg/kg/day). When comparing the effects observed in adults to those observed in the young, the HIARC considered the results of the pubertal assay. It is noted that delayed puberty was observed in both male and female offspring exposed to Atrazine during the pubertal period (30 days for the males and 20 days for the females) and that clear NOAELs were established for this endpoint in both sexes (6.25 mg/kg/day in males; 12.5 mg/kg/day in females). If the lowest offspring NOAEL from this study is protected by a factor of 3X, the extrapolated NOAEL is 2 mg/kg/day. Comparing this value to the adult NOAEL of 1.8 mg/kg/day from the 6-month LH Surge study (used to establish the Chronic RfD and for the intermediate and chronic oral, dermal, and inhalation exposure scenarios) indicates that the young are not likely to be an order of magnitude more sensitive than the adult. Therefore, the HIARC concluded that a half-log reduction in the default Special FQPA Safety Factor is considered to be sufficiently protective of the concerns for this CNS mode of action in the young.

HIARC also recommended that the additional Special FQPA Safety Factor of 3X would **not** be required for Acute dietary exposures (aRfD) because the open literature data demonstrate that while the neuroendocrine effects caused by Atrazine's mode of action could result from a single dose, this would only occur at very high doses (200-300 mg/kg which is significantly higher than the 10 mg/kg level used to establish the Acute RfD).

II. HAZARD IDENTIFICATION

1. Acute Reference Dose (aRfD) - FEMALES 13-50

Study Selected: Prenatal developmental study - RAT]

§ OPPTS 870.3700

MRID No.: 40566302 [Accession No. 254979]

NOTE: A weight of the evidence consideration used evidence provided by four studies: two developmental toxicity studies in rats, a rabbit developmental toxicity study, and a study examining the effects of maternal atrazine exposure during lactation on prostate effects in male offspring. The actual NOAEL and endpoint from which the reference dose is calculated is derived from one of the above-mentioned developmental studies in the rat - MRID 40566302.

Executive Summary: Developmental toxicity study in Charles River CD rats (MRID 40566302). Atrazine (96.7%) was administered to 27 rats/dose by gastric intubation at 0, 10, 70, or 700 mg/kg/day from days 6 through 15 of gestation.

Mortality was very high for the 700 mg/kg/day animals in this study. All but 6 of the 27 females in this group died during the gestation period. Other statistically significant findings in this group included: salivation; oral and nasal discharge; ptosis; swollen abdomens; blood on the vulva; enlarged stomachs and adrenal; and discolored lungs. Body weight gains and food consumption were statistically significantly reduced throughout most of the gestation period. Pregnancy rates (85.2% for controls vs 96.3% high dose) and the numbers of live fetuses at c-section (mean of 12.7 per litter for controls vs 13.4 for the high dose) for the high dose group were comparable to controls. There were few findings in either the low or mid dose animals.

The maternal LOAEL is 70 mg/kg/day, based on reduced body weight gain. The maternal NOAEL is 10 mg/kg/day.

Fetal weights were statistically significantly reduced in the high dose group. Skeletal examinations were not conducted in the high dose group due to the extremely low fetal weights. Visceral and external examinations were conducted. No group, including the high dose group, displayed any findings significantly different from control values. Skeletal anomalies were observed in the mid dose group.

The developmental LOAEL was found to be 70 mg/kg/day, based on delayed or no ossification at several sites. The developmental NOAEL is 10 mg/kg/day.

Executive Summary: In a developmental toxicity study (MRID 41065201) atrazine (97.6%) was administered by gavage to 104 mated female Sprague-Dawley rats, 26/dose, at dose levels of 0, 5, 25, 100 mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity findings were almost exclusively confined to the high dose group. Compared to control high dose dams displayed: reduced food consumption (decreased 13%, $p \leq 0.5$); reduced total body weight gain (reduced 18% during dosing period, $p \leq 0.5$); reduced corrected (minus uterine weight) weight gain (reduced 20% for entire gestation, $p \leq 0.5$); and increased alopecia 1/26 controls vs 5/26 high dose). One high dose animal died on gestation day 20 and salivation was noted as an observation in 18/26 high dose animals. The only observations seen outside the high dose group were: an abortion from one of the mid-dose animals on gestation day 19; a fluid-filled hollow right kidney in a mid-dose animal; and hollow discolored kidneys in a low dose animal.

The maternal LOAEL is 100 mg/kg/day based on reduced body weight gain and food consumption. The maternal NOAEL is 25 mg/kg/day.

The few malformations seen upon external examination of the fetuses were seen only in the control groups and clearly could not be compound related. Likewise, there was no increased incidence of visceral malformation in dosed groups vs control groups. There were no skeletal malformations observed but there was an increased incidence of incomplete ossification of various bones at the high dose. Hyoids (control fetal incidence of 11% vs 21.7% high dose), occipitals (7.7% vs 21.1%) and parietals (2.2% vs 8.4%) showed incomplete ossification. There was also an increased incidence ($p \leq 0.05$) of incomplete ossification of the interparietals in all dose groups compared to controls.

Fetal body weight, number of resorptions and implantations, and live fetuses/litter were not significantly affected by atrazine treatment. Exposure of gravid Sprague Dawley rats to atrazine under the conditions described in this study seemed to have few embryo/fetotoxic effects.

The developmental LOAEL is 100 mg/kg/day, based on increased incidence of delayed ossification of skull bones. The developmental NOAEL is 25 mg/kg/day.

Executive Summary: In a developmental toxicity study (Acc. No. 254979; MRIDs 00143006, 40566301) atrazine (96.3%) was administered by gavage to 76 mated female New Zealand White rabbits, 19/dose, at dose levels of 0, 1, 5, or 75 mg/kg/day, from days 7 through 19 of gestation.

Clinical signs seen in 75 mg/kg/day (high dose) animals that were considered to be related to compound treatment were stool changes (none, little or soft stool; 9/19 controls vs 19/19 high dose), and the appearance of blood in the cage or on the vulva (0/19 controls vs 4/19 high dose). Body weight gain was reduced in high dose dams and, at many time points, body

weight was below day zero values. At gestation days 14, 19, 21 and 25, mean maternal body weights were 12%, 19%, 18%, and 10% below control values ($p \leq 0.01$ for all four of these time points).

High dose animals displayed significantly reduced food consumption during treatment. During gestation days 12 to 17, the high dose average feed consumption was only 1-6 g of feed per animal per day compared to 175-182 g for the controls. The mid and low dose groups had no alterations that could be attributed to atrazine exposure.

The maternal toxicity LOAEL is 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs. The maternal toxicity NOAEL is 5 mg/kg/day.

Increased resorptions - mean of 1.3/dam in controls vs 4.8/dam in high dose - ($p \leq 0.01$), reduced live fetuses per litter - mean of 8.8/dam in controls vs 5.9/dam in high dose - ($p \leq 0.05$), and increased delayed ossification of appendicular elements were observed in the high dose group. The low and intermediate groups had no fetal findings that could be attributed to compound exposure. The findings in the high dose group were determined to be secondary to maternal toxicity and thus the LOAEL and NOAEL for embryo/fetotoxicity match the maternal LOAEL and NOAEL.

The developmental toxicity LOAEL is 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification. The developmental toxicity NOAEL is 5 mg/kg/day

Executive Summary: The availability of prolactin to the neonatal brain is known to affect the development of the tuberoinfundibular (TIDA) neurons and, as a consequence, lead to alterations in subsequent prolactin regulation. Without early lactational exposure to prolactin (derived from the dam's milk), TIDA neuronal growth is impaired and elevated prolactin levels are present in the prepubertal male. These observations, combined with the finding that alterations in prolactin secretion (*i.e.*, hyperprolactinemia) in the adult male rat have been implicated in the development of prostatitis, led us to hypothesize that early lactational exposure to agents that suppress suckling-induced prolactin release would lead to a disruption in TIDA development, altered prolactin regulation, and subsequent prostatitis in the male offspring. To test this hypothesis [MRID 45166902], suckling-induced prolactin release was measured in Wistar dams treated twice daily with the herbicide atrazine (by gavage, on PND 1-4 at 0, 6.25, 12.5, 25, and 50 mg/kg body weight), or twice daily with the dopamine receptor agonist bromocriptine (BROM, sc, at 0.052, 0.104, 0.208, and 0.417 mg/kg); BROM is known to suppress prolactin release. Similarly, atrazine has also been reported to suppress prolactin in adult females. Serum prolactin was measured on postnatal day [PND] 3 using a serial sampling technique and indwelling cardiac catheters. A significant rise in serum prolactin release was noted in all control females within 10 min of the initiation of suckling. Fifty-mg/kg atrazine inhibited suckling-induced prolactin release

in all females, whereas 25 and 12.5 mg/kg atrazine inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of atrazine was without effect. BROM, used here as a positive control, also inhibited suckling-induced prolactin release at doses of 0.104 to 0.417 mg/kg, with no effect at 0.052 mg/kg. To examine the effect of postnatal atrazine and BROM on the incidence and severity of inflammation of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of atrazine-treated dams (25 and 50 mg/kg). The 12.5 mg/kg atrazine and the two highest doses of BROM increased the incidence, but not the severity, of prostatitis. Combined treatment of ovine prolactin and 25 or 50 mg/kg atrazine on PND 1-4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in inflammation, seen after atrazine alone, resulted from the suppression of in the dam.

To determine whether or not there is a critical period for these effects, dams were dosed with 25 and 50 mg/kg on PND 6-9 and PND 11-14. Inflammation was increased in those offspring from dams treated on PND 6-9, but this increase was not significant. Dosing on PND 11-14 was without effect. These data demonstrate that atrazine suppresses suckling-induced prolactin release and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is PND 1-9.

Dose and Endpoint for Establishing aRfD: Developmental NOAEL = 10 mg/kg/day based on delayed or lack of ossification of several sites at 70 mg/kg/day (LOAEL), and supported by the decreased suckling induced PRL release and increased incidence of prostatitis (NOAEL 12.5 mg/kg/day, LOAEL 25 mg/kg/day).

Uncertainty Factor (UF): 100 (10x for interspecies extrapolation and 10x for intraspecies variations).

$\text{Acute RfD (Females 13-50)} = \frac{10 \text{ mg/kg}}{100} = 0.1 \text{ mg/kg}$

Comments about Study/Endpoint/Uncertainty Factor: Reproductive and developmental effects in various strains of rats that are associated with atrazine treatment include **pre-implantation and post-implantation losses, prostatitis in adult male offspring of treated lactating females, delays in vaginal opening and preputial separation, and disruption of the estrous cycle in young females.** A reduction in prolactin release in nursing dams is strongly associated with the development of prostatitis in male adult offspring. Decreases in serum lutenizing hormone (LH) or prolactin were not observed to occur at dose-levels that led to delays in vaginal opening (50 mg/kg/day) and preputial separation (13 mg/kg/day) in the same study but it is presumed that the variability in levels of these hormones in juvenile animals preclude obtaining definitive data. On the other hand, in a separate study, a twice daily dose of 12.5 mg/kg/day was sufficient to depress serum levels of prolactin in the

lactating dam. To the extent that decreased prolactin levels can serve as a marker for effects on neuroendocrine control, there is a linkage between pubertal development and an effect on the hypothalamic-pituitary axis.

Any of the four studies described above may be appropriate for selection of an endpoint for acute risk assessment. The developmental effects seen in the two rat and one rabbit developmental study are assumed to have the potential to occur after a single dosing. The effects seen in the open literature prostatitis paper occurs after only four days of dosing.

The lowest NOAEL seen in the above studies was 5 mg/kg/day, which is the developmental NOAEL from the rabbit developmental toxicity study (MRID 41065201). Though the NOAEL from this study would be acceptable for use as an acute RfD, HIARC notes that there was a large dose spread in this study. The mid dose tested (and the NOAEL) in this study was 5 mg/kg/day while the next highest dose tested (the highest dose tested and the LOAEL) was 75 mg/kg/day. This dose is a full 15 times higher than the mid dose tested. The large spread between 5 and 75 mg/kg/day raises the possibility that had intermediate doses between 5 and 75 been used then the NOAEL would have been higher.

Examination of the rat developmental toxicity studies indicates that intermediate doses in the rabbit study between 5 and 75 may not have shown any adverse effects. The NOAEL in both the rat studies are greater than 5 mg/kg/day (10 mg/kg/day for MRID 40566302 and 25 mg/kg/day for MRID 41065201). The effects seen in the rabbit and two rat developmental toxicity studies are similar with all three studies demonstrating delayed or no ossification in certain cranial bones at their respective LOAELS of 75 (rabbit), 70 (MRID 40566302) and 100 mg/kg/day (MRID 41065201). Other effects on which the developmental NOAEL were based in the rabbit study - reduced litter size and increased resorptions - were not seen in either of the rat studies. In this respect it should be noted that maternal effects were more severe at the LOAEL in the rabbit study than at the LOAELs in either of the two rat studies. The maternal LOAELs in the two rat studies were based on decreased food consumption and body. The maternal LOAEL in the rabbit study was based on clinical signs (none, little or soft stool, blood on the vulva), in addition to decreased food consumption and body weight.

HIARC also notes that an acute RfD based on a NOAEL of 10 mg/kg/day is supported by the prostatitis effects that have a NOAEL of 12.5 mg/kg/day.

2. Acute Reference Dose (aRfD) - General Population

An appropriate endpoint for the general population attributable to a single exposure was not available from the oral toxicity studies including the developmental toxicity studies in rats and rabbits.

3. Chronic Reference Dose (cRfD)

Study Selected: Six-month LH surge study - RAT

§ none; special study

MRID No.: 44152102

Executive Summary: In a study to evaluate the effect of long-term atrazine exposure on the proestrus afternoon luteinizing hormone [LH] surge (MRID 44152102) atrazine, 97.1% a.i., was administered to 360 female Sprague Dawley rats in the diet. Dose levels were 0 (negative control), 25, 50, and 400 ppm (0, 1.80, 3.65, 29.44 mg/kg/day) for 26 weeks (approximately six months).

Body weight, body weight gain and food consumption were significantly ($p \leq 0.05$) decreased in the high-dose animals compared to controls (body weight decreased 8.5% at the end of the study and food consumption decreased 3.75% for the entire study). The percentage of days in estrus were significantly increased ($p \leq 0.01$) during the 21-22 and 25-26 week time periods at the high dose. Percent days in estrus were also increased during the 21-22 and 25-26 week time periods at the mid-dose level, but the increase was only significant ($p \leq 0.05$) for the 21-22 week time period. The proestrus afternoon LH surge was severely attenuated at the high dose (LH levels were actually decreased compared to baseline at most sampling time points) and less so at the mid dose (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the high dose (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the high dose of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the high dose that could be attributed to compound exposure and there were no compound-related gross pathology findings at the mid or low dose. Selected tissues were saved for histopathology but those results have yet to be reported.

There were no compound related effects in mortality or clinical signs. The proestrus afternoon prolactin surge was not affected by compound exposure at any dose. The low dose had no effects on the estrous cycle, LH or prolactin surges.

The LOAEL is 3.65 mg/kg/day, based on estrous cycle alterations and LH surge attenuation as biomarkers of atrazine's ability to alter hypothalamic-pituitary function. The NOAEL is 1.8 mg/kg/day.

Dose and Endpoint for Establishing cRfD: **NOAEL = 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.**

Uncertainty Factor(s): 100 (10x for interspecies extrapolation and 10x for intraspecies variations)

$$\text{Chronic RfD} = \frac{1.8 \text{ mg/kg/day}}{100} = 0.018 \text{ mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: The attenuation of the LH surge is considered to be an indicator of atrazine's neuroendocrine mode of action or its potential to alter hypothalamic-pituitary function. This six-month study is considered adequate for use in selecting a chronic endpoint without an additional safety factor being added to account for study duration of less than 12 months. This is based on the fact that examination of estrous cycle data from other studies indicates that beyond 6 months of exposure, the differences in estrous cycle deterioration between treated animals and controls no longer widens as the control animals begin the normal reproductive aging process.

These biomarkers of atrazine's neuroendocrine mode of action (i.e., LH surge attenuation and estrous cycle disruption) are considered to be applicable to the general population including infants and children given that they result from atrazine's CNS mode of action. HIARC notes that this dose is the lowest NOAEL available in the toxicology database and therefore would be protective of other adverse effects, including those occurring in males, infants, and children. Therefore, a separate endpoint is not needed for this population (i.e., males, infants, and children).

4. Incidental Oral Exposure: Short-Term (1-30 days)

Study Selected: pubertal [screening] study - male RAT

§ none

MRID No.: none. Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

Executive Summary: Since atrazine, a chlorotriazine herbicide, has been shown previously to alter the secretion of luteinizing hormone (LH) and prolactin through a direct effect on the CNS, we hypothesized that exposure to atrazine in the EDSTAC male pubertal protocol (juvenile to peripubertal) would alter the development of the male rat reproductive system. We dosed intact male Wistar rats from postnatal day (PND) 23 to 53 and examined several reproductive endpoints. Atrazine (0, 6.25, 12.5, 25, 50, 100, 150 or 200 mg/kg) was administered by gavage and an additional pair-fed group was added to compare the effects of any decreased food consumption in the high dose group. Preputial separation was significantly delayed in the 12.5, 50, 100, 150 and 200 mg/kg atrazine dose groups. Preputial separation was also delayed in the pair-fed group, although significantly less than in the high dose atrazine group. The males were killed on PND 53 or 54 and pituitary, thyroid, testes, epididymides, seminal vesicles, ventral and lateral prostates were removed. Atrazine (50 to 200 mg/kg) treatment resulted in a significant reduction in ventral prostate weights, as did the pair-fed group. Testes weights were unaffected by atrazine treatment. Seminal vesicle and epididymal weights were decreased in the high dose atrazine group and the control pair-fed

group. However, the difference in epididymal weights was no longer significantly different when body weight was entered as a covariable. Intratesticular testosterone was significantly decreased in the high dose atrazine group on PND 45, but apparent decreases in serum testosterone were not statistically significant on PND 53. There was a trend for a decrease in luteinizing hormone as the dose of atrazine increased, however, dose group mean LH were not different from controls. Due to the variability of serum prolactin concentrations on PND 53, no significant difference was identified. Although prolactin is involved in the maintenance of LH receptors prior to puberty, we observed no difference in LH receptor number at PND 45 or 53. Serum estrone and estradiol showed dose-related increases that were significant only in the 200 mg/kg atrazine group. No differences were observed in thyroid stimulating hormone (TSH) and thyroxine (T4) between the atrazine groups and the control, however tri-iodothyronine (T3) was elevated in the high dose atrazine group. No differences in hormone levels were observed in the pair-fed animals. These results indicate that atrazine delays puberty in the male rat and its mode of action appears to be altering the secretion of steroids and subsequent effects on the development of the reproductive tract, which appear to be due to atrazine's effects on the CNS. Thus, atrazine tested positive in the pubertal male screen that EDSTAC is considering as an optional screen for endocrine disruptors.

Dose and Endpoint for Risk Assessment: NOAEL = 6.25mg/kg/day), based on a delay in preputial separation at the LOAEL of 12.5 mg/kg/day.

Comments about Study/Endpoint: This study is appropriate for this scenario since it demonstrates an endpoint in the young animal that is consistent with atrazine's mode of toxicity. The endpoint, delayed puberty, is relevant to the population of concern (infants and children), and delayed puberty also was demonstrated to occur in the female. Following exposure during PND 22-41, delayed puberty was observed in the female at 50 mg/kg/day [NOAEL of 25 mg/kg/day]. A possible explanation for a higher NOAEL in the female may be that the exposure duration in females [20 days] was shorter than in the males [31 days].

5. Incidental Oral Exposure: Intermediate-Term (1 - 6 Months)

Study Selected: Six-Month LH Surge Study - RAT

§ none

MRID No.: 44152102

Executive Summary: see under Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.

Comments about Study/Endpoint: The endpoints, estrous cycle alterations and LH surge attenuation, are considered indicative of atrazine's ability to disrupt hypothalamic-pituitary

function, and its potential to lead to various health consequences including, but not limited to, reproductive disruption. Although the endpoint selected [estrous cycle alterations and LH surge attenuation] for intermediate-term exposure of infants, children, young adults, and adults is derived from a 6-month study in adult rats, the endpoint is a reasonable surrogate for atrazine CNS-hypothalamic disruption in children. These biomarkers of atrazine's neuroendocrine mode of action (i.e., LH surge attenuation and estrous cycle disruption) are considered to be applicable to the general population including infants and children given that they result from atrazine's CNS mode of action. It should be pointed out that the population of concern includes teenage children, and some functional portions of the CNS, such as the hypothalamic controls of reproductive cycling, are not mature until the second decade [Developmental Toxicology, 2nd ed., edited by c. A. Kimmel and J. Buelke-Sam, Raven Press, Ltd. NY (1994)]. Additionally, since this dose is the lowest NOAEL available in the toxicology database, it would be protective of other adverse effects, including those occurring in males, infants, and children. Therefore, given the uncertainty of atrazine's potential effect during development *via* the mode of toxicity of atrazine, the use of the NOAEL from the 6-month study is considered protective of the population of concern [infants and children].

Different endpoints can be affected at different dose levels and at different times following dosing, and the pre-ovulatory LH surge appears to be the most sensitive biomarker. While this parameter was monitored in the adult studies, it has not been monitored in the studies on the young. Due to the lack of LH data for the young animal, the findings in the 6-month study are appropriate, and other data [1-day, 3-day, 21-day and 1-month studies] provide evidence that an effect on the LH surge can occur following exposure of any duration. Although it is recognized that the effects in the shorter duration studies were observed at higher dose levels, there is concern of the potential neuroendocrine effects of repeated atrazine exposure throughout all critical developmental periods, which have not been adequately characterized in the young animal.

6. Dermal Absorption

Dermal Absorption Factor: The committee recommended a dermal absorption factor of 6% (rounded up from 5.6%). This factor is based on a human study (MRID 44152114) in which 10 human volunteers were exposed to a single topical dose of [triazine ring-U-¹⁴C] atrazine (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 µg/cm² (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [¹⁴C] atrazine for the low and high doses, respectively. After 24 hours, the atrazine was removed and determination of percent absorbed occurred was determined 168 hours (7 days) after the commencement of exposure. The maximum percent absorbed in this study was 5.6% of the dose in the lower dose group. Because the maximum percent absorbed is being used and because an ample amount of time (168 hours) was allowed for absorption to occur, 6% is deemed to be a protective estimate of dermal exposure.

A rat (MRID 43314302) dermal penetration study was also available in which 21.6% absorption was observed. A comparison of the two studies reveals a similar dose used in each study. In the rat study, 0.1 mg/kg was applied to the skin for 10 hours. Absorption was measured at 82 hours following the application (10/82). The human study had a similar dose of 0.067 mg/kg left on the skin for 10 hours with measurement 168 hours following the start of exposure (10/168).

Exposure scenarios whose endpoints are derived from oral studies will use a 6% dermal absorption factor.

Dermal Absorption Factor: 6%, based on a human study.

7. Dermal Exposure: Short-Term (1- 30 days) Exposure

Study Selected: pubertal [screening] study - male RAT § none

MRID No.: none. Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

Executive Summary: see under Incidental Oral Exposure.

Dose and Endpoint for Risk Assessment: **NOAEL 6.25 mg/kg/day, based on a delay in preputial separation at the LOAEL of 12.5 mg/kg/day.**

Comments about Study/Endpoint: This study is appropriate for this scenario since it demonstrates an endpoint that is consistent with atrazine's mode of toxicity and is protective of this exposure duration. Since an oral dose is selected, 6% dermal absorption factor should be used for route-to-route extrapolation.

8. Dermal Exposure: Intermediate-Term (1 - 6 Months)

Study Selected: Six-Month LH Surge Study - RAT § none

MRID No.: 44152102

Executive Summary: see under Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.

Comments about Study/Endpoint: The endpoints, estrous cycle alterations and LH surge attenuation, are considered indicative of atrazine's ability to disrupt hypothalamic-pituitary function, and its potential to lead to various health consequences including, but not limited

to, reproductive disruption. See comments under Incidental Oral Exposure (1-6 Months). Since an oral dose is selected, 6% dermal absorption factor should be used for route-to-route extrapolation.

9. Dermal Exposure Long-Term (> 6 Months)

Study Selected: Six-Month LH Surge Study - RAT §none

MRID No.: 44152102

Executive Summary: see under Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.

Comments about Study/Endpoint: The attenuation of the LH surge is considered to be an indicator of atrazine's neuroendocrine mode of action or its potential to alter hypothalamic-pituitary function. This six-month study is considered adequate for use in selecting a chronic endpoint without an additional safety factor being added to account for study duration of less than 12 months. This is based on the fact that examination of estrous cycle data from other studies indicates that beyond 6 months of exposure, the differences in estrous cycle deterioration between treated animals and controls no longer widens as the control animals begin the normal reproductive aging process. Since an oral dose is selected, 6% dermal absorption factor should be used for route-to-route extrapolation.

10. Inhalation Exposure: Short -Term (1- 30 days)

Study Selected: pubertal [screening] study - RAT § none

MRID No.: none. Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

Executive Summary: see under Short-Term Dermal.

Dose/Endpoint for Risk Assessment: NOAEL 6.25 mg/kg/day, based on a delay in preputial separation at the LOAEL of 12.5 mg/kg/day.

Comments about Study/Endpoint: This study is appropriate for this scenario since it demonstrates an endpoint in the young animal that is consistent with atrazine's mode of toxicity. The endpoint, delayed puberty, is relevant to the population of concern.

11. Inhalation Exposure: Intermediate-Term (1- 6Months)

Study Selected: Six-Month LH Surge Study - RAT

§ none

MRID No.: 44152102

Executive Summary: see under Chronic RfD

Dose/Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.

Comments about Study/Endpoint: The endpoints, estrous cycle alterations and LH surge attenuation, are considered indicative of atrazine's ability to disrupt hypothalamic-pituitary function, and its potential to lead to various health consequences including, but not limited to, reproductive disruption. See comments under Incidental Oral Exposure (1-6 Months).

12. Inhalation Exposure: Long-Term (> 6 Months)

Study Selected: Six-Month LH Surge Study - RAT

§ none

MRID No.: 44152102

Executive Summary: see under Chronic RfD

Dose/Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day, based on estrous cycle alterations and LH surge attenuation at the LOAEL of 3.65 mg/kg/day.

Comments about Study/Endpoint: The attenuation of the LH surge is considered to be an indicator of atrazine's neuroendocrine mode of action or its potential to alter hypothalamic-pituitary function. This six-month study is considered adequate for use in selecting a chronic endpoint without an additional safety factor being added to account for study duration of less than 12 months. This is based on the fact that examination of estrous cycle data from other studies indicates that beyond 6 months of exposure, the differences in estrous cycle deterioration between treated animals and controls no longer widens as the control animals begin the normal reproductive aging process.

13. Margins of Exposure

The target Margins of Exposure (MOEs) for **occupational** exposure risk assessments are as follows:

Route Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Dermal	100	100	100
Inhalation	100	100	100

The target MOEs for **residential** exposure risk assessments will be determined by the FQPA Safety Factor Committee.

14. Recommendation for Aggregate Exposure Risk Assessments

As per FQPA, 1996, when there are potential residential exposures to the pesticide, aggregate risk assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. The toxicity endpoints selected for these routes of exposure may be aggregated as follows:

A common toxicological endpoint [puberty delay and/or estrous cycle/LH alterations] was selected for the short- and intermediate-term oral, dermal (oral equivalent), and inhalation (oral equivalent) routes. Therefore, these routes can be combined for aggregate exposure risk assessment for the appropriate populations.

A common endpoint [LH alterations] was identified for long-term oral, dermal (oral equivalent), and inhalation (oral equivalent) routes. Therefore, these routes can be combined for aggregate exposure risk assessment for the appropriate populations.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No. 00158930; 42085001; 42204401; 44544701

In a combined 2-year feeding/oncogenicity study (MRID 40629302) atrazine, 95.9% +/- 2.3% was administered to Sprague-Dawley rats, 20/sex/dose, in the diet, at dose levels of 0, 10, 70, 500, and 1000 ppm, (0, 0.5, 3.5, 25, 50 mg/kg/day) for 2 years.

Toxicologic alteration appeared to be confined to the 500 and 1000 ppm dose groups.
25 mg/kg/day: Both male and female rats showed reductions in body weight gain, and food consumption. Body weight for males and females at 104 weeks was 8% and 19% below 104 week control values. Food consumption at 104 weeks was not significantly below control, but at weeks 1, 13 and 26 for males and week 1 for females, food consumption was

statistically significantly (SS) reduced. Food consumption was reduced 11% at week 1, 11% at week 13 and 9% at week 26 in males and 8% at week 1 in females. Males displayed no unusual histopathology findings in this group, but females had SS increases of myeloid hyperplasia in the bone marrow of the femur (25 control incidences vs 38) and sternum (21 vs 33), and splenic extra medullary hematopoiesis (12 vs 22).

50 mg/kg/day: Both sexes showed reductions in body weight gain (19% vs controls at 104 weeks for males, 27% at 104 weeks for females) and food consumption (reduced vs controls 20%, 16%, 14% and 11% at weeks 1, 13, 26 and 52 for males and 18%, 8%, and 7% at weeks 1, 13, and 26 for females). Female survival was 50% at 104 weeks in controls and 26% in 1000 ppm animals. Interestingly, survival was increased in 1000 ppm males - 44% control survival vs 67% 1000 ppm at 104 weeks. Altered hematology and clinical chemistry findings were noted in females and included: decreased hemoglobin concentration; hematocrit; RBC; and serum glucose. Males did not display these alterations but did display decreased serum triglyceride levels throughout the course of the study. Organ-to-body weight ratios were decreased in high dose animals which may have been the result of decreases in body weight gain. Histopathology findings in females dosed 1000 ppm consisted of SS increases in incidences of (% increase vs controls in parenthesis): retinal degeneration (83%); centrilobular necrosis in the liver (300%); degeneration of the rectus-femoris muscle (160%); transitional epithelial hyperplasia in the bladder (150%) and the kidney (82%); splenic extra medullary hematopoiesis (133%); and myeloid hyperplasia in both the femur (108%) and sternum bone marrow (119%). Histopathology findings in males dosed 1000 ppm consisted of SS increases in incidences of: degeneration rectus femoris muscle (366%); prostate epithelial hyperplasia (141%); calculi in the kidney pelvis (106%); and acinar hyperplasia of the mammary gland (200%).

An oncogenicity study run concurrently with the toxicity study determined that there was a dose-related increase in mammary adenocarcinomas (p value for the trend < 0.00005) in 70, 500 and 1000 ppm females. Adenocarcinomas incidences were: controls - 15/66; 70 ppm - 26/68; 500 ppm - 27/65; 1000 ppm - 35/64.

The dose response curve in this study appeared to be adequate as the low doses (10 and 70 ppm) showed few toxic effects; the mid-dose level showed some toxic effects (reduced body weight gain and food consumption); while the high dose showed the same effects as the mid dose plus hematology/clinical chemistry and histopathology findings.

The LOAEL for non-cancer effects is 500 ppm (25 mg/kg/day), based on reduced body weight gain and food consumption. The NOAEL is 70 ppm (3.5 mg/kg/day).

In a carcinogenicity toxicity study (MRID 42227001), atrazine was administered to 600 Fischer- 344 rats, 60/sex/dose in the diet at dose levels of 0, 10, 70, 200, 400 ppm (0, 0.49, 3.43, 9.87 and 20.17 mg/kg/day for males and 0, 0.61, 4.35, 12.71, and 26.18 mg/kg/day for females) for 104 weeks.

Administration of atrazine did not affect animal survival nor, with the exception of thinness in the high-dose group, were any clinical signs apparent. Body weight and body weight gain were significantly reduced in 200 and 400 ppm exposed animals of both sexes throughout most of the study. Food consumption in the high dose group males was significantly reduced throughout the study, and significantly reduced in high dose females for the first 13 weeks of exposure. No findings were seen at necropsy which could be attributed to compound exposure. There were no significant increases in either neoplastic or non-neoplastic findings which could be attributed to compound exposure.

The LOAEL is 3.9 mg/kg/day based on decreased body weight gain. The NOAEL is 0.55 mg/kg/day.

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate based on decreased body weight gain.

Executive Summary: In a carcinogenicity study (MRID 44544701) intended to provide information about the mode of action for oncogenicity in atrazine exposed Sprague-Dawley rats, atrazine, [97.1% a.i.] was administered to 800 female Sprague-Dawley rats. The rats were divided into 2 groups of 400 each. One group was ovariectomized (OVX) while the other was left intact. Atrazine was mixed with the diet at dose levels of 0 (control) 25, 50, 70 and 400 ppm (0, 1.5, 3.1, 4.2, 24.4 mg/kg/day for intact animals and 0, 1.2, 2.5, 3.5, and 20.9 mg/kg/day for ovariectomized animals) for 2 years.

Hematology, clinical chemistry, and urinalysis were not assessed in this study. Food consumption in the dose groups compared to the controls was not altered by compound exposure. The trend for survival was statistically significantly decreased in the dosed groups compared to the controls. Survival was as follows: 43.3% in controls; 31.7% - 25 ppm; 28.8% - 50 ppm; 31.6% - 70 ppm; 21.7% 400 ppm. Body weight was SS reduced in the first half of the study in the 400 ppm group (other groups were not significantly altered), but by the end of the study body weights were similar to control values. Organ weights for pituitary, uterus and the ovaries were taken in this study. No organ weights in either the intact or ovariectomized group were altered by compound exposure.

The only gross necropsy finding which was altered by compound exposure was the occurrence of mammary masses in the intact dosed animals. Dosed animals showed a higher incidence of mammary masses (many of which were confirmed by histopathology to be tumors).

There were no non-neoplastic findings at histology that were increased in dosed OVX animals compared to controls. OVX animals in all groups displayed very high incidences of juvenile uterus and castration cells in the pituitary which would be expected in an OVX animal. The only finding in dosed intact animals which was increased in incidence over controls were ovarian cysts which were slightly increased at 70 and 400 ppm compared to

controls. There were though, many findings which were prevalent in the intact animals, yet were not seen (or were seen at lower levels) in the OVX animals. Mammary gland galactoceles were seen in anywhere from 65 to 78% of the intact animals, depending on the dose group, but the highest percentage of galactoceles in the OVX group was 24% in the 50 ppm group. Mammary gland secretory activity was seen in from 34 to 46% of the intact animals but was not seen in any OVX animals. Uterine dilation was seen in from 8-18% of the intact animals and uterine cystic endometrial hyperplasia was seen in about 50% of the intact animals. No OVX animals displayed uterine dilation and only about 20% displayed cystic endometrial hyperplasia. Intact animals also had increased incidence of pituitary findings compared to OVX animals. From 65 to 73% of the intact animals were found to have sinusoid ectasia/angiectasis but the range in OVX animals was 31 to 45%. The differences in mammary gland, uterine and pituitary findings between OVX and intact animals may provide information about the mode of action of atrazine's carcinogenicity.

Neoplastic histopathology findings were mostly limited to the pituitary and the mammary gland. Neither OVX nor intact animals showed an increase in pituitary tumors compared to their respective controls, but intact animals did show a 20-30% greater incidence of pituitary adenomas compared to OVX animals.

There were few mammary tumors in the interim sacrifice animals, which is not surprising given that these animals were sacrificed after only one-year. Excluding the interim sacrifice and looking only at those animals which were sacrificed at 24 months and those which died prematurely, there was an increase in mammary tumor incidence at all intact dose groups compared to controls. In ascending order of dose the percentage of animals with any type of mammary tumors was: 38.3% in controls; 53.3%; 71.2%; 56.6% and 68.3% at 400 ppm. Looking at carcinomas alone values are: 18.3%; 36.7%; 33.9%; 20%; and 41.7%. Fibroadenomas alone were: 26.6%; 40%; 52.5%; 45%; and 40%. If interim sacrifice data are included the incidence rates for all types of tumors combined becomes: 30%, 42.5%; 56.4% 47.5% and 53.8%. The increased incidence is statistically significant at 50, 70 and 400 ppm.

A decrease in the time-to-tumor is also evident from exposure to atrazine. In the control group 50% of the tumors occurred in the last 6 months of the study. The percentage of tumors which appeared in the last 6 months of the study in the dose groups were: 35.8%; 37.5% 36.5% and 33.4%. The number of tumors which occurred in the first year of the study was slightly increased at 25, 50 and 760 ppm and greatly increased at 400 ppm: 9% in controls; 10.7%; 10%; 12% and 17.9% in the 400 ppm group.

An increased incidence of in mammary tumor in intact animals is determined to occur at doses as low as at 50 ppm (3.1 mg/kg/day), based on a statistically significant increase in combined fibroadenomas, carcinomas and adenomas.

The purpose of this study was to examine mammary tumor carcinogenesis in female Sprague-Dawley rats. Thus, a large part of this review focuses on carcinogenicity. One of the

more striking aspects of the study was the complete lack of mammary tumors in OVX animals. Not a single mammary tumor of any sort was seen in any OVX animal. The lack of mammary tumors in OVX animals provides evidence that the mode of action of atrazine is neither a direct genotoxic nor estrogenic effect on the mammary gland. Rather, an indirect hormonally-mediated effect involving the ovary is implied.

At the 50 ppm and above doses, there was a treatment related increase in mammary tumor incidence when compared to controls. Dosing was considered adequate based on decreases in body weight. Additionally, there was a decreased time-to-tumor at doses of 25 ppm and above.

EXECUTIVE SUMMARY: Atrazine technical was given in the diet to groups of 60 (female only) Sprague-Dawley rats for 24 months (MRID 42204401) at concentrations of 0, 70, or 400 ppm (approximating 0, 3.79, 23.01 mg/kg/day respectively). Water and food were available *ad libitum*. Test doses were selected following a 2-year study using 0, 10, 70, 500 or 1000 ppm of atrazine in the diet (MRID 00158930).

A slight reduction (a negative trend) in survival found amongst the dosed groups was considered to be equivocal because the data were statistically significant ($p < 0.05$) by the Gehan Breslow test but not by the Cox-Tarone test. No treatment-related increases in clinical signs were noted in the study. Body weight gains were statistically significantly reduced relative to controls (approx. 12 to 13%) only at 400 ppm during weeks 0-76. Food consumption was only minimally reduced at the highest dose. Slight alteration in red blood cell shapes and incidence of nucleated RBCs were transient in occurrence. Spleen weights were slightly increased but the increase was not statistically significant. Other organ weight, and organ:body weight values from the 400 ppm group were not significantly different from controls. nonneoplastic lesion findings were comparable in controls and treatment groups. Palpation times for tumors confirmed histologically indicated an early onset of mammary tumors. Total numbers of tumors over the length of the study were statistically significantly increased for the combined incidence of fibroadenomas and carcinomas only when adjustments for survival were made.

The NOAEL for systemic toxicity is 70 ppm (calculated by the reviewer to be approximately 3.79 mg/kg/day) based on body weight gains of this group being 12-13% less than controls as well as statistically significant decreases in body weights in the 0-76 week period. Also, a reduction in survival considered to be equivocal is reported at 400 ppm. An MTD and effect level was determined in a previous chronic feeding study (MRID 00158930).

Discussion of Tumor Data: Several chronic bioassays in the Sprague Dawley rat (MRIDs shown above) have demonstrated that chronic atrazine exposure is associated with an increased incidence and/or an earlier onset of mammary tumors. There is also limited evidence (primarily from a single chronic bioassay) that atrazine exposure may be associated

with an earlier onset of pituitary adenomas.

Adequacy of the Dose Levels Tested: The dose levels tested were adequate for the assessment of the carcinogenic potential of atrazine.

2. Carcinogenicity Study in Mice

MRID No. : 40431302

Executive Summary: In an oncogenicity study (MRID 40431302), atrazine, (purity not given) was administered to CD-1 mice, 59-60/sex/dose, in the diet at dose levels of 0, 10, 300, 1500 and 3000 ppm (male/female mean daily dose 0/0, 1.4/1.6, 38.4/47.9, 194.0/246.9, 385.7/482.7 mg/kg/day) for 91 weeks. Female mice in the 300, 1500 and 3000 ppm groups received a daily atrazine dose about 25% higher than their counterpart males. No dose-related increases in neoplasms were observed. The dose response curve seemed adequate since toxic effects, such as a decrease in mean body weight of both sexes and an increase in cardiac thrombi in the females, are seen at both 1500 and 3000 ppm, while no dose-related toxic effects are seen at 10 and 300 ppm. In addition to the toxic effects just mentioned, the 3000 ppm animals of both sexes also displayed decreases in food consumption and decreases in RBC, hematocrit, and hemoglobin concentration. Female mice, but not males, at 3000 ppm showed decreased mean group brain and kidney weights and decreased percentages of neutrophils and lymphocytes. There was also an increase in mortality ($p < 0.05$) in 3000 ppm females, but not males, with only 25% of the females surviving vs 39-43% of the females surviving in the other female dose groups.

The cardiac thrombi found at both 1500 and 3000 ppm may have contributed to unscheduled female deaths during the course of the study. The incidence of unscheduled death in mice with cardiac thrombi is statistically significantly different from the incidence of unscheduled death in mice from control groups. The occurrence of cardiac thrombi must be considered a severe effect.

The LOAEL is 1500 ppm (222.0 mg/kg/day), based on decreased body weight gain in both sexes and increased cardiac thrombi in the females. The NOEL is 300 ppm (43 mg/kg/day).

Discussion of Tumor Data: There was no treatment-related increase in tumor.

Adequacy of the Dose Levels Tested Dosing was considered adequate due to the occurrence of decreased body weight gain and cardiac thrombi.

3. Classification of Carcinogenic Potential. The December 13, 2000 CARC reaffirmed the classification of atrazine as “Not Likely To Be Carcinogenic To Humans” based on the overall weight of evidence that:

1. The mode of carcinogenic activity in the female SD rat is supported by the data.
2. The mode of carcinogenic activity in the female SD rat essentially involves an acceleration of the reproductive aging process.
3. The mode of action for the carcinogenicity of atrazine is unlikely to be expressed in humans; no human conditions can be established that support a potential for atrazine to lead to carcinogenicity in humans.
4. Other modes of action are not supported by the available data and, in particular, mutagenic and estrogenic activity do not appear to significantly contribute to atrazine's carcinogenic potential.
5. Although a few epidemiological studies suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer, these cancers do not appear to be plausible based on atrazine's mode of action. Therefore, the human studies by themselves do not make a strong case for an association.

IV. MUTAGENICITY

The HIARC concluded that there is not a concern for mutagenicity resulting from exposure to *atrazine*. Atrazine has not been found to be mutagenic in bacteria and does not cause unscheduled DNA synthesis in primary rat hepatocytes. Atrazine did not induce clastogenicity in the mouse micronucleus assay. Atrazine was negative in a mouse Dominant-Lethal Assay.

(i) Gene Mutation

In a reverse gene mutation assay in bacteria (MRID 40246601), strains TA 98, 100, 1535 and 1537 of *S. typhimurium* were exposed to atrazine (98.2% a.i.), in dimethylsulfoxide, at concentrations of 0, 20, 78, 313, 1250, and 5000 µg/plate. Tests were conducted in the presence and absence of mammalian metabolic activation S9 fraction of Tif:RAIf rats treated with Aroclor 1254. Atrazine was tested up to the limit concentration, 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

(ii) Structural Chromosomal Aberrations

A mouse bone marrow micronucleus test was conducted using Tif:MAGF mice (MRID 40722301). The test consisted of two parts. The first portion consisted of 24 male and 24 female mice being dosed with 2250 mg/kg atrazine (98.2% a.i.). Eight animals of each sex were then sacrificed at 16,

24 or 48 hours following treatment. The second portion of the study 24 mice, 8/sex/dose, were treated with atrazine (98.2% a.i.) at doses of 562.5, 1175, 2250 mg/kg. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle in both portions of the study was carboxymethyl cellulose. Exposure in both portions of the study was accomplished by a single gastric intubation. There were no signs of cytotoxicity in bone marrow erythropoiesis seen either portion of the study. However, the high dose was clearly toxic since 7 of the 32 females which received the high dose died prematurely. Atrazine was tested at an adequate doses being that these were doses that induced death in mice. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micro nucleated polychromatic erythrocytes in bone marrow after any treatment time or dose.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

(iii) Other Genetic Effects

In an unscheduled DNA synthesis assay (MRID 42547105), primary rat hepatocyte cultures were exposed to atrazine, (97.1% a.i.), in dimethyl sulfoxide at concentrations of 15, 46, 139, 417, 835, and 1670 µg/ml for 16-18 hours. Atrazine was tested up to precipitating concentrations, 139 µg/ml. The positive controls did induce the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by nuclear silver grain counts, was induced.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

In a mouse dominant lethal assay (MRID 42637003), groups of 30 male Tif: MAGf (SPF) mice were treated orally by gavage with Atrazine technical (97.1% a.i., batch #SG8029BA10) at doses of 0, 500, 1000, 2000, or 2400 mg/kg body weight in a volume of 10 mL/kg. The vehicle was corn oil. Starting immediately after dosing, each male was mated with 2 untreated females per interval for days 1-4, days 4-8, and days 8-12. Each male was then mated with 2 untreated females per week for weeks three through eight.

Atrazine technical was tested at an adequate dose. There were signs of toxicity after dosing as evidenced by piloerection and decreased locomotor activity. The females were sacrificed on gestation day 13-15 and the uteri examined for the number of alive, early, and late dead embryos and resorptions. Cyclophosphamide served as the positive control. There was no significant difference between the control group and treated groups with respect to post-implantation mortality of embryos. Under the conditions of this study atrazine technical did not induce dominant lethal mutations in male mice at doses as high as 2400 mg/kg.

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test guideline 84-2 for rodent dominant lethal data.

V. HAZARD CHARACTERIZATION

Atrazine is a herbicide most commonly used on corn and sorghum to control broadleaf grasses. The toxicological database for atrazine is complete and acceptable. Atrazine has low acute toxicity, and it is not a dermal sensitizer.

Guideline subchronic, dermal, chronic, developmental, and reproduction studies did not indicate any particular target organ for toxicity except pituitary and mammary gland tumors in females of Sprague Dawley rats. However, special studies have indicated that atrazine disrupts hypothalamic-pituitary gonadal axis via a central nervous system [CNS] target. Neuroendocrine alterations of the hypothalamic-pituitary axis of rodents following atrazine exposure have been described both in studies submitted by the registrant and in studies conducted by EPA labs. These alterations are seen in chronic studies at low doses and in shorter term studies at higher doses. Atrazine's effect on ovarian cycling and the pre-ovulatory LH surge (as well as its effects on pregnancy, puberty, suckling induced prolactin release which leads to prostatitis) are viewed as neuroendocrinopathies or biomarkers indicative of atrazine's ability to alter hypothalamic-pituitary function in general. It should be noted that atrazine's neuroendocrine effects have been demonstrated in several strains of rats (Sprague Dawley, Long Evans, Wistar).

The mutagenicity database for atrazine is extensive, and atrazine is not considered mutagenic. Special studies have also been conducted to determine the estrogenic potential of atrazine, and these studies have demonstrated that atrazine lacks direct estrogenic activity.

The Cancer Assessment Review Committee (CARC) has classified atrazine as "Not Likely To Be Carcinogenic To Humans".

VI. DATA GAPS / REQUIREMENTS

There is one datagap. A 28-day study *via* the inhalation route of exposure is required to assess the estrous cycle/LH surge in females for comparison with the 28-day oral exposure study. There are no other data gaps for atrazine according to the Subdivision F Guideline requirements. HIARC recommends, *but does not require*, that special studies examining atrazine's associations with delayed puberty and prostatitis in offspring of dams exposed shortly after parturition, be conducted. Should such studies be conducted, it is recommended that study protocols be approved by HED prior to commencement of any such study.

In addition, HIARC recommends, but does not require, that special studies examining the CNS alterations following atrazine exposure be performed.

VII. ACUTE TOXICITY*Acute Toxicity of Atrazine (PC Code 080803)*

Guideline No.	Study Type	MRID #(s)	Results	Toxicity Category
81-1	Acute Oral	00024709	LD ₅₀ = 1869 mg/kg	III
81-2	Acute Dermal	00024709	LD ₅₀ >2000 mg/kg	III
81-3	Acute Inhalation	43016502	LC ₅₀ = >5.8 mg/L	IV
81-4	Primary Eye Irritation	00024709	PIS = 0.0/110	IV
81-5	Primary Skin Irritation	00024709	PIS = 0.2/8.0	IV
81-6	Dermal Sensitization	00105131	not a sensitizer	-

There are no acute toxicity data available for DACT

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Summary of Toxicology Endpoint Selection for ATRAZINE/DACT (PC Code 080803)

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Dietary Risk Assessments			
Acute Dietary <u>females 13-50 years of age</u>	NOAEL = 10 UF = 100 Acute RfD = 0.1 mg/kg/day	1X	rat developmental toxicity study LOAEL = 70 mg/kg/day based on delayed or lack of ossification of several sites. Supported by decreased suckling-induced prolactin release and increased incidence of prostatitis in male offspring at 25 mg/kg/day [NOAEL 12.5 mg/kg/day]
Acute Dietary <u>general population including infants and children</u>	NOAEL = [] UF = [] Acute RfD = [] mg/kg/day	NA	no appropriate endpoint attributable to a single exposure is available
Chronic Dietary <u>all populations</u>	NOAEL= 1.8 UF = 100 Chronic RfD = 0.018 mg/kg/day	3X	six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Incidental Oral Short-Term (1 - 30 Days) Residential Only	NOAEL= 6.25 MOE= TBD	3X	pubertal screening study LOAEL = 12.5 mg/kg/day based on delay in preputial separation.

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Incidental Oral Intermediate-Term (1 - 6 Months) Residential Only	NOAEL= 1.8 mg/kg/day MOE = TBD	3X	[six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Non-Dietary Risk Assessments			
Dermal ^A Short-Term (1 - 30 days)	Dermal or Oral NOAEL= 6.25 mg/kg/day		pubertal screening study LOAEL = 12.5 mg/kg/day based on delay in preputial separation.
Residential	MOE = TBD	3X	
Occupational	100	NA	
Dermal ^A Intermediate-Term (1 - 6 Months)	Dermal or Oral NOAEL= 1.8 mg/kg/day		six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Residential	MOE = TBD	3X	
Occupational	100	NA	
Dermal ^A Long-Term (> 6 Months)	Dermal or Oral NOAEL= 1.8 mg/kg/day		six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Residential	MOE = TBD	3X	
Occupational	100	NA	
Inhalation ^A Short-Term (1 - 30 days)	Inhalation or Oral NOAEL= 6.25 mg/kg/day		pubertal screening study LOAEL = 12.5 mg/kg/day based on delay in preputial separation.
Residential	MOE = TBD	3X	
Occupational	100	NA	

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Inhalation ^A Intermediate-Term (1 - 6 Months)	Dermal or Oral NOAEL= 1.8 mg/kg/day		six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Residential	MOE = TBD	3X	
Occupational	100	NA	
Inhalation ^A Long-Term (>6 Months)	Dermal or Oral NOAEL= 1.8 mg/kg/day		six-month LH surge study - rat LOAEL = 3.65 mg/kg/day based on estrous cycle alterations and LH surge attenuation
Residential	MOE = TBD	3X	
Occupational	100	NA	
Cancer	Classification: Not Likely		

Convert from oral dose using a dermal absorption rate of 6% or an inhalation absorption rate of 100% [default].
TBD = To Be Determined. Target MOEs for residential exposures will be determined by the FQPA Safety Factor Committee.



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HED File Code	21100 HIARC
Memo Date:	04/05/2002
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