AGENDA
FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING
April 26 – 29, 2010

FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/
OPP Docket Telephone: (703) 305-5805
Docket Number: EPA-HQ-OPP-2010-0125

The Hamilton Crowne Plaza Hotel
The Hamilton Ballroom
1001 14th Street, NW
Washington, DC 20005
202-682-0111

Reevaluation of the Human Health Effects of Atrazine: Review of Experimental Animal and *In Vitro* Studies and Drinking Water Monitoring Frequency

Please note that all times are approximate (see note at end of Agenda).

Day 1
Monday, April 26, 2010

1:00 P.M. **Opening of Meeting and Administrative Procedures** – Joseph Bailey, Designated Federal Official, Office of Science Coordination and Policy, EPA

1:05 P.M. **Introduction and Identification of Panel Members** – Kenneth Portier, Ph.D., FIFRA Scientific Advisory Panel Session Chair

1:15 P.M. **Welcome and Opening Remarks** – Steven Bradbury, Ph.D., Acting Director, Office of Pesticide Programs, EPA

1:25 P.M. **Welcome and Introductions** – Tina Levine, Ph.D., Director, Health Effects Division, Office of Pesticide Programs, EPA

1:30 P.M. **Introduction: Status of the Reevaluation of the Health Effects of Atrazine** – Anna Lowit, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

1:45 P.M. **Reevaluation of Human Health Effects of Atrazine: Review of Experimental Animal and In Vitro Studies and Drinking Water Monitoring Frequency** – Elizabeth Mendez, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

2:00 P.M. **A Proposed MOA for Atrazine and Atrazine Metabolites** – Ralph L. Cooper, Ph.D., Endocrinology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, EPA.

3:00 P.M. Break

3:15 P.M. **Review of Atrazine Immunotoxicity** – Robert Luebke, Ph.D., Cardiopulmonary and Immunotoxicity Branch, Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, EPA.

3:45 P.M. **Neurological Effects of Atrazine** – John Liccione, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

4:15 P.M. **Summary of Non-Cancer Mammalian Toxicity Reevaluation** – Elizabeth Mendez, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

4:30 P.M. **Approaches to Evaluating Water Sampling Strategies and Frequency of Monitoring** – Nelson Thurman, M.S., Environmental Fate and Effects Division, Office of Pesticide Programs, EPA

5:00 P.M. **Evaluating the Performance of Sampling Strategies for Estimating Maximum Concentrations of Different Durations** – Mary Frankenberry, Environmental Fate and Effects Division, Office of Pesticide Programs, EPA

5:25 P.M. **Artificial Neural Network Modeling of Atrazine Occurrence Patterns** – Michael J. Messner, Ph.D., Standards and Risk Reduction Branch, Office of Ground Water and Drinking Water, EPA

5:50 P.M. **Water Sampling Summary** – Nelson Thurman, M.S., Environmental Fate and Effects Division, Office of Pesticide Programs, EPA

5:55 P.M. **Summary and Conclusion –** Anna Lowit, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

6:00 P.M. **Adjourn**

Day 2
Tuesday, April 27, 2010

8:30 A.M. **Opening of Meeting and Administrative Procedures** – Joseph Bailey, Designated Federal Official, Office of Science Coordination and Policy, EPA

8:35 A.M. **Introduction and Identification of Panel Members** – Kenneth Portier, Ph.D., FIFRA Scientific Advisory Panel Session Chair

8:45 A.M. **Follow-up from Previous Day's Presentations**

9:15 A.M. **Public Comments**

10:00 A.M. **Break**

10:15 A.M. **Public Comments**

12:15 P.M. **Lunch Break**

1:30 P.M. **Charge to Panel – Question 1**

In Vivo & In Vitro Experimental Toxicology in Mammals

In 2003, the human health risk assessment was based on a mode of action in which exposure to atrazine leads to a reduced release of gonadotropin releasing hormone (GnRH) from the hypothalamus thereby lessening the afternoon pituitary luteinizing hormone (LH) surge in female Sprague Dawley rats. As a result, the estrus cycle lengthens. This, in turn, leads to increased estrogen levels and an increased incidence of mammary tumors in female Sprague Dawley rats. EPA determined that atrazine's cancer mode of action (i.e., premature reproductive aging) in the Sprague-Dawley rat is not likely to be operative in humans (a conclusion consistent with the SAP recommendation in 2000). The Agency will be evaluating the cancer classification further as the experimental toxicology data are integrated with the epidemiology literature; new experimental toxicology studies (Section 3.6 of the draft issue paper) do not suggest a change to the Agency's previous conclusion that atrazine is "not likely to be carcinogenic to humans."

Although the cancer mode of action may not be operative in humans, it is not unreasonable to assume that atrazine might cause adverse effects on hypothalamic-pituitary function in humans. Thus, the same endocrine perturbations that induce tumors in rats may play a role in at least some reproductive/developmental effects (not associated with reproductive aging) that may be relevant to humans. Accordingly, the Agency identified disruption of estrous cyclicity and delays in puberty onset (males and females) occurring as a consequence of disruptions to the hypothalamic-pituitary-gonadal (HPG) axis as the critical endpoints of concern (Sections 2.0 & 3.2 of the draft issue paper).

The Agency continues to believe that this disruption of the HPG axis is critical to affecting estrous cyclicity and delayed puberty onset. However, as discussed in Questions 1.2-1.6 (See Figure 3 of the draft issue paper), new information has been published that indicates a more expanded understanding of how atrazine may perturb the pathway leading to reproductive effects. These new data indicate that atrazine disrupts the hypothalamic-pituitary-adrenal (HPA) axis altering the central nervous system's control of the pituitary and adrenal which, in turn, disrupts the HPG axis. Furthermore, a hypothesis for a direct effect of atrazine on the adrenal cortex and the gonads which may impact steroidogenesis directly is also supported by the available data.

Question 1.1 – With the caveat that the review of the epidemiological literature is still on-going, please comment on the Agency's preliminary conclusion that new experimental data from *in vitro* and *in vivo* laboratory animal studies do not support a change in the conclusions from the 2003 risk assessment that atrazine is unlikely to be a human carcinogen.

2:15 P.M. Charge to Panel – Question 1 (continued)

Question 1.2 – Based on an evaluation of the studies examining the mode of action of atrazine on neuroendocrine function (Section 3.2 of the draft issue paper), the Agency has preliminarily concluded that atrazine affects both the HPG axis and the HPA axis. With respect to the temporal concordance, recent studies show that atrazine induces a rapid (within minutes) increase in ACTH and adrenal cortical hormones (corticosterone and progesterone) in both male and female rats (Fraites et al., 2009; Laws et al., 2009; Pruett et al., 2009), while changes in the HPG axis, such as the suppression of the LH surge, may take up to 2-3 days (Cooper et al., 2009). This sequence of HPA alterations followed by HPG changes indicates that the suppression of the surge may be, in part, mediated by activation of the HPA axis (i.e., a corticosterone suppression of the GnRH and LH release). In addition, with respect to dose-response concordance, atrazine-induced increases in ACTH, corticosterone and adrenal progesterone are seen following a single dose of atrazine (50 mg/kg). In contrast, the HPG is not altered following a single dose up to 200 mg/kg. However, when treated for multiple days, the dose necessary to alter the HPA axis is lower than or equal to the one needed to affect the HPG axis.

Please comment on the Agency's preliminary hypothesis for the mode of action involving atrazine's alteration of both the HPA and HPG axes. Does the document adequately and clearly describe the hypothesis in the context of a "toxicity pathway" (*i.e*. cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects)? Does the document clearly describe the

data used to test the proposed hypothesis? To what extent do the available data establish key events in the proposed mode of action hypothesis? What are the strengths and limitations of the data available on this hypothesis?

Please include in your comments a discussion of the Agency's interpretations of the data linking the initial perturbations in HPA axis to changes in the HPG axis.

Please comment on the evidence that the initial perturbations in the HPA axis may lead to impairment in reproductive function and/or developmental consequences. Which event(s) is/are viewed as critical in leading to health consequences? Are there data on other substances that would inform this question?

3:15 P.M. Break

3:30 P.M. Charge to Panel – Question 1 (continued)

Question 1.3 – The Agency has preliminarily concluded that atrazine directly targets cells within the HPA axis (Sections 3.2 & 3.3.2.3 of the draft issue paper) and that the increased activity of the adrenal axis does not reflect nonspecific stress. Evidence for this conclusion is based on the following observations.

a. Several studies report increased adrenocorticotropic hormone (ACTH) and corticosterone levels immediately following a single exposure to atrazine and its two metabolites (deisopropyl-atrazine, DIA; deethyl-atrazine, DEA) but not diamino-s-chlorotriazine (DACT; Laws et al., 2009; Fraites et al., 2009; Pruett et al., 2009).

b. A single intravenous administration of DEA *in vivo* (Fraites et al., 2009) also causes an immediate increase in ACTH and adrenal hormone release, indicating that a chlorotriazine-induced gastrointestinal distress is not driving the hormonal response.

c. The adrenal corticosterone response to continued oral exposure to atrazine did not habituate in rats (Fraites et al., 2009; Laws et al., 2009) or mice (Pruett et al., 2009).

d. Although, atrazine induced a rapid increase in ACTH, prolactin secretion was not affected in the same animals (Laws et al., 2009). This is unusual in that an increase in both ACTH and prolactin are typically observed in response to a number of physiological and psychological stressors.

Please comment on the extent to which the available evidence supports the preliminary conclusion that atrazine and its intermediate metabolites (DIA and DEA) induce changes in the HPA axis as a result of a direct action on the HPA tissue and such changes are not due to a generalized or non-specific stress response. Are there data on other substances that would support this conclusion?

4:15 P.M. Charge to Panel – Question 1 (continued)

Question 1.4 – Based on a review of *in vitro* studies evaluating the effects of atrazine on estrogen production, the Agency has preliminarily concluded that atrazine does not have a direct effect on the catalytic activity of aromatase. However, with continued exposure (> 24 hrs) atrazine can cause increased estrone and estradiol production in the H295R and JEG-3 cell lines (Sanderson et al., 2000; Laville 2006; Higley et al., 2010). These changes in estrogen production have been associated with increased cAMP and CYP19 mRNA (Sanderson et al., 2000, 2001) that are part of a complex mode of action through which atrazine up-regulates the gene expression of aromatase and possibly other enzymes within the steroidogenic pathway (Section 3.3.2.3 & Figure 3, Arrow 7of the draft issue paper). The hypothesis that atrazine alters multiple steroids is supported by increased testosterone concentrations in H295R cells following exposure to atrazine (Higley et al., 2010), and *in vitro* studies demonstrating that atrazine affects a number of signal transduction pathways and/or transcription factors in a variety of cell lines (Suzawa and Ingraham, 2008; Albanito et al., 2008). Up- regulation of the expression of a group of major steroidogenic genes has been observed in JEG-3 cells (Suzawa and Ingraham, 2008*). In* vivo, there is little evidence that atrazine alters aromatase *per se* (Modic, 2004) and

the evidence associating atrazine exposures to increases in serum estrogens appears to be related to a general increase in gonadal and adrenal progesterone and androstanedione (Modic 2004; Laws et al., 2009).

Please comment on the extent that the available data do or do not support the Agency's preliminary conclusion that atrazine has a general stimulatory effect on steroidogenesis, as opposed to a direct effect on aromatase.

5:00 P.M. Adjourn

Day 3
Wednesday, April 28, 2010

8:30 A.M. **Opening of Meeting and Administrative Procedures** – Joseph Bailey, Designated Federal Official, Office of Science Coordination and Policy, EPA

8:35 A.M. **Introduction and Identification of Panel Members** – Kenneth Portier, Ph.D., FIFRA Scientific Advisory Panel Session Chair

8:45 A.M. **Charge to Panel – Question 1 (continued)**

Question 1.5 – A series of studies evaluating the effect of either peripubertal or gestational exposure of atrazine in the male rat indicate that atrazine decreases testosterone concentrations and may lower androgen dependent tissue weights but that it has little effect on basal LH secretion in the male. This response generally occurs at doses of 50 mg/kg and above. In contrast, atrazine *in vitro* has been found to consistently increase steroid hormone production. Thus, there is an apparent discrepancy between the *in vivo* and *in vitro* data. However, our understanding of the interplay between the adrenal and the gonadal axes may provide an explanation. At the doses used in the *in vivo* studies, atrazine induces an activation of the adrenal axis (increased ACTH and corticosterone) and the increase in corticosterone does not habituate (i.e., it does not lessen with repeated dosing). There are a number of studies in the literature demonstrating that prolonged corticosterone stimulation will impair testosterone production by a direct action on steroidogenesis in the Leydig cells. Therefore, the *in vivo* effects on testosterone synthesis are likely the result of an adrenal hormone-mediated down regulation of HPG axis (through corticotropin releasing hormone [CRH] modulation of GnRH pulsatility) or directly on the testis (through a glucocorticoid receptor mediated change in steroidogenesis).

Please comment on the degree to which the proposed mode of action (Figure 3 of the draft issue paper) provides a biologically plausible explanation for the decrease in serum and testicular testosterone identified in the *in vivo* studies. Please comment on the extent that the available data do or do not support this hypothesis.

10:00 A.M. Break

10:15 A.M. Charge to Panel – Question 1(continued)

Question 1.6 - Based on a review of the neurotoxicity studies (Section 3.4 of the draft issue paper), the Agency has preliminarily concluded that several recent studies provided further support for the concern that dopaminergic neurotransmission may be affected by atrazine, a concern that was raised by studies prior to 2003. However, the Agency has also concluded that several aspects of these studies of the dopaminergic neuronal pathways, in particular the changes noted in stereological evaluations and the observed changes in behavior, should be considered as preliminary findings. This conclusion is based on the notable limitations identified in the data including: (1) lack of clear dose-response relationships, (2) lack of inclusion of suitable positive controls to confirm the competency and reliability of the procedures

utilized in examining dopaminergic systems in the brain, (3) limited data to corroborate stereological findings, (4) limited or no supporting histological and behavioral assessments, and (5) no consideration of the potential role of the HPA axis (e.g. alterations in corticosterone). EPA has further determined that two non-dopaminergic neurotoxicity studies--one on brain somatostatinergic systems and the other on neurobehavior in mice -- also have significant limitations (*e.g.*, lack of details on source and purity of atrazine, and age and body weights of the mice; poor quality of amino cupric silver staining photomicrographs; limited presentation of reverse-transcriptase polymerase chain results; lack of data to corroborate conclusion of neuronal degeneration; reference citations did not support statements made in the text; no explanation of biological plausibility of alterations in somatostatinergic receptor subtypes; inappropriate statistical evaluation of data (litter vs. pup); discrepancies in number of pups examined for behavioral endpoints, and lack of objective and validated behavioral tests). Please comment on these preliminary conclusions regarding the neurotoxicity findings.

The Agency has preliminarily concluded that the available studies indicate that the neurotoxicity endpoints examined are not more sensitive than those evaluated for neuroendocrine function following atrazine exposure (e.g., attenuation of LH surge and estrous cycle disruptions in female Sprague-Dawley rats which form the basis for the current chronic RfD). In addition, the Agency has concluded that there is no association between atrazine exposure and development of Parkinson's Disease on the basis of non-specificity of effects on brain dopaminergic systems, lack of histological and behavioral features characteristic of Parkinson's Disease, and results of epidemiological studies. Please comment on this preliminary conclusion.

11:00 A.M. Charge to Panel – Question 1(continued)

Question 1.7 – Based on a review of two studies of the potential effects of atrazine on the prostate (Section 3.3.2.2 of the draft issue paper), the Agency has concluded that the results of the Rayner et al. (2007) study of pregnant rats treated during gestation support previous observations (Stoker et al., 1999) that atrazine treatment to the rat dam either perinatally or early postnatally can increase prostate weights (due to an increase in inflammatory infiltrate) in the male offspring. This effect on the offspring was shown to be due to a suppression of prolactin in the atrazine-exposed dams during lactation, and is consistent with the mode of action of atrazine on neuroendocrine function. The Agency also preliminarily concluded that further research is needed to provide more convincing evidence that atrazine-mediated suppression of prostate cancer in the probasin/SV40T antigen transgenic rat (androgen-dependent prostate cancer rodent model) may possibly be due to caloric restriction rather than endocrine-related as postulated by Kandori et al. (2005). Please comment on these preliminary conclusions regarding the recent prostate findings.

Please comment on the extent to which the available data support the proposed mode of action for prostatitis and the appropriateness of the rodent model in the context of human health.

12:00 noon Lunch
1:15 P.M. Charge to Panel – Question 1 (continued)

Question 1.8 – Based on data obtained in studies to assess the effects of atrazine on the immune system following developmental and adult exposure, as well as *in vitro* mechanistic studies, EPA concluded that atrazine has the potential to affect the immune system (Section 3.5 of the draft issue paper). However, the Agency has also concluded that underlying mechanisms of atrazine-mediated immunotoxicity and its relevance to potential adverse health effects in humans are still not thoroughly understood. It is well established that products of the endocrine system modulate immune function. However, adult rodent studies have not determined whether immune system effects are caused by direct effects of atrazine and/or its metabolites or whether they are the result of modulated endocrine hormone production, which in turn affects immune cells. Two published studies indicate that exposure to atrazine during immune system development may result in altered immune function in offspring, although no conclusions were drawn on the potential adversity of the effects, because immunosuppression was observed in one study and immunoenhancement was observed in the other. Please comment on potential explanations for the disparate findings reported by Rooney *et al.* and Rowe *et al.* Many

immunotoxicologists consider immunomodulation, *i.e.,* suppression or enhancement of immune function, as a potentially adverse alteration of homeostasis, because both have been associated with disease states. Thus, while the results Rooney et al. and Rowe et al. may appear to be contradictory, together the data indicate unintended immunomodulation at approximately the same dose in two species. Please comment on characterizing the effects of gestational atrazine exposure as immunomodulation, when describing the immunotoxicological outcome of developmental immunotoxicity studies.

Appendix B of the draft issue paper describes experiments conducted by EPA scientists on the potential developmental immunotoxicity of atrazine. The results of the experiments provide conflicting results. Additional studies did not provide a suitable explanation of the differences. The Agency believes both sets of data are of high quality. However, in the context of hazard assessment, such differences are difficult to interpret. Please comment on the information contained in Appendix B and provide suggestions for interpreting such data as part of the atrazine re-evaluation.

The available data do not indicate that atrazine-induced immunotoxicity is a more sensitive endpoint than the atrazine-induced effects on neuroendocrine function, (e.g., attenuation of LH surge and estrous cycle disruptions which form the basis for the current chronic RfD) in female Sprague-Dawley rats. Please comment on the degree to which these preliminary conclusions are supported by the available data.

2:00 P.M. Charge to Panel – Question 1 (continued)

Question 1.9 – After the April 2010 SAP, the Agency will evaluate the weight of evidence (WOE) for atrazine by integrating the experimental toxicology data with the epidemiological studies. As part of this work, the Agency will consider the available data on hormonal changes and functional outcomes that may be used as endpoints for deriving PoDs across different durations of exposure and for different populations (including potentially sensitive ones). Important studies evaluating the dose-response relationships for hormones involved in the HPA axis are still on-going. These studies are expected to provide high quality data that can be used to characterize the entire dose response curve. With the caveat that these data are not available now, given the current understanding of atrazine's mode of action, please comment on what would be appropriate endpoints to consider for use in deriving points of departure in a possible future risk assessment.

Given that the duration of the toxicological concern is a key factor in evaluating the frequency of drinking water monitoring, please include in your response a consideration of the magnitude and duration of changes in key events in the toxicity pathway that are sufficient perturb normal function and comprise human health. In defining the exposure window of interest, please comment on the toxicokinetic/dynamic considerations with respect to atrazine's effects on the HPA/HPG axis.

3:00 P.M. Break
3:15 P.M. Charge to Panel – Question 2

Approaches to Evaluating Water Sampling Strategies & Frequency of Monitoring

In conjunction with the toxicological review presented in the issue paper, the Agency has also discussed methods for re-evaluating the sampling frequency that is necessary for determining, with confidence, concentrations of the pesticide in water that sources drinking water. These have included different methods for estimating pesticide concentrations between known sampling events and examining the performance of different sampling strategies for averaging periods of different durations. The Agency seeks feedback from the Panel with regard to how the uncertainty and variability in both the monitoring data and in the toxicity data *(i.e.,* point of departure) can be integrated to characterize and to interpret the potential significance of atrazine concentrations in drinking water.

Question 2.1 – Given the nature of the temporal patterns of pesticide occurrence in surface waters described in Section 5.2, including serial correlations from day to day, periodicity in elevated

concentrations within seasons and from year to year, below quantitation data, and uncertainty in the shape of the pesticide distributions in surface waters--what statistical approaches should the Agency consider in determining confidence bounds on exposure estimates from monitoring data? Please comment on how the approach may vary depending on the duration of concern.

4:00 P.M. Charge to Panel – Question 2 (continued)

Question 2.2 – The first two simulation methods presented in Section 5.5 are applicable to the specific data sets they describe, although some generalities regarding shape patterns appear to exist. Given this information, please comment on the strengths and weaknesses of the approaches and on the practical merits of pursuing them or some other numerical approach with a larger set of higher concentration systems. Please comment on how the methods for determining confidence bounds might apply given these considerations.

5:00 P.M. Adjourn

Day 4
Thursday, April 29, 2010

8:30 A.M. **Opening of Meeting and Administrative Procedures** – Joseph Bailey, Designated Federal Official, Office of Science Coordination and Policy, EPA

8:35 A.M. **Introduction and Identification of Panel Members** – Kenneth Portier, Ph.D., FIFRA Scientific Advisory Panel Session Chair

8:45 A.M. Charge to Panel – Question 2 (continued)

Question 2.3 – As described in Section 5.4.2, the Agency is considering the use of a confidence interval or prediction interval approach to characterize the uncertainty of exposure estimates derived from monitoring data of varying sampling frequencies. Please comment on the strengths and weaknesses of either placing confidence bounds on the rolling average estimates and comparing the upper limit from monitoring against the level of concern (LOC) or, conversely, placing confidence bounds on the LOC.

9:45 A.M. Charge to Panel – Question 2 (continued)

Question 2.4 – Please comment on the relative merits of the various modeling approaches the Agency described in Section 5.4.1 and 5.6 for interpolating pesticide concentrations between sampling points and, in particular, on the strengths and weaknesses of these methods as the frequency of samples decreases. Considering the health endpoint(s) being considered for atrazine, particularly data for the HPA axis, and the exposure time frame needed to induce the health effect(s) which is shorter than that used in the 2003 risk assessment, please comment on the advantages and disadvantages of each model for evaluating the likely occurrence and exposure via drinking water of short, moderate, and long duration concentrations. Please comment on the Agency's proposed approach for evaluating these methods, as described in Section 5.7.1. To what extent should the Agency consider other factors, such as the shape of the chemograph (Section 5.5.3), weather patterns, stream flow, and/or pesticide use patterns in evaluating the modeling approaches?

10:30 A.M. Break
10: 45 A.M. Charge to Panel – Discussion continued as needed
12:00 noon Lunch

1:15 P.M. **Charge to Panel – Discussion continued as needed**
3:00 P.M. **Break**
3:15 P.M. **Charge to Panel – Discussion continued as needed**
5:00 P.M. **Adjourn**

Please be advised that agenda times are approximate; when the discussion for one topic is completed, discussions for the next topic will begin. For further information, please contact the Designated Federal Official for this meeting, Joseph Bailey, via telephone: (202) 564-2045; fax: (202) 564-8382; or email: bailey.joseph@epa.gov