Report of a Peer Workshop on Toxicological Assessment and Development of Reference Doses for Acetanilide Degradates

May 11-12, 2009

Peer Consultation Organized by
Toxicology Excellence for Risk Assessment

July 21, 2009

Northern Kentucky University, METS Center

Cincinnati, Ohio

EXECUTIVE SUMMARY

An independent peer expert workshop was convened on May 11 and 12, 2009, to review pertinent toxicology data for the derivation of Reference Doses (RfDs) for four acetanilide (alachlor and acetochlor) degradates: 1) alachlor tertiary-ethanesulfonic acid (ESA), 2) alachlor tertiary-oxanilic acid (OXA), 3) acetochlor ESA, and 4) acetochlor OXA. The workshop was convened by Toxicology Excellence for Risk Assessment (*TERA*), under the auspices of the Alliance for Risk Assessment (*ARA*). *TERA* was contracted by Monsanto and Dow AgroSciences to prepare a data package that summarizes the toxicity and toxicokinetic data for the parent compounds as well as the acetanilide degradates for the workshop, to independently organize and conduct the peer workshop, and to identify and select an independent panel. The data package was developed to facilitate an independent analysis by the expert panel for development of non-cancer oral risk values (i.e., RfDs) for the acetanilide degradates. The panel was comprised of scientists with expertise in toxicology studies and development of RfDs or similar health-based guidance values, particularly for use in setting water standards. The members reviewed the data package prior to and during the workshop. The meeting was open to the public and was webcast on the Internet.

The panel suggested additional data relevant to the development of the RfDs for the degradates that was incorporated into the panel deliberations. The panel discussed whether sufficient data exist for each of the acetanilide degradates for the development of the RfDs. Panel members unanimously agreed that all four degradates have more than the U.S. EPA minimum database requirement for developing an RfD, but also noted several key deficiencies in the overall database – such as lack of data for a second species, absence of functional reproductive studies and developmental toxicity studies in a second species, and the absence of chronic studies. Panel members unanimously concluded that these deficiencies preclude development of a high confidence RfD for any of the degradates, but data are sufficient for lower confidence RfD, with use of an uncertainty factor (UF) to cover data gaps. In spite of the complete databases for the parent compounds, the panel unanimously suggested that use of the data for the degradates would be preferred due to the notable differences in toxicity and toxicokinetic profiles between the degradates and their parent molecules.

The panel discussed the potential critical effects and potential point of departure estimates for the acetanilide degradates. Panel members considered an array of endpoints including body weight (BW) changes (and related body weight metrics), thyroid effects, reproductive and developmental toxicity, effects on hematology/clinical chemistry parameters, etc., to identify potential critical effects as well as effective doses associated with these effects. Panel members agreed that BW decreases were a treatment-related adverse effect in some studies for the degradates. The panel also agreed that there were no consistent treatment-related adverse effects on the thyroid for the degradates. After a thorough evaluation of the hematological findings observed in the drinking water study at the meeting and post-meeting, the panel concluded that

the changes were marginal and not of clinical significance and may have been related to changes in drinking water intake. No treatment-related adverse effects on reproductive or developmental endpoints that would be suitable for RfD development were identified for the degradates, although the absence of developmental toxicity studies for two of the degradates and the possibility for reproductive effects following longer-term dosing (based on the finding of testicular effects in dogs for acetochlor) were considered in the selection of UF values.

The panel discussed the uncertainty factors (UF) to apply for the derivation of the RfD. Panel members unanimously agreed to use the same UF for all four degradates because the chemical structures are similar, all have similar toxicity profiles. The panel judged that a composite UF of 1000 (10_H x 10_A x $10_{S\ \&\ D}$) for each degradate was reasonable, while noting that an argument could be made for an UF of 3000 (10_H x 10_A x $30_{S\ \&\ D}$), where the extra 3-fold represented combined uncertainties in the duration extrapolation and database insufficiency areas.

The panel recommended an RfD for each of the degradates that was derived as follows.

- For acetochlor ESA, the panel unanimously identified a NOAEL of 3000 ppm (225.4 and 259.1 mg/kg-day in males and females, respectively) and a LOAEL of 12,000 ppm (919.4 and 1073.2 mg/kg-day in males and females, respectively) in the 90 day feeding study, based on decreased body weight gain, decreased food consumption, and decreased food utilization (Lees, 2000). Applying the composite UF of 1000 yields an RfD of 2 E-1 mg/kg-day.

- For acetochlor OXA, the panel unanimously identified a NOAEL of 3000 ppm (230.2 and 268.0 mg/kg-day in males and females, respectively) and a LOAEL of 12,000 ppm (955.2 and 1082.7 mg/kg-day in males and females, respectively) observed in a 90 day feeding study based on decreased body weight gain and decrease food utilization (Williams, 2000). Applying the composite UF of 1000 yields an RfD of 2 E-1 mg/kg-day.

- For alachlor ESA, neither the 91-day drinking water study (Siglin, 1993; Heydens et al., 1996) nor the 90 day-feeding study (Kirkpatrick, 2002) identified any effects that the panel members considered to be adverse. Panel members unanimously selected the high dose in the dietary study of 12,000 ppm (788 and 926 mg/kg-day in males and females, respectively) to serve as the appropriate NOAEL, since it is lower than that seen in the drinking water study. Application of a UF of 1000 yields an RfD of 8 E-1 mg/kg-day for this degradate.

- For alachlor OXA panel members did not consider the effects observed in the 90-day study (Lemen et al., 2000) to be adverse. The panel unanimously selected the high dose in this study of 13,000 ppm (834.6 and 1008.3 mg/kg-day in males and females,

respectively) to serve as the appropriate NOAEL. Application of a UF of 1000 yielded an RfD of 8 E-1 mg/kg-day.

The panel members unanimously agreed that low to medium confidence exists in the RfD for each degradate and that additional studies that might reduce the overall uncertainty factor would be a bioassay in a second mammalian species and comparative toxicokinetics information in humans. Panel members also concluded that data are inadequate to identify the mode of action for the observed BW changes or clinical signs, except perhaps for the proposed effect of irritation on the gastric effects observed in the 28-day study for alachlor OXA. In the absence of such data on mode of action, a cumulative risk assessment approach would not be supported for the four degradates.

BACKGROUND

Toxicology Excellence for Risk Assessment (*TERA)* convened an independent peer expert workshop under the auspices of the Alliance for Risk Assessment (*ARA*). *TERA* is an independent non-profit organization with a mission to protect public health through the best use of toxicity and exposure information in the development of risk assessments. See www.tera.org/peer for information about the *TERA* Peer Review program and for downloadable reports from this meeting. The *ARA* is a collaborative initiative of non-profit organizations dedicated to protecting public health by improving the process and efficiency of risk assessment, and to increasing the capacity for developing risk values to meet growing demand. See http://www.allianceforrisk.org/ for more information about the *ARA*.

The subject of this independent peer expert workshop was to review pertinent toxicology data for the derivation of Reference Doses (RfDs) for the following alachlor and acetochlor degradates: 1) alachlor tertiary-ethanesulfonic acid (ESA), 2) alachlor tertiary-oxanilic acid (OXA), 3) acetochlor ESA, and 4) acetochlor OXA as addressed in the charge questions to the panel. The data package (http://www.tera.org/ART/Degradates/index.html) was prepared by *TERA* staff as a resource to facilitate the deliberations of the independent expert panel. *TERA* was not charged with providing recommendations or conclusions for review or acceptance by the expert panel. Monsanto and Dow AgroSciences selected and contracted with *TERA* to independently organize and conduct this peer workshop. *TERA* is being paid for labor and the direct expenses related to this independent peer expert workshop under a contract with Monsanto.

The independent panel was comprised of scientists with expertise in the key disciplines necessary to evaluate the data and address the questions described in the Panel Charge. The panel members of the peer workshop are all experienced in the review of toxicology studies, and development of Reference Doses (RfDs) or similar health-based guidance values, particularly for use in setting water standards. The Panel included the following members.

John Christopher, Ph.D., DABT
Dept. of Toxic Substances Control, California Environmental Protection Agency

Michael Dourson, Ph.D., DABT, ATS
Toxicology Excellence for Risk Assessment

Lebelle Hicks, Ph.D., DABT
Maine Board of Pesticides, Department of Agriculture

Santhini Ramasamy, Ph.D., DABT
Office of Water, U.S. Environmental Protection Agency

Steve Roberts, Ph.D., ATS
Center for Environmental and Human Toxicology, University of Florida

TERA independently identified candidates for the panel and was solely responsible for the selection of the panel members. *TERA* offered to cover travel expenses and offered an honorarium to partially compensate panel members for their time to review the materials and participate in the meeting. The travel expenses and honorarium offers were declined by Dr. Hicks while the other panel members accepted neither, one or both offers. Each panel member disclosed information regarding potential conflicts of interest and biases related to the subject of the peer work shop. Panel members noted that the opinions given represented their own scientific judgments and should not be construed to represent the opinions of their employers. Short biographical sketches and disclosure statements for panel members were provided. No changes to these disclosure statements were made during the meeting.

DISCUSSION OF CHARGE QUESTIONS

Charge Question 1: Are there additional relevant data that should be considered?

Several panel members suggested additional information for consideration. One panelist suggested that data on environmental fate and transport could be more fully explored to assess the degree of exposure, which would impact the consideration as to whether there is a need for RfDs for the degradates or only for the parent chemicals. This panelist noted that data for acetochlor (Baran et al., 2004) suggest that the degradates are more persistent and may have a higher concentration in soil than parent chemicals. Thus, using a single RfD for the parents would overestimate the toxicity if degradates are less toxic, and an RfD for at least one degradate should be developed if possible. A second panelist indicated that additional data in several areas for the parent and other related chemicals should be considered. First, review of the toxicity of metolachlor, including comparative toxicity of metolachlor and metolachlor ESA (including, in particular, rat and dog subchronic toxicity studies). Second, review of metabolic and soil degradation pathways for the parent chemicals to verify whether or not that the degradates are formed in vivo. Third, comparison of toxicity for triazine herbicides (chlorine-containing) versus hexazinone (non-chlorine containing) to evaluate the impact of the reactive chlorine atom versus a related molecule that lacks the chlorine moiety. During the discussion of several charge questions panel members identified additional useful sources of data or analyses that were not included in the pre-meeting data package, including additional analyses of temporal changes in body weights and food consumption findings, data on the toxicity of metolachlor, data regarding

metabolism profiles of the parent compounds and the degradates, and information on SAR modeling.

Two panel members noted that the opening presentation by *TERA* as well as the pre-meeting data package did not address genotoxicity or carcinogenicity. The Chair clarified that this reflects the focus of the review for development of an RfD, rather than a cancer assessment, and thus such data were not included in the meeting package, but were available if needed by the panel during the meeting.

New data raised during the deliberations were added to the data package (available at: http://www.tera.org/ART/Degradates/index.html) and discussions of such data are reflected in the summary of the remaining charge questions.

Charge Question 2: Are there sufficient data for each of the acetanilide degradates?

A panel member opened the discussion noting that according to U.S. EPA methods a single sub-chronic study is the minimum database for developing an RfD and that all four degradates have more than this minimum data base. Panel members noted several key deficiencies in the overall database – such as lack of data for a second species, absence of functional reproductive studies and developmental toxicity studies in a second species, and also noted the absence of chronic studies. Several panelists noted a concern that due to the limitation in the data base one might encounter a situation where a critical effect level from the available subchronic studies adjusted by a series of uncertainty factors might yield an RfD that is lower than for the respective parent chemicals. Panel members commented that this would not be appropriate because several lines of evidence suggest that the parent chemicals are more toxic. For example, one panel member noted that the EPA Office of Pesticide Programs (U.S. EPA, 2006, 2007) assessment concluded that the degradates are less potent than parent chemicals. Other panel members agreed adding that this would be expected based on the absence of the reactive chlorine atom in the structure of the degradates and the lower absorption from the GI tract; specifically, the relative toxicity of the triazines with reactive chlorine are greater. Overall, the panel concluded that the data are inadequate for high confidence RfD for any of the degradates, but are sufficient for lower confidence RfD, with use of an uncertainty factor (UF) to cover data gaps.

The panel also discussed in detail the comparative toxicokinetics and toxicity findings and how such information would inform conclusions regarding each endpoint both in terms of hazard characterization, as well as decisions related to point of departure and selection of uncertainty factors. Since data on systemic targets were available for all four degradates from the subchronic studies in rats, much of the discussion centered on the consideration of parent data on database insufficiencies related to reproductive and developmental effects and the absence of systemic toxicity studies for a second species.

Panel members identified issues related to comparative toxicokinetics of the parents versus the degradates of particular importance in evaluating these considerations. One panel member commented that a key point would be whether the degradates are metabolized to reactive quinoneimine like the parent chemicals. The sponsor confirmed that the degradates do not undergo this reaction, and the panel discussed that this might be because the degradates would not have the same affinity for the monooxygenase (CYP) that catalyzes the p-hydroxylation step. A panelist added that this conclusion regarding differences in metabolism is supported by the

observations from elimination studies, since degradates are largely excreted unchanged compared to the parent chemicals which are extensively metabolized. This panel member also noted that there are large differences in absorption between the parent molecules and their degradates that would affect relative toxicity. The panel also explored the relationship between the in vivo metabolites of the parent chemicals and the structures of the degradates. Panel members reviewed metabolism pathway data provided at the meeting including data for the related pesticide, metolachlor (these additional references were added to the meeting package and are available on the report website). These additional data and information provided by the sponsors indicated that the metabolism of the parent chemicals in vivo versus degradation in soil is very different and that the degradates are not formed in vivo. Based on the conclusion that the degradates are not the same as in vivo metabolites, a panelist asked if they could be considered as structural or functional analogs. The sponsors indicated that there are significant differences in polarity between the in vivo metabolites and the degradates. On the other hand, the sponsor did suggest that the parent molecules are similar to each other (acetochlor and alachlor). Moreover, the toxicity of their degradates also appeared comparable to each other. The conclusion that the toxicity of parent chemicals (including any toxicity caused by in vivo metabolites) is greater than the toxicity of the degradates from direct exposure was supported by the comparative toxicity findings for acetochlor and alachlor versus their degradates as well as for metolachlor and its degradates. Moreover, this conclusion was consistent with conclusions in the recent EPA assessments.

Charge Questions 3 and 4: What are the potential critical effects and potential point of departure estimates for the acetanilide degradates?

The panel evaluated each potential key study as well as the array of endpoints examined to identify the potential critical effects as well as effective doses associated with these effects. For endpoints that were judged to be adverse and treatment related the panel also reviewed benchmark dose modeling results provided in the pre-meeting data package if the data were amenable to such modeling. The panel deliberations are summarized here by toxicological effect.

Body Weight

Body weight (BW) changes were a common finding among the studies for the degradates. The panel reviewed the dose-response data for body weight and when available adjusted body weight or body weight gain as well as correlated findings related to food consumption and food utilization. The panel agreed that BW decreases were a treatment-related adverse effect for some studies and conclusions regarding each study are noted below. Several general comments were made by panelists regarding the evaluation of such data. In general the panel considered a dose-related decrease in BW reaching 10% as an adverse effect. In some cases changes that reached this level were observed but were not reported as statistically significant. However, a panelist noted that the data package did not specify the nature of the statistical tests and remarked that a trend test would likely have identified a significant effect in some of the cases that were not marked as statistically changed. It was also noted that evaluating the trends in body weight gain over the course of the subchronic study durations is important in making this determination and where curves showing such data were not available in the pre-meeting data package the sponsors were asked to develop such charts from the actual study data for evaluation by the panel.

Alternative metrics for body weight effects were also considered. The panel members asked for clarification of the interpretation of "adjusted" body weight as provided in the studies for some of the degradates. The sponsor clarified that this adjustment reflects a covariate adjustment based on initial starting body weight of the animals, and is not a measure of body weight gain. A panelist noted that if this is the case then adjusted body weight would be a better basis for the analysis. Panel members also considered the data on food consumption and food utilization in the dietary studies in interpreting the body weight findings. Panel members noted that in several studies food consumption was also decreased. This might due to effects on palatability or food spillage. A panelist noted that depending on how the diet was presented food may be lost through the cage, and for this reason food consumption data as highly variable, does not necessarily reflect a toxic effect, and may not be reliable. This panelist asked if there were any data on food spillage. The sponsors noted that no quantification of food spillage was available and would need to verify if any written comments were included in the reports regarding food spillage. Panel members indicated that confirmation of food wastage would improve interpretation of the food utilization and food consumption data.

Based on these considerations, the following conclusions were reached by all the panel members regarding NOAELs and LOAELs for BW for each degradate 90-day study:

- Acetochlor ESA Dietary study (Lees, 2000). The LOAEL was 12,000 ppm (919.4 and 1073.2 mg/kg-day in males and females, respectively) for body weight effects based on a decrease in body weight of 10% or greater in males at 14 weeks, and significantly decreased food utilization in males and females. It was noted that at the mid-dose of 3000 ppm (225.4 and 259.1 mg/kg-day in males and females, respectively) the 4% change in adjusted body weight was statistically significant, but the degree of change was not considered biologically significant. Thus, the mid-dose of 3000 ppm was considered a NOAEL by all panel members.
- Acetochlor OXA Dietary study (Williams, 2000). The LOAEL was 12,000 ppm (1082.7 mg/kg-day) for body weight effects in males. The panel noted that the adjusted body weight was decreased at the mid- and high doses and food utilization was affected at the high dose in both sexes. However, the changes observed were less than 10% at mid- and high dose. One panelist pointed out that the females have a greater response than males, with a clear effect on reduced food utilization and adjusted body weight. Also examination of the body weight curves shows an effect that is dose related in females at high dose. The low- and mid-dose body weights were greater than controls, while at the high dose body weight was decreased versus controls. This observation confirms that the mid-dose is a NOAEL. Another panel member agreed, but felt the severity of high dose effects would be considered marginal. All panel members felt that the mid-dose of 3000 ppm (230.2 and 268.0 mg/kg-day in males and females, respectively) is a NOAEL.

- Alachlor ESA Drinking Water study (Siglin, 1993; Heydens et al., 1996). The panel noted that there was a 5% decrease in body weight at week 13 in the high-dose of 10,000 ppm (896 and 1108 mg/kg-day for male and females, respectively) and discussed whether such a change should be considered adverse in light of potential effects secondary to water intake. The panel reviewed the pattern of body weight change over the course of the study along with the pattern of water intake. One panelist concluded that the observations during the initial periods of the study are consistent with an effect on water

palatability. This panelist added that for the dietary study no treatment related adverse effect was observed at the high dose and the observed decrease in body weight in this study is not biologically significant compared to the use of a 10% change as a BW effect benchmark. It was also noted that taste and odor have not been considered biologically adverse, rather they are organoleptic effects. Another panel member suggested that the high dose should be considered a LOAEL based on the combined effects of clinical signs (including few and/or small feces, urine staining, dehydration, emaciation, rough coat and dark material around the eyes) and decreased body weight gain in males and females. The panelist noted that this was also the conclusion of EPA's 2007 assessment. All the panel members agreed that in males the high dose was a NOAEL for body weight effects and all but one panelist considered the high dose a NOAEL for body weight effects in females as well. One panelist considered the high dose a LOAEL, pending further evaluation of the effects of palatability (discussed later).

- Alachlor ESA Dietary study (Kirkpatrick, 2002). All panel members agreed that the high dose of 12,000 ppm (788 and 926 mg/kg-day in males and females, respectively) was a NOAEL based on minimal changes in body weight.
- Alachlor OXA Dietary study (Lemen, 2000). All panel members agreed that the high dose of 13,000 ppm (834.6 and 1008.3 mg/kg-day in males and females, respectively) was a NOAEL based on the based on minimal changes in body weight.

Thyroid Effects

The panel discussed the interpretation of thyroid-related effects for the acetanilide degradates, despite reservations voiced by some panel members that species differences between rats and humans in thyroid hormone physiology would cloud the interpretation of any observed effects. The panel evaluated potential patterns in the data for the acetochlor degradates noting that there were some statistically significant changes in hormones and enzyme activities, but the changes were not consistent with the expected mode of action for a thyroid stimulant. Also in comparing control groups between comparable studies the degree of changes are within the control variability. Moreover, there were no consistent changes in thyroid weights in the corresponding 90-day studies. Based on these inconsistencies the panel members agreed that there were no treatment related adverse effects on the thyroid for the 28-day or 90-day studies for acetochlor degradates. For alachlor degradates, the panel concluded that there were no effects on thyroid weights that were clearly treatment related (due to lack of a consistent dose-response or effects only on relative weight and not absolute weights). Since no hormone evaluations were available for the alachlor degradates, the panel also reviewed the pathology reports for the 90-day studies to supplement the organ weight data. The panel concluded that these studies did not identify any treatment related thyroid histopathology. Overall the panel agreed that there were no consistent treatment-related adverse effects on the thyroid for the acetochlor or alachlor degradates and thus no point of departure estimates were developed.

Reproductive and Developmental Toxicity

The panel members reviewed the available studies related to reproductive and developmental endpoints. The panel discussed the observation of rales in the developmental toxicity studies for the degradates and agreed that the rales found with alachlor ESA would be consistent with a

short-lasting irritation effect from gavage dosing and not appropriate as the basis for a chronic RfD.

The panel discussed the findings related to developmental endpoints. Several panelists concluded that there were no developmental effects at the highest doses tested for acetochlor OXA (1000 mg/kg-day) and alachlor ESA (900 mg/kg-day). Thus the highest doses tested in these two studies would be a NOAEL for developmental toxicity. However, panelists felt that whether these two studies are adequate to assess developmental toxicity for all four degradates and to address the absence of test data for a second species needed additional discussion. Several panelists noted that data are available for the parent compounds. Panelists noted that the parent compounds show developmental effects only at or above maternally toxic doses, that data are available in rats and rabbits, and that the latest EPA assessment concluded that a FQPA factor of 1 was considered sufficient. A panelist added that based on the data for the parent chemicals, which are likely more toxic, requiring additional developmental toxicity studies would be an unnecessary burden just to avoid a database uncertainty factor. Other panel members commented that these data would suggest that there is limited concern for developmental toxicity for the degradates, but several were concerned that the data were unclear as to how far one could take this conclusion for the untested degradates. The ability to rely on the data for parent chemicals was discussed at length and this discussion is summarized in the context of Charge question 2 above since the conclusions affected the panels' decision regarding other endpoints beyond developmental toxicity. The impact of this consideration on identifying whether a data gap exists for each degradates was also discussed further in the context of Charge Question 5.

The panel also discussed the potential for concerns regarding reproductive effects. One panelist opened the discussion by noting that there was no evidence for effects on reproductive organs in the 5 different 90-day studies for degradates. The observation of small changes in ovary and testes weights led the panel to review in detail the histopathology reports for alachlor ESA (Siglin, 1993; Heydens et al., 1996), particularly in light of the reported testicular effect observed in dogs for acetochlor (Broadmeadow, 1988). The panel also received an independent review of the histopathology report from a reproductive toxicologist (see in-meeting record evaluation of the study data by Dr. Raymond York). Based on this more detailed review the panel agreed that there was no evidence for hypospermia in the testes and epididymis in the high dose male rats or uterus dilation in high dose female rats exposed to alachlor ESA. The small differences were not sufficient to be considered treatment-related or adverse effects. The panel also considered the question as to whether these subchronic studies addressed database uncertainties or whether the reproductive studies available for the parent chemicals, or related chemicals such as metolachlor, added to the database for the degradates as noted in the summary of Charge Question 5.

Effects on Hematology/Clinical Chemistry Parameters

The panel members agreed that the hematology and clinical chemistry findings for all the degradates, except for the alachlor ESA drinking water study, did not show a consistent pattern of changes and were generally of small degree and not likely to be toxicologically relevant. The panel reviewed in detail the findings of the alachlor ESA drinking water study (Siglin, 1993; Heydens et al., 1996). The panel concluded that the clinical chemistry changes related to potential liver markers were not of significant concern based on a review of the corresponding

histopathology report, which did not find evidence for treatment related liver toxicity. The panel also considered the observations related to hematology. One panelist commented that the pattern of hematology changes was characteristic of anemia. All the changes in various parameters were consistent with this diagnosis, although the degree of the correlation was not strong. The panel also evaluated this endpoint in the context of the decrease in drinking water intake due to palatability to ascertain whether the observations are secondary to dehydration. A review of the detailed clinical findings on this specific issue occurred among panel members after the meeting (email correspondences are available on the website). The conclusion of this post-meeting review was that while the panel considered potential point of departure estimates for the observations and concluded that the $BMDL_{1SD}$ of 381 mg/kg-day could be an interpreted as an appropriate point of departure, the hematological changes were marginal and not of clinical significance, due to the magnitude of changes (small and within control ranges of similar degradates studies) and the potential for confounding effects related to decreased water consumption. Thus, the high dose was considered to be a NOAEL.

Other Potential Critical Effects

Several studies identified clinical signs of toxicity as a potential co-critical adverse effect. In the context of the potential RfDs, clinical signs played the most significant impact in the context of alachlor ESA based on findings in the drinking water study (Siglin, 1993; Heydens et al., 1996). In this study clinical signs were observed at the highest dose 20,000 ppm in water (896 mg/kg-day for males and 1108 mg/kg-day for females). This finding was supported by the observation of clinical signs at a feed concentration of 20,000 ppm (2217 mg/kg-day in males and 2378 mg/kg-day in females) in the 28-day study (Siglin, 1993), but not in a 90-day feeding study with dietary concentrations of 12,000 ppm (788 mg/kg-day in males and 926 mg/kg-day in females). The lack of consistency between the drinking water and dietary studies for alachlor ESA was further examined by the panel at the meeting as well as in post meeting deliberations with a specific focus on whether the observed clinical signs could be attributed to dehydration or infection. Review of the study by veterinary ophthalmologists indicated that ocular and periocular findings were not related to test material, but were instead manifestations of abnormalities typically associated with the Fisher 344 rat, as well as viral infection. The panel evaluated the remaining effects for consistency with a dehydration effect after the meeting (email correspondences are available on the website). The conclusion of this post-meeting review was that the panel considered the clinical findings as due to dehydration. Nearly all effects occurred during the first two weeks of the study, when water consumption was markedly lower; as water consumption returned to normal, these clinical signs abated. Thus, the high dose was considered to be a NOAEL.

Gastric hyperplasia and esinophilic inclusion were observed in a 28-day rat study for alachlor OXA at the high dose of 20,000 ppm (1539.32 mg/kg-day for the males and 1595.26 mg/kg-day for the females) (Stout and Thake, 2000). No such effects were observed in the corresponding 90-day studies for alachlor degradates. The sponsor noted that this observation could reflect a dose-related effect, since the 28-day studies tested higher doses than the 90-day studies, and suggested that the effect could be attributed to local irritation. A panelist concurred that this would be consistent with high dose effects with an acidic compound. Other panel members asked whether the effects of alachlor OXA are related to the gastric tumors induced by the parent chemical, alachlor. The sponsored clarified that the location in the stomach as well as the

underlying cellular targets and mode of action for alachlor versus the degradates show that the effects on the stomach for the alachlor and its degradates are not similar. A summary of the mode of action arguments was provided in an Appendix in the pre-meeting data package. The panel agreed that observed changes represent an adverse treatment-related effect consistent with a local toxicity associated with high dietary doses with an acidic chemical, rather than a general systemic effect. Thus, these effects were not considered as an appropriate basis for the RfD.

The panel also considered the possibility of other effects. One such effect was raised by a technical observer comment regarding the ocular developments and cardiac changes in the developmental studies for the parent molecules. With regard to the ocular changes, the commenter noted that the strain of rat used in the testing for the degradates have unpigmented eyes, unlike those tested with the parent chemicals, and as such effects on ocular pigment observed with the parent chemicals would not be observable with the degradates. This commenter also noted concerns about effects observed in dogs, since no studies in dogs had been completed for any of the degradates. The panel members indicated appreciation for these comments and noted that these considerations were some of the issues that would fall into the selection of the database uncertainty factor selection and stated that this is one reason to focus on degradates separately from the parents in the determination of the critical effect.

Charge Question 5: For derivation of the RfD for each acetanilide degradate, what uncertainty factors should be applied?

Two of the panelists gave a brief technical commentary on the U.S. EPA use of a 10,000-fold or 3000-fold composite uncertainty factor (UF), including the EPA method of coalescing UFs when 4 or 5 areas of uncertainty are outstanding (3 areas = 1000; 4 areas = 3000; 5 areas = 10,000). In addition, an observer technical comment was raised regarding the panels' thoughts on the basis of the uncertainty factors for the State of Wisconsin. The panel commented that an additional factor will likely be needed for the presence of data gaps for these degradates, although read-across information would not be precluded. Such considerations were discussed in greater detail as noted below. The panel chair continued the discussion by stating a presumption that the UFs for the degradates for human variability in sensitivity (H) and experimental animal to human variability (A) should both be judged as 10-fold in the absence of data that would allow the estimation of Chemical Specific Adjustment Factors (CSAFs). The panel chair stated a further presumption that a UF of 1-fold for LOAEL to NOAEL (L) should be used since each degradate had at least one adequate study that defined either a NOAEL or BMDL. The panel unanimously agreed with these two presumptions. Thus, UFs for these 3 areas of uncertainty were unanimously judged by the panel to be 10_H, 10_A, and 1_L.

The panel then discussed the UFs for subchronic to chronic duration (S) and database completeness (D) in greater detail, since none of the degradate studies was longer than about 90 days, and none of the individual degradates had a complete database (minimally defined by EPA as: 2 systemic toxicity studies of at least subchronic duration in different species, 2 developmental toxicity studies in different species, and 1 reproductive study). The panelists initially started out at slightly different judgments on these two factors. One panelist judged that the factors should be 10_S and 3_D for a combined factor of 30. Another panelist thought that the reverse was a better judgment that is a 3_S and 10_D for a combined factor of 30. Both panelists offered their reasoning, which was carefully considered by the other panelists. Afterwards, this

latter judgment was initially echoed by two other panelists. The fifth panelist queried if one can take any information from the parent compounds for consideration of these UFs.

During an extended discussion, arguments were made for a combined factor of $30_{S \& D}$ including:

- The lack of a reproductive toxicity study for any degradate;
- The lack of a second species developmental toxicity study for any degradate; and
- The lack of a second species standard subchronic systemic toxicity study for any degradate.

However, arguments were also made for a factor of 3_S and 3_D (or $10_{S \& D}$) including:

- Degradates are poorly absorbed, are metabolized very little, and are not expected to be highly reactive molecules,
- Available studies appeared to have characterized the toxicity reasonably well, since clear evidence of additional target organ effects were not apparent,
- A number of studies have examined distributions of ratios of NOAELs among different durations, and the median is a value of 3; parent ratios for subchronic to chronic toxicity appear to be 2 or less,
- U.S. EPA used an FQPA factor of 1-fold for this class of chemicals indicating a more or less complete database protecting infants and children (EPA considers database issues strongly in its determination of appropriate use of the FQPA---EPA, 2002),[1]
- Toxicity tests for each degradate were done close to the limit dose, and
- Read across toxicity seems likely among these degradates, since the toxicities, when seen, appear to be similar.

A panelist stated that when EPA had 4 full areas of uncertainty, it generally used a factor of 3000 in its determination of an RfD (as mentioned above), and when it had a database with 3 to 4 areas of uncertainty then a factor less than 3000 was often judged to be acceptable. For example, prior EPA RfD/RfC work group decisions (many of which are still on EPA's IRIS) usually judged that a factor of 1000 was appropriate if an extra factor of 3 was for developmental or reproductive toxicity data gaps. All panel members agreed that the outstanding uncertainties reflected between 3 and 4 areas as defined by EPA.

The panelists then focused on whether reproductive toxicity would likely be a significant data gap that would impact the selection of the UF for database insufficiency. As noted earlier, the panel concluded that the examination of reproductive organs in the available subchronic studies

[1] See U.S. EPA. (2002). U.S Environmental Protection Agency. Determination of the appropriate FQPA safety factor(s) in tolerance assessment. Office of Pesticide Programs. Washington, D.C. February, 28.

did not suggest a concern for reproductive endpoints. This conclusion was supported by the examination of organ weight and histopathology findings by a reproductive toxicologist (Dr. York, as mentioned above). The panel also compared effect levels for reproductive toxicity versus the most sensitive systemic effects for the parent chemicals. They noted that in the alachlor reproductive toxicity study in rats the NOAEL was 10-fold higher than the chronic dog NOAEL (which was based on anemia). In the acetochlor reproductive toxicity study in rats, the critical NOAEL was 30-fold higher than the chronic dog NOAEL (which was based on testicular and other effects). Thus, for acetochlor, reproductive effects in rats were not the critical effect. Reproductive effects in dogs were a critical effect for acetochlor and such effects remain a possibility for the degradates (in the absence of specific tests for this endpoint for the degradates). Review of the available data for metolachlor led the panel to conclude that reproductive or developmental toxicity was not the critical effect for this chemical.

A second consideration discussed by the panel regarding the database uncertainty factor was the absence of a longer-duration systemic toxicity study in a second species, since only rat studies were available for the degradates. The panel specifically commented on the need for a 1-year dog study, since some data suggest that the dog is the more sensitive species and effects occur at one year that are not found in the 90 day studies. One panelist noted that U.S. EPA no longer requires a chronic (1-yr) dog study as part of the required data set for pesticides, rather a 13-week study is deemed sufficient. Another panelist added that the value added by conducting such a study was analyzed by the U.S. EPA Science Advisory Panel in 2005. EPA found that one does not gain a lot of information going from 13-week to 1-year for the dog study (minutes from the SAP meeting are available from the EPA website).

The panel compared effect levels across species for the parents to guide the importance of this consideration. Charts showing the comparison of effect levels across species and study duration for acetochlor and alachlor and the impact of the combination of these considerations were presented to the panel. (This analysis is available on the meeting website). For example, based on comparison of NOAELs for acetochlor a factor of 8 would cover the difference between the 90-day rat NOAEL and the chronic dog study NOAEL. The same comparison based on LOAELs resulted in a ratio of 16-fold. For alachlor an evaluation of NOAELs yielded a ratio of 15, while the same comparison using LOAELs was 49-fold. In addition, the panel reviewed similar data on effect levels for various species and study durations for metolachlor and its ESA degradate, for which a subchronic dog study was available. These comparisons led the panel to conclude that the database for three related parent chemicals is complete and ratios for effect levels across durations and species address some uncertainty in the degradates database. In no case of the parent compounds did reproductive or developmental toxicity drive the assessment, and in all three, dog and rat were roughly quantitatively similar, at the same study exposure length. A panelist added that the four degradates are less reactive, less absorbed and less metabolized when compared with the parent compounds. In such a case one would not expect more variability in toxicity among degradates than in their parent compounds.

The chair then raised the issue of whether or not all sensitive individuals would be protected with a choice of a 1000 UF (10_H x 10_A x $10_{S\ \&\ D}$). One panelist stated that we do not have enough information to modify 10_H, and thus the lack of information on off-spring still necessitates this factor. All panelists agreed with this statement. In addition, another panelist stated that an FQPA factor of 1-fold was applied to all three parents for the FQPA evaluation, indicating that

the U.S. EPA conclusion that the toxicity database is complete and no indication of effect in either rats or rabbits to in utero or post-natal exposures, and also no indication of developmental neurotoxicity.

The panelists were comfortable with using the same UF for all four degradates, because the chemical structures are similar, all have similar toxicity, and nothing stands out as unique. The panel was comfortable developing 4 separate RfDs, one for each degradate, since choices of NOAELs were different. The panelists judged that a composite UF of 1000 (10_H x 10_A x $10_{S\ \&\ D}$) for each degradate was reasonable, although argument could be made for a UF of 3000 (10_H x 10_A x $30_{S\ \&\ D}$), where the extra 3-fold represented combined uncertainties in the duration extrapolation and database insufficiency areas, as previously discussed. The panel did not consider an uncertainty factor of less than 1000 to be appropriate.

After a brief additional discussion of the outstanding uncertainties, including lack of a reproductive study and information in a second species, but balancing available information on similar chemicals, panelists thought that confidence in each of these RfDs was low to medium, meaning that as new data for these degradates are developed, the value of these RfDs is more likely to change (usually upward).

Charge Question 6: For each acetanilide degradate what is the appropriate RfD?

Based on the previous discussions of critical effects, appropriate effect levels, and uncertainty factors, the following RfDs were developed:

Acetochlor ESA

The critical effects for acetochlor ESA are decreased body weight gain, decreased food consumption, and decrease food utilization at the high dose of 12,000 ppm (919.4 and 1073.2 mg/kg-day in males and females, respectively) in the 90 day feeding study (Lees, 2000). The No Observed Adverse Effect Level (NOAEL) is 3000 ppm (225.4 and 259.1 mg/kg-day in males and females, respectively). An uncertainty factor of 1000 is applied to this NOAEL. This factor reflects the default value of 10 fold for experimental animal to human extrapolation (10_A), in lieu of chemical-specific information that would allow the development of a Chemical Specific Adjustment Factor (CSAF); the default value of 10 fold for within human variability (10_H), in lieu of chemical-specific information that would allow the development of a CSAF, and a combined value of 10-fold for uncertainties in both the lack of a full database to determine the critical effect and the lack of a chronic study ($10_{S\&D}$) as a basis of the RfD. This latter factor is best judged as 10, although it could be as high as 30, because the available toxicology data for the parent compound suggest only a modest change between subchronic and chronic NOAELs (~2-fold), and the available information suggests that neither developmental nor reproductive toxicity is the critical effect.

- The resulting RfD is 2 E-1 mg/kg-day.
- Low to medium confidence in this RfD exists. Additional studies that might reduce the overall uncertainty factor would be a bioassay in a second mammalian species and comparative toxicokinetics information in humans.

Acetochlor OXA

The critical effects for acetochlor OXA are decreased body weight gain and decrease food utilization at the high dose of 12,000 ppm (955.2 and 1082.7 mg/kg-day in males and females, respectively) in the 90 day feeding study (Williams, 2000). The No Observed Adverse Effect Level (NOAEL) is 3000 ppm (230.2 and 268.0 mg/kg-day in males and females, respectively). An uncertainty factor of 1000 is applied to this NOAEL. This factor reflects the default value of 10 fold for experimental animal to human extrapolation (10_A), in lieu of chemical-specific information that would allow the development of a CSAF; the default value of 10 fold for within human variability (10_H), in lieu of chemical-specific information that would allow the development of a CSAF; and a combined value of 10-fold for uncertainties in both the lack of a full database to determine the critical effect and the lack of a chronic study ($10_{S\&D}$)as a basis of the RfD. This latter factor is best judged as 10, although it could be as high as 30, because the available toxicology data for the parent compound suggest only a modest change between subchronic and chronic NOAELs (~2-fold), and the available information suggests that neither developmental nor reproductive toxicity is the critical effect.

- The resulting RfD is 2 E-1 mg/kg-day.
- Low to medium confidence in this RfD exists. Additional studies that might reduce the overall uncertainty factor would be a bioassay in a second mammalian species and comparative toxicokinetics information in humans.

Alachlor ESA

Although several differences were noted, no statistically significant, or other, effects were judged to be adverse after an extensive review of individual animal data, including clinical signs and chemistries, for alachlor ESA in either a 91-day drinking water study (Siglin, 1993; Heydens et al., 1996) or in a 90 day-feeding study (Kirkpatrick, 2002). [Note well: a statistically significant decrease in body weight gain of less than 10% without a concurrent decrease in food utilization was judged not to be an adverse effect in the drinking water study; clinical signs were judged to be due to taste aversion resulting in dehydration early in the study; and clinical chemistries while statistically significant at the high doses were modest and within control ranges of other studies.] The high dose in the dietary study of 12,000 ppm (788 and 926 mg/kg-day in males and females, respectively) serves as the appropriate NOAEL as the basis of the RfD, since it is lower than that seen in the drinking water study. An uncertainty factor of 1000 is applied to this NOAEL. This factor reflects the default value of 10 fold for experimental animal to human extrapolation (10_A), in lieu of chemical-specific information that would allow the development of a CSAF; the default value of 10 fold for within human variability (10_H), in lieu of chemical-specific information that would allow the development of a CSAF; and a combined value of 10-fold for uncertainties in both the lack of a full database to determine the critical effect and the lack of a chronic study ($10_{S\&D}$) as a basis of the RfD. This latter factor is best judged as 10, although it could be as high as 30, because the available toxicology data for the parent compound suggest only a modest, if any, change between subchronic and chronic NOAELs (~equal), and the available information suggests that neither developmental nor reproductive toxicity is the critical effect.

- The resulting RfD is 8 E-1 mg/kg-day.
- Low to medium confidence in this RfD exists. Additional studies that might reduce the overall uncertainty factor would be a bioassay in a second mammalian species and comparative toxicokinetics information in humans.

Alachlor OXA

Although several differences were noted, no statistically significant, or other, effects were judged to be adverse for alachlor OXA in a 90 day-feeding study (Lemen et al., 2000). The high dose in this latter study of 13,000 ppm (834.6 and 1008.3 mg/kg-day in males and females, respectively) serves as the appropriate NOAEL. An uncertainty factor of 1000 is applied to this NOAEL. This factor reflects the default value of 10 fold for experimental animal to human extrapolation (10_A), in lieu of chemical-specific information that would allow the development of a CSAF; the default value of 10 fold for within human variability (10_H), in lieu of chemical-specific information that would allow the development of a CSAF; and a combined value of 10-fold for uncertainties in both the lack of a full database to determine the critical effect and the lack of a chronic study ($10_{S\&D}$)as a basis of the RfD. This latter factor is best judged as 10, although it could be as high as 30, because the available toxicology data for the parent compound suggest only a modest, if any, change between subchronic and chronic NOAELs (~equal), and the available information suggests that neither developmental nor reproductive toxicity is the critical effect.

- The resulting RfD is 8 E-1 mg/kg-day.
- Low to medium confidence in this RfD exists. Additional studies that might reduce the overall uncertainty factor would be a bioassay in a second mammalian species and comparative toxicokinetics information in humans.

The RfDs for the parent compounds, from the Tolerance Reassessment Eligibility Decision (TRED) Document for acetochlor (U.S. EPA, 2006) and the Reregistration Eligibility Decision (RED) for alachlor (U.S. EPA, 1998), are as follows:

- Acetochlor = 2 E-2 mg/kg/day, based on clinical signs (excessive salivation) and microscopic findings in the liver, testes and kidney in dogs.

- Alachlor = 1 E-2 mg/kg/day, based on hemosiderosis and hemolytic anemia in dogs.

Charge Question 7: Are there sufficient mode of action data to justify a cumulative risk assessment approach?

The panel considered potential commonalities in critical effects and their underlying modes of action. One panelist noted that for two degradates no adverse effects were observed and for the other two the most common finding was an effect on body weight. Another panelist added that it is not clear what the underlying cause of the clinical effects were since weight loss without other target organ findings was the critical effect, so one cannot be sure that the four degradates are causing the same underlying toxicity. Another panel member concluded that all four degradates are weak toxicants, with no clear data to explain the mode of action for the observed

body weight changes or clinical signs. Thus, the data are inadequate to identify the mode of action for any of the effects, except perhaps for the proposed effect of irritation on the gastric effects observed in the 28-day study for alachlor OXA. The panel members all agreed that in the absence of such data on mode of action, a cumulative risk assessment approach would not be supported for the four degradates.

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