



Exponent
1800 Diagonal Road
Suite 500
Alexandria, VA 22314

telephone 571-227-7200
facsimile 571-227-7299
www.exponent.com

March 21, 2014

Via Email and FedEx

Lauren Zeise, Ph. D.
Deputy Director
Office of Environmental Health Hazard Assessment
1001 I Street
Sacramento, California 95814

Re: Opposition to Notice of Intent to List: Atrazine, Propazine, Simazine, and the chlorometabolites DACT, DEA, and DIA

Dear Ms. Zeise:

California's Office of Environmental Health Hazard Assessment (OEHHA) has recently published a Notice of Intent to List: Atrazine Propazine, Simazine, and the chlorometabolites DACT, DEA, and DIA under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Prop 65) as "known to the state to cause reproductive toxicity." I have been asked by Syngenta, based upon my knowledge about the potential reproductive and developmental toxicology of atrazine and my experience at US EPA, to review and comment on the evidence presented in the Notice of Intent to List.

This letter expresses my opposition to the proposed listing of atrazine based on the lack of sufficient data to support the stated reproductive effects. The pertinent regulation requires that the determination of sufficiency take into account:

"the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible."

OEHHA did not fully take into account these factors and the current state of knowledge regarding atrazine. In particular, when the reproductive effects reported in rats are considered in light of the mechanism of action (MOA) for atrazine and pharmacokinetics from the studies that compare gavage and dietary exposure to atrazine, it is clear that the

reproductive effects seen in rodents are not relevant to humans. These factors are described in greater detail below, and demonstrate that the potential for reproductive toxicity from atrazine exposure in humans lacks biological plausibility.

Mechanism of Action: Suppression of LH Surge in Sprague Dawley Rats

The Notice of Intent to List cites several US EPA documents from 2002 and 2006 that describe a common MOA for neuroendocrine effects in rats associated with the chlorotriazine herbicides, including atrazine. Although the US EPA has relied on this MOA as a point of departure in risk assessment, the hormonal alteration is not, in and of itself, an adverse effect and is described by the Agency as “a precursor event for the reproductive effects” in rats, not humans (US EPA 2011, p. 14). Furthermore, since the 2002 and 2006 reports, new data have been developed and are being actively considered by the Agency. The new data provide even more information on the MOA to show that the reproductive effects seen in rats are not relevant to humans because:

- Significant differences exist between the rat and human female reproductive hormone cycle;
- Reproductive effects are observed only in studies where atrazine is administered by gavage resulting in high plasma concentrations that could not occur in humans from dietary, drinking water or occupational exposure to atrazine.

The common MOA for chlorotriazine herbicides relied on by EPA for risk assessment is suppression of the luteinizing hormone (LH) surge that is part of the hormonal cascade that comprises the rat estrous cycle. This suppression of the LH surge has been observed in several animal models, but the Sprague Dawley (SD) strain of rat is the most sensitive. The observed differences in response to atrazine raise the question about whether this strain of rat is appropriate for assessing potential human health effects.

The estrous cycle in the rodent corresponds to the menstrual cycle in humans with ovulation or release of an egg for possible fertilization as the end result. The estrous cycle in the rat is only four days in length and is linked to circadian signals from the brain in conjunction with feedback from circulating levels of the hormone, estradiol. Normally, during the four day cycle the brain sends a signal that produces release of gonadotropin releasing hormone (GnRH), which in turn triggers the LH surge (Plant et al. 2012). The LH surge induces ovulation and if the animal does not become pregnant, the cycle repeats itself. SD rats begin to lose reproductive capability at 9 months or at one-third of their lifetime. This change is predominantly due to the reduced hypothalamic GnRH stimulation of the pituitary secretion of LH and follicle stimulating hormone. Estrogen levels remain elevated, resulting in a higher estrogen to progesterone ratio.

In humans, the menstrual cycle is approximately four weeks in duration with the LH surge occurring for two to three days prior to ovulation. Unlike the rat, the LH surge in humans

appears to occur independently from a GnRH signal and is primarily the result of positive feedback from estradiol (Simpkins et al. 2011). The timing and control of ovulation in humans is not linked with a circadian signal and the primary site for feedback from estradiol is the pituitary, not the preoptic area of the hypothalamus which is the feedback site in rats (Plant et al. 2012). The loss of reproductive capability of human females is related to the reduced number of eggs in the aging ovaries, not the reduction in GnRH levels seen in aging SD rats (Chapin et al. 1996). Menopause is also associated with lower estrogen production leading to a lower estrogen to progesterone ratio – the direct opposite of what is seen in SD rats. Thus, there are several significant differences in the induction of the LH surge between rats and humans.

When rats are administered certain doses of atrazine, the LH surge is suppressed and the animals exhibit a lengthened estrous cycle or remain in persistent estrus, one of the phases of the estrous cycle. Under these conditions ovulation does not occur and these rats exhibit an accelerated reproductive aging (described as senescence). The suppression of the LH surge by atrazine is very dependent on the timing, duration, and route of exposure. Although reproductively aged female SD rats have been shown to be sensitive to the effects of atrazine, younger animals are more resilient to the influences of atrazine on GnRH (Ashby et al. 2002), which was acknowledged by the US EPA Scientific Advisory Panel (SAP):

An extensive hazard database, spanning all life stages from conception to adulthood for atrazine, indicates no unique susceptibility in the developing organism. Additionally, the proposed point of departure, based upon attenuation of the LH surge, appears to be protective against adverse reproductive/developmental outcomes such as delays in onset of puberty, disruption of ovarian cyclicity and inhibition of suckling-induced prolactin release. (EPA SAP 2011, p 14)

Recent studies have shown that multiple doses are required to suppress the LH surge and that a single, high dose just prior to the LH surge induces an increased (not decreased) response in plasma LH levels (Goldman et al. 2013). The route of exposure is particularly important since high bolus doses of atrazine are associated with suppression of the LH surge, but extremely high dietary exposures are required to produce a similar effect. Although all of the details of the cellular mechanism related to the suppression of LH surge are not known, several pieces of evidence suggest that the disruption of the LH surge is not relevant to humans.

First, alterations by the triazines and certain metabolites in the LH surge appear to be the result of an effect on the hypothalamus, reducing the release of GnRH (Cooper et al. 2007, Fraites et al. 2009). Because the signal for initiating the LH surge in humans is a feedback mechanism driven by estradiol released by the ovary, the reduction in GnRH is unlikely to impact the LH surge in humans. Second, atrazine does not directly affect LH secretion from the pituitary in the rat (Cooper et al. 2000). Given the fact that the ovary and pituitary play

a more central role in the hormonal control of the menstrual cycle in humans, this suggests that atrazine should not affect the LH surge in humans. Third, the causes of reproductive aging are different between rats and humans. Because exposure to atrazine suppresses the LH surge, leading to pre-mature reproductive aging in rats, this would not be relevant to human females since aging is not a consequence of changes in LH. Finally, in comparative studies of gavage and dietary exposure to atrazine, only the bolus doses from gavage have been demonstrated to alter the LH surge in rats. The direct administration by gavage allows for the rapid absorption and metabolism of atrazine, producing high plasma concentrations of atrazine and metabolites. As discussed in greater detail below, the pharmacokinetics of atrazine demonstrate that the gavage route of exposure is not appropriate for modeling the potential exposure to atrazine in humans.

OEHHA specifically identifies estrous cycle alterations as one of the endpoints indicative of reproductive toxicity. The estrous cycle alterations include persistent estrus, which leads to premature senescence in female rats. These changes are a direct result of the suppression of the LH surge, and as outlined above, cannot be considered relevant to humans.

Considering the current body of evidence on the MOA for atrazine, it is apparent that the suppression of the LH surge is not relevant or biologically plausible in humans. The US EPA SAP similarly concluded that:

It seems unlikely that humans would ever experience the sorts of internal exposures necessary in rats to produce suppression of the LH surge. (EPA SAP 2011, p. 84)

Therefore, the sufficiency of the data presented by OEHHA as evidence for the biological plausibility of reproductive toxicity from exposure to atrazine in humans cannot be supported.

Reproductive Toxicity and Consideration of Experimental Study Design

OEHHA specifically identifies several male and female reproductive endpoints as the basis for the Notice of Intent to list atrazine as a reproductive toxicant. These endpoints include: prolonged estrus in the dams (as discussed above), delayed ossification of certain cranial bones of fetuses, delayed vaginal opening (VO) in female pups, and delayed preputial separation (PPS) in male pups. As acknowledged by EPA at the Scientific Advisory Panel in July 2011, all of these effects occur at doses greater than that associated with suppression of the LH surge:

The Agency will continue to use changes in LH secretion as the basis of the atrazine risk assessment. As such, any of the identified adverse outcomes would be protected since they occur at doses higher than those eliciting changes in LH (US EPA 2011, p. 13)

The reproductive effects noted by OEHHA have been observed in gavage studies, but have not been reported in dietary studies at comparable doses. The precursor event, suppression of the LH surge, has been only been observed in dietary studies at doses greater than 400 ppm, which is considered higher than the maximum tolerated dose and is associated with significant effects on body weight and body weight gains (Chapin et al. 1996, Simpkins et al. 2011). The changes in body weight are considered signs of systemic toxicity and in a reproductive study would clearly represent maternal toxicity which, as stated in the Prop 65 regulations, should be considered in judging the biological plausibility for humans.

A number of recent studies have been conducted to better understand the pharmacokinetics (PK) of atrazine (Coder et al. 2011, Hui et al. 2011, Press et al. 2012, Stuhler et al. 2011). These studies have shown that administration by gavage results in rapid, high plasma concentrations of atrazine and its metabolites, while dietary intake at similar doses resulted in lower peak and total plasma concentrations of atrazine and its metabolites (see Figure 8 in the Syngenta Science paper, Breckenridge et al. 2013). The gavage route of exposure results in a bolus dose to the animal, which is immediately available for absorption and distribution. In contrast, animals exposed to atrazine in the diet have a slow steady exposure, resulting in a lower, more constant plasma levels. Given the rapid elimination of atrazine and metabolites, they do not build up in the body, so there is no opportunity for plasma concentrations to reach the levels achieved by gavage (Coder et al. 2011, Foradori et al. 2014, Hui et al. 2011, Press et al. 2012, Stuhler et al. 2011). As stated by the SAP, “[i]t is important to be cognizant that the toxicological doses of atrazine being discussed (e.g., 12.5 – 100 mg/kg/day) are not relevant to probable exposure levels in the "real" environment. (US EPA SAP 2011, p. 40). Thus, when one takes into account that the reproductive effects of atrazine are associated with gavage exposures and high plasma concentrations, it is not biologically plausible for these types of effects to be seen in humans, given the substantially lower exposures to atrazine for humans.

It is also important to note that in two- and three-generation reproductive studies, no effects on reproduction, including mating and fertility have been reported (DeSesso et al. 2014). Thus, long-term dietary exposures, which are more representative of potential human exposures, do not support reproductive effects from atrazine.

In addition to the consideration of the route of exposure, a couple of additional factors should be taken into account when evaluating the relevance of the developmental toxicity highlighted by OEHHA. First, the observation of delayed ossification of cranial bones is not considered an adverse effect, such as a malformation. Second, as noted by US EPA (2006), these developmental delays were seen in conjunction with a decreased body weight gain, which suggests maternal toxicity. Furthermore, in a recent review of all developmental toxicity studies, Scialli et al. (2014) concluded that “[o]verall, data show that neither

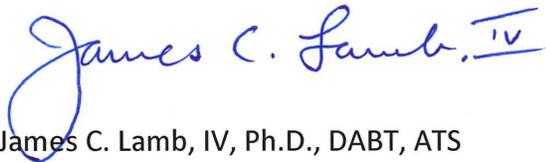
March 21, 2014

Page 6

[atrazine] or its metabolites statistically significantly affected rat or rabbit embryo-fetal development even at dose levels producing maternal toxicity.”

I conclude that atrazine should not be listed by the State of California on the basis of reproductive toxicity. This conclusion is based on the study design parameters and other toxicological factors that are associated with the observation of reproductive effects in rats. When the route of administration, frequency and duration of exposure, and maternal toxicity are taken into consideration, the biological plausibility of these effects occurring in humans at typical or higher occupational exposures is not credible.

Sincerely,

A handwritten signature in blue ink that reads "James C. Lamb, IV". The signature is written in a cursive style with a large initial "J" and a distinct "IV" at the end.

James C. Lamb, IV, Ph.D., DABT, ATS
Principal Scientist and Center Director
Center for Toxicology and Mechanistic Biology

References

Ashby, J., Tinwell, H., Stevens, J., Pastoor, T. and Breckenridge, C.B. 2002. The effects of atrazine on the sexual maturation of female rats. *Regul Toxicol Pharmacol.* Jun;35(3):468-73.

Chapin, R.E., Stevens, J.T., Hughes, C.L., Kelce, W.R., Hess, R.A. and Daston, G.P. 1996. Endocrine modulation of reproduction. *Fundam Appl Toxicol.* Jan;29(1):1-17.

Coder et al. 2011. Syngenta Crop Protection, LLC., Syngenta Task Number 0033837. WIL 639080. Atrazine: An oral (Gavage and dietary) study of the effects of atrazine on the spontaneous luteinizing hormone surge in female Sprague-Dawley rats. Final Report Date: April 7, 2011.

Cooper, R.L., Laws, S.C., Das, P.C., Narotsky, M.G., Goldman, J.M., Lee, Tyrey E. and Stoker, T.E. 2007. Atrazine and reproductive function: mode and mechanism of action studies. *Birth Defects Res B Dev Reprod Toxicol.* Apr;80(2):98-112.

DeSesso, J.M., Scialli, A.R., White, T.E.K. and Breckenridge, C.B. 2014. Multigeneration Reproduction and male developmental toxicity studies on atrazine in rats. *Birth Defects Research (Part B)* 00:1–16.

Fraites, M. J. P., Narotsky, M. G., Best, D. S., Stoker, T. E., Davis, L. K., Goldman, J. M., Hotchkiss, M. G., Klinefelter, G. R., Kamel, A., Qian, Y., Podhorniak, L. and Cooper, R. L. 2011. Gestational atrazine exposure: Effects on male reproductive development and metabolite distribution in the dam, fetus, and neonate. *Repro Tox.*, 32, 52-63.

Goldman, J., Davis, L. K., Murr, A. S. and Cooper, R. L. 2013. Atrazine-induced elevation or attenuation of the LH surge in the ovariectomized, estrogen-primed female rat: role of adrenal progesterone. *Reproduction*, 146, 305-14.

Hui, X., Wester, R.C. and Maibach, H.I. 2011. Pharmacokinetics of [14C]-atrazine in rhesus monkeys, single-dose intravenous and oral administration. *Toxicol. Environ. Chem.*, 93, 370-382.

Plant, T. M. 2012. A comparison of the neuroendocrine mechanisms underlying the initiation of the preovulatory LH surge in the human, Old World monkey and rodent. *Front. Neuroendocrinol.*, 33, 160-168.

Press, R. 2012. Syngenta Crop Protection LLC., Syngenta Task Number 0121327; Covance Study Number 8273284. Characterization of the metabolic profile of 14c atrazine after a single intravenous dose to adult female Cynomolgus monkeys, Protocol Date: October 10, 2012.

Scialli, A., DeSesso, J. D. and Breckenridge, C. B. 2014. Developmental toxicity studies with atrazine and its major metabolites in rats and rabbits. *J. Birth Defects Research (Part B)* 00:1–16.

Simpkins, J. W., Swenberg, J. S., Weiss, N., Brusick, D., Eldridge, J. C., Stevens, J. T., Handa, R. J., Hovey, R. C., Plant, T. M., Pastoor, T. P. and Breckenridge, C. B. 2011. Atrazine and breast cancer: A framework assessment of the toxicological and epidemiological evidence. *Toxicol. Sci.*, 123, 441-459.

Stuhler, J. D. 2011. Syngenta Crop Protection LLC., Syngenta Task Number 0006147; Covance 8244640. Determination of the Pharmacokinetics of Atrazine after Single Doses to female *Cynomolgus* monkeys. April 7, 2011.

US Environmental Protection Agency (US EPA). 2011. Reevaluation of the human health effects of atrazine. Review of Cancer Epidemiology, Non-cancer experimental animal and in vitro studies and drinking water monitoring. Health Effects Division, Environmental Fate and Effects Division in collaboration with the Office of Research and Development. Presented on July 26-29, 2011.

US Environmental Protection Agency (US EPA). 2006. Triazine cumulative risk assessment. HED Human Health Risk Assessment in Support of the Reregistration Eligibility Decisions for Atrazine, Simazine and Propazine. PC Codes: 080808, 080803, 080807. DP 317976. Health Effects Division (7509C), Benefits and Economic Analysis Division (7503C), and Environmental Fate and Effects Division (7507C). March 28, 2006. Office of Prevention, Pesticides and Toxic Substances. United States Environmental Protection Agency. Washington, DC.

US Environmental Protection Agency (US EPA). 2002. The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity. USEPA, OPP, Health Effects Division, March, 2002.

US Environmental Protection Agency Scientific Advisory Panel (US EPA SAP). (2011). Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held July 26-28th, 2011 on the Re-Evaluation of the Human Health Effects of Atrazine: Review of Non-Cancer Effects, Drinking Water Monitoring Frequency and Cancer Epidemiology. October 26, 2011.