Derived Reference Doses (RfDs) for the Environmental Degradates of the Herbicides Alachlor and Acetochlor: Results of an Independent Expert Panel Deliberation

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Abstract

An independent peer expert panel was convened under the auspices of the Alliance for Risk Assessment (*ARA*) to review toxicology data and derive oral Reference Doses (RfDs) for four environmental degradates of the acetanilide herbicides, alachlor and acetochlor. The degradates included in this evaluation were 1) alachlor tertiary-ethanesulfonic acid (ESA), 2) alachlor tertiary-oxanilic acid (OXA), 3) acetochlor ESA, and 4) acetochlor OXA. Each degradate was judged to have sufficient data for developing low to medium confidence RfD, with use of an additional uncertainty factor (UF) to cover data gaps. Body weight decreases were identified as the most sensitive treatment-related adverse effect for RfD development. A composite UF of 1000 (10 for human variability in sensitivity, 10 for interspecies differences in sensitivity, and 10 for subchronic to chronic and database deficiency combined; i.e., 10_A x 10_H x $10_{S\&D}$) for each degradate was considered reasonable, while noting that an argument could be made for an UF of 3000 (10_A x 10_H x $30_{S\&D}$). Based on the available data, an oral RfD of 0.2 mg/kg-day is recommended for both acetochlor ESA and acetochlor OXA and an oral RfD of 0.8 mg/kg-day is recommended for both alachlor ESA and alachlor OXA.

Keywords: acetanilide degradates, alachlor ESA, alachlor OXA, acetochlor ESA, acetochlor OXA, oral, RfD, uncertainty factor.

1. Introduction

Alachlor is a chloroacetanilide herbicide that was first registered for use in 1969 for control of grasses and broadleaf weeds on corn, soybeans, sorghum, peanuts, and beans (U.S. EPA, 1998). Acetochlor has a similar structure and toxicology profile to alachlor and was registered in 1994 for pre-emergence control of weeds on corn, but registered uses in the U.S. have been expanded to include direct application on sorghum and rotational crops of soybeans, wheat, nongrass animal feeds, sugar beets, dried shelled beans and peas, sunflowers, potatoes, cereal grains, an forage, fodder, and straw of cereal grains (U.S. EPA, 2007a). The major dissipation routes for both alachlor and acetochlor appear to be microbially-mediated degradation, runoff, and leaching. The aerobic soil metabolism products ethanesulfonic acid (ESA) and oxanilic acid (OXA) (Figure 1) from both parent chemicals are the most commonly detected environmental degradates in groundwater, and are most often found in ground and surface water at higher concentrations than the parent chemicals.

The U.S. EPA reviewed available toxicology studies and judged that alachlor is likely to be carcinogenic to humans at high doses, but not likely at low doses. A margin of exposure approach was recommended for its cancer dose-response assessment (U.S. EPA 1998, 2007b). For the noncancer assessment, the U.S. EPA developed an oral Reference Dose (RfD) of 0.01 mg/kg-day based on a chronic (1-year) dietary toxicity study in the dog with a No Observed Adverse Effect Level (NOAEL) of 1 mg/kg-day, a Lowest Observed Adverse Effect Level (LOAEL) of 3.0 mg/kg-day based on liver toxicity (hemosiderosis and hemolytic anemia), and a composite uncertainty factor (UF) of 100-fold (a factor of 10 for human variability in sensitivity and a factor of 10 for interspecies differences).

U. S. EPA (2007a) classified acetochlor as having "Suggestive Evidence of Carcinogenic Potential," and determined that a margin of exposure approach based on non-cancer endpoints is protective of both non-cancer and cancer effects, and derived an oral RfD of 0.02 mg/kg-day. This oral RfD was derived based on a chronic (1-year) dietary toxicity study in the dog with a NOAEL of 2 mg/kg-day, a LOAEL of 10 mg/kg-day based on clinical signs (increased salivation) and histopathology in the kidneys, liver, and testes, and a composite UF of 100-fold (a factor of 10 for human variability in sensitivity and a factor of 10 for interspecies differences).

The U.S. EPA considers alachlor and acetochlor degradates as unlikely to be carcinogenic (U.S. EPA, 1998, 2006a). The ESA and OXA degradates are also considered by the U.S. EPA to be significantly less toxic than the parent chemicals (U.S. EPA, 1998, 2006a). In addition, the U.S. EPA has not developed RfDs for these degradates because their risk assessments have demonstrated large margins of exposure (U.S. EPA, 1998, 2006a, 2007a). However, these degradates have been the subject of significant regulatory action and public health debate in several states, due to their frequent detection in groundwater and lack of benchmark values for comparison to monitoring data. The lack of federal RfDs, maximum contaminant levels (MCLs), or Health Advisories for the degradates has spurred additional, and often disparate, evaluations of their toxicity. This paper reports on the deliberations of a diverse expert panel that was convened to determine the appropriate oral RfDs for the ESA and OXA environmental degradates of alachlor and acetochlor.

2. Methods

2.1. Reference Dose Methodology

The U.S. EPA defines an RfD as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." Although many of the underlying assumptions, judgments of critical effect, and choices of uncertainty factors are similar among health agencies in estimating these sub-threshold doses, the approach used in this analysis followed current U.S. EPA's Reference Dose (RfD) methods (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 2002a). These methods were applied to derive the RfD for alachlor and acetochlor degradates based on potential chronic exposures via the oral route.

The first step in defining the RfD is to identify the critical effect(s) via a robust hazard characterization, including an evaluation of the mode of action and human relevance based on the weight of evidence (Sonich-Mullin et al., 2001; Boobis et al., 2006; Meek, 2008). U.S. EPA (2009) and Haber et al. (2001) define critical effect(s) as the first adverse effect(s), or its known precursor, that occurs as dose rate or exposure level increases. When this definition was first developed by the U.S. EPA in the late 1980s, the precursor was understood to be the immediate precursor, and not some precursor distant to the first adverse effect (Dourson, personal communication, 2009) since the intent of the RfD was to estimate the threshold boundary in sensitive humans for the onset of adverse health effects, rather than the onset of any adaptive biological events.

In the determination of critical effect, it is crucial that distinctions be drawn between adverse effects and adaptive effects. An adaptive effect enhances an organism's performance as a whole and/or its ability to withstand a challenge; an adverse effect is a biochemical change, functional impairment, or pathological lesion that impairs performance and reduces the ability of an organism to respond to additional challenge (Barnes and Dourson, 1988; U.S. EPA, 2009). Thus, a critical step in the dose-response assessment of these herbicide degradates involves determination of the critical effect from treatment-related adverse effects relevant to human health.

The second step in the determination of RfDs is reflected in the choices of appropriate species, study, and the point of departure. For this evaluation, the panel also used U.S. EPA methods as cited above, including a review of existing experimental animal data and the use of the NOAEL, LOAEL, or preferably, the benchmark dose (BMD) (U.S. EPA, 2000) for endpoints where this modeling was possible.

The third step in the determination of an RfD is the judgment of the appropriate composite uncertainty factor (UF) based on a review of the information supporting the choice of critical effect, and issues associated with extrapolation from experimental animals to humans, including sensitive human subpopulations. As before, the panel used U.S. EPA methods cited above that describe five potential areas of uncertainty for this judgment. In brief, these areas are within human variability (UF_H), experimental animal to human extrapolation (UF_A), shorter-term to chronic extrapolation (UF_S), LOAEL to NOAEL extrapolation (UF_L), and incompleteness of studies in the database for determination of the critical effect (UF_D). The panel also considered that two uncertainty factors addressing biological variability (i.e., UF_H and UF_A) can be replaced with adjustment factors based on chemical-specific toxicokinetic and toxicodynamic data (IPCS,

2005).

2.2. Expert Panel Workshop

An expert panel[1] of five state, federal, academic, and non-profit risk assessment scientists highly experienced in the areas of dose-response assessment and pesticide toxicology met publicly over two days to develop oral RfDs for the acetanilide degradates. To facilitate the work of the panel, scientists from Toxicology Excellence for Risk Assessment (*TERA*) compiled toxicology and other relevant data for the parent chemicals (acetochlor and alachlor) and their degradates, and provided a data package to the panel three weeks prior to the meeting. The package included charge questions to the panel, issue descriptions, data summary tables from relevant studies, key findings on the selection of potential critical effects, and benchmark dose modeling results. Care was taken to not provide the panel with final recommendations for the critical effects, points of departure or uncertainty factors, so that the panel could independently select them. A more thorough summary of key studies, supporting dose-response modeling, and full study reports were also made available to the panel members. The summary information mailed to the panel is available at http://www.tera.org/ART/Degradates/index.html.

Using the charge questions, meeting data package, responses to panel questions by sponsors, and comments from public observers, and the panel was successful in reaching a consensus on the development of RfDs for the four acetanilide degradates. The detailed

[1] Panel members and associated Conflict of Interest (COI) forms and meeting materials are available at http://www.tera.org/ART/Degradates/index.html.

workshop report summarizing panel deliberations (including presentations), and post meeting correspondence are also available at http://www.tera.org/ART/Degradates/index.htlm./

3. Results

Step 1. Hazard Characterization and Identification of Critical Effects

A complete database for development of an RfD with high confidence exists for both alachlor and acetochlor (U.S. EPA, 1998, 2006a, 2007a, b). In contrast, a complete database is not available for any of the four degradates included in this evaluation. The studies for the degradates, studies for the parent chemicals, data for related chloroacetanilide herbicides (e.g., metolachlor), and mechanistic and mode of action studies were examined to identify potential critical effects as part of the hazard characterization process. Based on the review of the available toxicokinetic studies for the parent chemicals (U.S. EPA, 1998, 2006a, 2007a,b) and the degradates, the panel concluded that, compared to the parent chemicals, the degradates are less likely to be biologically reactive because they: (1) are more polar, (2) have relatively low absorption, (3) undergo little biotransformation, (4) are rapidly eliminated, (5) are not likely to undergo conjugation reactions due to lack of a reactive dehalogenation site as is present in the parent chemicals, and (6) the presence of oxamic acid or ethane suflonate moieties that prevent the chemicals from being metabolized to a reactive quinoneimine like the parent chemicals (Kraus et al., 1995; U.S. EPA 1998, 2006a, 2007a,b; Albin and Kraus, 2000a,b; Heydens et al., 2000). The degradates are not formed *in vivo* as the metabolic/degradation pathways are different in soil than in mammals. Although toxicokinetic data were not available for alachlor OXA, based on structure activity relationships, the panel judged that toxicokinetics of the other degradates predict that

alachlor OXA, following a single oral administration, would also be poorly absorbed, rapidly eliminated, and excreted largely untransformed. The available data suggest that the toxicity of the parent chemicals are similar to each other and the toxicity of their degradates also appeared comparable to each other. However, due to clear differences in toxicity profiles (i.e., clear target organ effects for parents and lack of target organ effects for their degradates) and toxicokinetic profiles data, direct bridging data from the parent chemicals to the degradates was deemed unreliable. The panel concluded 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. This conclusion is 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 U.S. EPA assessments. For these reasons, the panel considered developing the RfDs directly for the degradates as a better option than using the parent chemicals as toxicological surrogates.

Key findings related to candidate critical effects from from animal studies on the degradates are described in the following section.

3.1.1. Decreased Body Weight

Subchronic and chronic studies with the parent chemicals, acetochlor and alachlor, have shown that body weight change is a key effect for these chemicals (U.S. EPA, 1998, 2006a, 2007a, b). The NOAELs and LOAELs for decreased body weight for the parent chemicals and the degradates are presented for the rat in Figures 2a and 2b.

Like the parent chemicals, body weight changes were a common finding among the 28-

day and 90-day studies available for the degradates. A review of the dose-response data for body weight, adjusted body weight (provided for some studies for the degradates) or body weight gain as well as correlated findings related to food consumption and food utilization indicate that body weight decreases were a treatment-related adverse effect for some 90-day studies. In this analysis, a dose-related decrease in body weight reaching 10% was considered as an adverse effect. Group mean body weight over time for the degradates are presented in Figures 3a – 3e (only the most sensitive sex from each subchronic study is shown) and a summary of the study design and dosing regimen for each of the subchronic studies for the four degradates is provided in Table 1.

A 90-day dietary study and a 91-day drinking water study were available for alachlor ESA. In the 90-day dietary study (Kirkpatrick, 2002) the high doses, 788 mg/kg-day (males) and 926 mg/kg-day (females) (Figure 2a) were considered NOAELs. Effects at the LOAEL were only minimal changes in body weight and no change in food consumption. Data on food utilization were not provided in the study.

In the drinking water study (Siglin, 1993; Heydens et al., 1996) for alachlor ESA, there was a 5% decrease in body weight at week 13 in both sexes at the high dose. The relevance of the early (week 1) observations of decreased body weight in the high dose was considered in light of potential effects secondary to water intake (see Figure 3a). A review of the pattern of body weight change over the course of the study along with the pattern of water intake indicated initial rapid decreases in body weight as well as water intake with gradual recovery of both, suggestive of a palatability issue. The observed decrease in body weight in the drinking water study was considered not biologically significant compared to the use of a 10% change as a body weight effect benchmark and the potential confounding palatability issues in the study. In

addition, decreases in food or water consumption due to taste and odor have not been considered biologically adverse but are regarded as organoleptic effects (i.e., objectionable taste and odor). The high dose in the drinking water study was initially 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 slightly decreased body weight gain in males and females. However, the panel judged both the body weight changes and clinical signs as partially due to dehydration and the high doses, 896 mg/kg-day (males) and 1108 mg/kg-day (females), were ultimately regarded as NOAELs in the alachlor ESA drinking water study (Figure 2a).

For alachlor OXA, there were no statistically significant changes in body weight or food consumption (no data were provided on food utilization) (Figure 3b), indicating the high doses of 834 mg/kg-day (males) and 1008 mg/kg-day (females) were NOAELs (Figure 2a).

For acetochlor ESA, the 90-day dietary study yielded NOAELs of 225 mg/kg-day (males) and 259 mg/kg-day (females), with corresponding LOAELs of 919 and 1073 mg/kg-day (Figure 2b) for body weight effects based on a decrease in body weight of 10% or greater in males at 14 weeks (Figure 3c), and significantly decreased food utilization in males and females. In this study, a 4% change in adjusted body weight was noted at the mid-dose of 3000 ppm (males – 225 mg/kg-day; females – 259 mg/kg-day), but the degree of change was not considered biologically significant.

For acetochlor OXA (Williams, 2000b) significant reductions in body weights, adjusted for initial body weight differences, were observed in both sexes at the high dose when data were combined for the main and satellite groups. The reductions in the body weight were maximally 7% for males and 5% for females by the end of the study period. Adjusted body weight (not a

measure of body weight gain but a calculated value reflecting a covariate adjustment based on initial starting body weight of the animals) was also decreased at the mid dose and food utilization was affected at the high dose in both sexes. The body weight decreases were less than 10% at mid- and high-dose levels. Female rats responded similarly to males at the high dose, but with a clear effect on reduced food utilization and adjusted body weight. Examination of body weight curves shows an effect that is dose-related in females at high dose (Figure 3d). The low- and mid-dose body weights were greater than controls, while at the high dose the body weight was decreased versus controls. This observation confirms that the mid doses, 230 mg/kg-day (males) and 268 mg/kg-day (females) are NOAELs (Figure 2b). Although the severity of high-dose effects in females was considered marginal, the panel judged it to be a LOAEL based on the dose-response trend and effects on food utilization. Therefore, the high doses of 955 mg/kg-day (males) and 1082 mg/kg-day (females) were considered LOAELs for this study.

Overall, the available data demonstrate a consistent pattern of effects on body weight changes, when considering the overall database for the parent chemicals (see Figures 2a and 2b) and their environmental degradates. The degradates are clearly much less potent than the parent chemicals in repeat-dose studies in rats, with LOAELs that are at least five-times higher than those of the parent chemicals and NOAELs that range from five- to 60-times higher for the degradates compared to the parent chemicals (see Figure 2). While decreased food consumption was a relatively consistent finding among the chemicals, decreased food utilization was also common suggesting that palatability was not the sole reason for the decreases in body weight. However, the underlying mode of action for decreased body weights is unclear, and relative impacts of different mechanisms were not readily apparent from the available data.

3.1.2. Effects on Thyroid Homeostasis

The U.S. EPA (1998, 2006a) and the European Union (EU, 2005) have concluded that the parent chemicals, acetochlor and alachlor, cause a slight increase in thyroid follicular tumors in rats, but at doses at or above the maximum tolerated dose (MTD) and through a mode of action that is generally considered to be of minimal relevance to humans. The thyroid tumors are considered to result from induction of hepatic uridine diphosphate glucuronyl transferase (UDPGT), with subsequent decrease in circulating tri-iodothyronine (T3) and thyroxine (T4), and a subsequent increase in thyroid stimulating hormone (TSH). Increased levels of TSH are thought to be responsible for the chronic stimulation of the thyroid follicular cell epithelium which results in follicular cell hypertrophy, hyperplasia, and ultimately neoplasia. The data available support the ability of both acetochlor and alachlor to cause thyroid stimulation, which led to the examination of thyroid effects for the alachlor and acetochlor degradates. Thyroid effects (weight changes, hormone levels, etc.) were examined only in 28-day studies for acetochlor ESA and acetochlor OXA. Changes in thyroid weights and/or histopathology were evaluated in the 90-day studies for all four of the degradates. Tables 1a and 1b present the effects of the acetochlor degradates (for which data were available) on biochemical parameters critical to thyroid homeostasis in rats.

In the 28-day dietary study with acetochlor ESA (Lees, 2000a) (Table 2a), thyroid weights were unaffected. Free T3 levels and TSH levels increased in a dose-related manner in males, but these increases did not reach statistical significance and plasma levels of free T4 levels did not change in a dose-dependent manner. T4-UDPGT activity was statistically significantly increased in both males and females at the high dose. No dose-related changes in

liver or thyroid weights or histopathology were observed in the 90-day dietary study (Lees, 2000b).

In the 28-dietary study available for acetochlor OXA in rats (Williams, 2000a), no liver weight changes were observed. A slight but statistically significant ($p<0.05$) increase was noted in absolute thyroid weights in males in the mid- and high-dose groups. The relative thyroid weights in males were also statistically significantly increased at all dose levels, but there was no clear dose-response relationship. In addition, the thyroid weights from the treated males in this study were lower than those of the control animals in the concurrent 28-day rat study with acetochlor ESA (Lees, 2000a). Furthermore, no thyroid weight changes were noted in females and there were no correlative histopathological findings. Total and free plasma T3 levels (Table 2b) decreased in males, but not in females, at the high dose. TSH levels were reduced at the high dose, but without statistical significance in either sex. The direction of this change (decrease) in TSH levels is not consistent with increased thyroid growth stimulation. A statistically significant decrease in hepatic T4-UDPGT activity was observed in females whereas an increase was observed in males but without statistical significance at the high dose. The direction of this change (decrease) in the females is also not consistent with increased thyroid growth stimulation. These results suggest possible slight or equivocal changes in some thyroid hormone parameters at the high dose, but without any correlative treatment-related effects on thyroid weights or histopathology in this study or the 90-day study for this degradate (Williams, 2000b), and the toxicological significance of these changes is not clear.

No short-term (28-day) studies were available that evaluated thyroid parameters (weight or hormone levels) for alachlor ESA. However neither of the two subchronic (90-day) studies available for alachlor ESA, the drinking water study (Siglin, 1993; Heydens et al., 1996) and the

dietary study (Kirkpatrick, 2002), indicated that the test article was associated with any significant changes in liver or thyroid/parathyroid weights or histopathology.

A 28-day study is available for alachlor OXA (Stout and Thake, 2000), but thyroid effects were not evaluated. Liver weights were increased in males only at the high dose of 20,000 ppm (1539 mg/kg-day). A 90-day dietary study (Lemen et al., 2000) reported a slight increase in absolute thyroid weight in males at the high dose of 834 mg/kg-day, but a decrease in absolute thyroid weight in females at the high dose of 1008 mg/kg-day. There were no changes in absolute liver weight, although a slight but significant reduction in relative liver weight was observed in high-dose females. There were no changes in liver or thyroid histopathology.

The panel noted that for the acetanilide degradates, some statistically-significant changes in hormones and enzyme activities were observed, but the changes were not consistent with the expected mode of action for a thyroid stimulant. Also, in comparing control groups among comparable studies for these degradates, the degree of changes are within the control variability. Moreover, there were no consistent changes in thyroid weights or signs of histopathology in the corresponding 90-day studies. Based on these inconsistencies, the panel concluded 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, there were no clear treatment-related effects on the thyroid weights 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 pathology reports for the 90-day studies were reviewed to supplement the organ weight data. These studies did not identify any treatment-related thyroid histopathology. Overall, the panel concluded that there were no consistent treatment-related adverse effects on the thyroid for the acetochlor or alachlor degradates.

3.1.3. Effects on Hematology/Clinical Chemistry Parameters

A full battery of hematology and clinical chemistry data from the dietary subchronic studies conducted with each of the degradates were evaluated. Small and sporadic changes were noted for selected parameters in some of these studies, but none of the findings showed a consistent pattern of changes indicative of underlying toxicity. The panel judged such findings as not likely to be toxicologically relevant. Among the subchronic studies, findings of potential toxicological interest were most notable for the 90-day drinking water study for alachlor ESA (Siglin, 1993; Heydens et al., 1996). Hematology and clinical chemistry changes related to potential liver damage were observed at the mid and high dose in males and/or females in the 90-day alachlor ESA drinking water study (Table 2). No changes in liver weight were observed in this study. Statistically significant differences in clinical chemistry parameters were observed. However, differences were not considered biologically meaningful because similar changes were not observed at the higher treatment levels, there was no dose-response relationship, the degree of change was small and/or the differences did not correlate with any abnormal histopathology.

Hemolytic anemia was observed in the one-year dog study with alachlor (U.S. EPA, 2007a), leading the panel to carefully examine the hematological effects observed in the alachlor ESA drinking water study. Statistically significant differences in hematology data (Table 3) consisted of decreased hemoglobin, hematocrit and erythrocyte counts, and increased mean cell hemoglobin (MCH) in males, and an increased total leukocyte count in females. The observed changes in hematology parameters were small (2-5%) and did not follow any apparent dose-

response relatioship. In addition, these hematological effects did not correlate with histopathological changes. The pattern of hematology changes observed in the study might have been considered characteristic of a mild hemolytic anemia although the strength of the evidence was not strong. The panel considered whether the apparent mild hematological effects were likely due to a direct toxicological mode of action or whether the effects might be secondary to changes in blood volume due to decreased drinking water intake. This alternative hypothesis was considered in light of the absence of hematological effects at similar doses in the dietary studies. Examination of the dose and temporal pattern of hematological effects with drinking water intake suggested to the panel that dehydration could have been at least partly responsible.

Overall, the panel felt that the hematological changes were marginal in nature 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. Therefore, the high dose in the alachlor ESA drinking water study was considered a NOAEL.

3.1.4. Reproductive and Developmental Toxicity

In light of the reported testicular effect observed in dogs following dietary exposure to acetochlor (U.S. EPA, 2006a), the potential for reproductive effects was closely examined for the degradates. Although no reproductive toxicity studies are available, there was no evidence for effects on reproductive organs in the 90-day studies for any of the degradates. Small changes in ovary and testes weights were observed in the 91-day drinking water study with alachlor ESA

(Siglin, 1993; Heydens et al., 1996). Detailed review of the histopathology reports (Siglin, 1993) available for that study indicated that 2/10 male rats in the high-dose (10,000 ppm) group had hypospermatogenesis. One of these two male rats was found dead (autolysis had already began on several of the organs) on study day 13 and the animal had consumed negligible water (13 g/kg-day) during the first week of the study, indicating that this rat was exposed to very little test article. The panel agreed that this animal should not have been included in the incidence tables for hypospermatogenesis and that only one male in the high dose group had slight/mild (grade 2) hypospermatogenesis.

Minimally dilated lumen of the uterus (grade 1; lowest stage that can be recorded) was also observed in two female rats in the control group and a slight/mild (grade 2) and a moderate (grade 3) finding was observed in two and one female rats, respectively, in the high dose group. As the finding was not considered treatment-related, the uterus in the low- and mid-dose groups was not examined histologically. Since the stage of estrous cycle of each female was not determined at necropsy, it is difficult to determine if there is any correlation with cyclicity. The panel, in consultation with an independent reproductive toxicity expert (Raymond York, Ph.D., DABT, ATS, ERT, personal communication, 2009), agreed with the report author that the small differences were not treatment-related.

Developmental toxicity studies were available for acetochlor OXA and alachlor ESA (Holson, 1995a, b; Heydens et al., 1996), but not for acetochlor ESA or alachlor OXA. Sprague-Dawley female rats, 25 animals/group, were administered alachlor ESA (90% purity) in corn oil, by gavage, at a dose of 0, 150, 400 or 1000 mg/kg-day (adjusted doses – 0, 135, 360 and 900 mg/kg-day) once daily from gestation days (GDs) 6 through 15 and animals were killed on GD 20 (Holson, 1995b; Heydens et al., 1996). All maternal animals survived to the scheduled

necropsy and no internal findings related to treatment were observed at any dose level. Rales were observed with alachlor ESA during the daily examinations, at the time of dosing and one hour following dosing. The rales were considered to be consistent with a short-lasting irritation effect from gavage dosing with an acidic compound and not appropriate as the basis for a chronic oral RfD. There were no effects on intrautetine growth and survival at any dose level and no treatment-related fetal malformations or developmental variations were observed in this study. The panel concluded that alachlor ESA did not cause any adverse effects in pregnant rats or their offspring at any dose. Therefore, the NOAEL for maternal and developmental toxicity was 900 mg/kg-day, the highest dose tested. The panel also reviewed the developmental toxicity of metolachlor ESA and noted that both the maternal and developmental NOAELs for this degradate are greater than 1000 mg/kg-day (Doubovetzky, 1999), the highest dose tested, suggesting that developmental toxicity is not a concern for these acetanilide degradates.

For acetochlor OXA, Sprague-Dawley female rats (25 animals/group) were administered the test material in distilled water, by gavage, at a dose of 0, 250, 500 or 1000 mg/kg-day once daily from GDs 6 through 19 and were sacrificed on GD 20 (Holson, 2000). There was potential maternal toxicity evidenced by maternal mortality in two of 25 dams at a dose level of 1000 mg/kg-day. Necropsy revealed no test article-related internal findings at any dose level. There were no effects on intrauterine growth and survival of pups at any dose level evaluated. Some malformations and developmental variations were observed in fetuses in this study, but were considered to be spontaneous in origin and not related to test article administration. The panel concluded that the NOAEL for maternal toxicity was 500 mg/kg-day while the NOAEL for developmental toxicity was 1000 mg/kg-day. The panel also concluded that that there were no developmental effects at the highest doses tested for alachlor ESA (900 mg/kg-day) and

acetochlor OXA (1000 mg/kg-day) and that the highest doses tested in these two studies are NOAELs for developmental toxicity.

Because data available in two species, rats and rabbits, show that the parent chemicals caused developmental effects only at or above maternally toxic doses, the panel further discussed whether the developmental studies available for the two degradates were adequate to assess developmental toxicity for all four degradates and to address the absence of test data for a second species. The panel agreed that the data available for the two degradates suggested limited concern for developmental toxicity for any of the degradates. This was based on the overall structural similarity, similar toxicity among the degradates, and uniformly low gastrointestinal absorption. Moreover, given that developmental toxicity is not the critical adverse effect for the more toxic parent chemicals, and that the latest U.S. EPA (2006b) assessment concluded that a Food Quality Protection Act (FQPA) factor of 1 was considered sufficient for the parents, the panel concluded that there is limited concern for developmental toxicity for the degradates, but recognized that lack of data for the untested degradates continues to represent a data gap.

3.1.5. 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 were considered significant for alachlor ESA, based on findings in the drinking water study (Siglin, 1993; Heydens et al., 1996). In the drinking water study, clinical signs (including few and/or small feces, urine staining, rough coat, dehydration, emaciation and material around the eyes) were observed at the highest dose 20,000 ppm (males – 896 mg/kg-day; females –1108 mg/kg-day). This finding was supported by the

observation of clinical signs (decreased activity, dehydration, rough coat, hunched posture, unkempt appearance, emaciation, dark material around the nose and mouth, and increased incidence of urine staining and few and/or small feces) at a feed concentration of 20,000 ppm (males – 2217 mg/kg-day; females – 2378 mg/kg-day) in the 28-day study (Siglin, 1993), but not in a 90-day feeding study with dietary concentrations up to 12,000 ppm (males – 788 mg/kg-day; females – 926 mg/kg-day) (Kirkpatrick, 2002). The lack of consistency between the 90-day dietary and the 91-day drinking water studies for alachlor ESA was further examined in terms of whether the observed clinical signs could be attributed to dehydration or infection. Ocular and periocular findings (such as dark material and hair loss around the eyes) were noted in both control and alachlor ESA treated animals in the drinking water studies (Siglin, 1993; Heydens et al., 1996). These changes tended to occur at a higher overall incidence in high-dose males and in females at all dose levels, but without a dose-response relationship. Ocular findings were not considered by the panel to be related to test material, but were instead manifestations of abnormalities typically associated with the Fisher 344 rat used in these studies, as well as viral infection (Heydens et al., 1996). When the remaining clinical signs were evaluated for consistency with dehydration, based on the nature of the effects as well as dose-response and temporal patterns, the clinical signs were considered to be most consistent with dehydration as the underlying cause. 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 doses in the drinking water study of 20,000 ppm, 896 mg/kg-day (males) and 1108 mg/kg-day (females), were considered to be NOAELs for clinical signs.

Gastric hyperplasia (primarily in the fundic region of the stomach) and eosinophilic inclusion (primarily in the fundic region, but also present to some extent in pyloric epithelium)

were observed in male and females in a 28-day rat dietary study for alachlor OXA at the high dose of 20,000 ppm (males – 1539 mg/kg-day; females – 1595 mg/kg-day) (Stout and Thake, 2000). No such effects were observed in the corresponding 90-day studies for the alachlor degradates. The panel concluded that it is likely that this observation is dose-related, since the 28-day studies tested higher doses than the 90-day studies. The finding could also be attributed to local irritation, which would be consistent with exposure to high doses of alachlor OXA due to its acidity. The investigators considered the gastric changes to have been the result of altered mucus production in the epithelium of the glandular stomach. These observations were examined critically in light of the finding that chronic dietary treatment of rats with the parent chemical, alachlor caused gastric tumors in the rat (U.S. EPA, 1998, 2007a). However, gastric histopathological changes observed in this 28-day study with alachlor OXA are different (hyperplasia of mucus cells and eosinophilic inclusions) than those that are associated with the development of gastric tumors (mucosal atrophy and loss of parietal cells) in the rat stomach after treatment with the parent alachlor. Based on the available data, the panel concluded that the observed changes with alachlor OXA represent an adverse treatment-related effect consistent with a local toxicity associated with high dietary doses of an acidic chemical, rather than a general systemic effect. Thus, these effects were not considered as an appropriate basis for the RfD.

Step 2: Choice of Appropriate Species, Study, and Point of Departure

A detailed critical examination of each potential key study as well as the array of endpoints described above led the panel to conclude that decreased body weight (and body

weight-related metrics) was a treatment-related adverse effect in some studies for the degradates. No consistent treatment-related adverse effects were observed on the thyroid for these degradates. Hematological findings observed in the drinking water study for alachlor ESA (Siglin, 1993; Heydens et al., 1996) were marginal, 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 were identified for these degradates. Table 4 shows the critical effects and the point of departure for each degradate. For alachlor ESA, several differences were noted but no statistically significant effects were judged to be adverse after an extensive review of individual animal data, including clinical signs and chemistries in either a 91-day drinking water study (Siglin, 1993; Heydens et al., 1996) or in a 90-day feeding study (Kirkpatrick, 2002). The high dose in the 90-day dietary study serves as the appropriate NOAEL and the basis of the RfD, since it is lower than the high dose used in the drinking water study. For alachlor OXA, the highest dose was judged as the appropriate NOAEL, because only minimal changes in body weight were observed in the 90-day dietary study (Lemen et al., 2000) without statistically significant differences between controls and treated animals. The critical effects for acetochlor ESA are decreased body weight gain, decreased food consumption, and decreased food utilization noted in the 90-day dietary study, with NOAELs at the mid dose (Lees, 2000b). For acetochlor OXA, NOAELs at the mid dose were identified for decreased body weight gain and decreased food utilization in the 90-day dietary study (Williams, 2000b).

Endpoints with statistically significant changes or trends were also chosen for modeling using U.S. EPA's Benchmark Dose Software (BMDS) 1.4 (U.S. EPA, 2000). However, BMD runs did not result in values that were more reliable than identified NOAELs and LOAELs, due principally to the lack of a clear dose-response. In some cases, where acceptable fit to the data

was achieved, the Benchmark Dose (Lower Confidence Limit) (BMDL) was not judged as an appropriate point of departure because the maximum effect observed at the highest dose was substantially less than the typical benchmark response (BMR) of 10% decrease in body weight relative to controls. This was a concern, because the large extrapolation beyond the data adds uncertainty to the BMDL estimate and the large extrapolation had the potential for calculating a BMDL above the threshold for co-critical effects that were not amenable to modeling. The BMD outputs are available for examination at http://www.tera.org/ART/Degradates/index.html.

Step 3: Areas of Uncertainty in Deriving an RfD

The U.S. EPA (2002a) has suggested five different uncertainty factors to address issues of variability and uncertainty when deriving RfDs and the panel deliberations surrounding each of these five areas are provided below.

3.4.1. Interspecies Variability (UF_A)

Because no human health effects data or comparative data on toxicokinetics or toxicodynamics between rats and humans is available, a factor of 10 was considered by the panel to be appropriate for UF_A, for all degradates.

3.4.2. Intraspecies Variability (UF_H)

The panel's evaluation suggests that not enough information was available to modify the value of 10 for UF_H and information does not exist that would allow the development of a chemical-specific adjustment factor (CSAF) for human variability. The factor of 10 for human variability was considered adequate, since the limited absorption and metabolism of the degradates suggest that variability in toxicokinetics might be lower than for other chemicals (including the parent chemicals). This latter conclusion was consistent with the fact that an FQPA factor of 1-fold was applied to all three related herbicides (alachlor, acetochlor, and metolachlor), indicating that the toxicity database is complete, and there is no indication of selective toxicity in either rats or rabbits to *in utero* or post-natal exposures, and also that there is no indication of developmental neurotoxicity in either parent chemicals or degradates.

3.4.3. Subchronic to Chronic (UF_S)

Because only subchronic studies are available for these degradates, but data were identified that suggest a lack of progression with exposure duration (see the discussion of this issue with the database uncertainty factor below), the factor of 10 was reduced by the panel to a factor of 3. Moreover, this factor was reduced in the context of applying multiple UFs as described below.

3.4.4. LOAEL to NOAEL Extrapolation (UF_L)

Each degradate had at least one adequate study from which a NOAEL was selected as the point of departure. Therefore, a LOAEL to NOAEL extrapolation is not necessary. Thus, a value of 1 was considered by the panel to be appropriate for the UF_L.

3.4.5. Database (UF_D)

This factor was reduced in the context of applying multiple UF as described below.

3.5 Selection of Chemical-Specific Combined Uncertainty Factors

The UF for subchronic to chronic duration and the UF for database completeness were reviewed in significant detail, since none of the degradate studies was longer than about 90 days and none of the individual degradates had a complete database as defined by U.S. EPA. Possible combinations of these two factors were considered since both address issues related to deficiencies in the overall database.

The impact of lack of a reproductive toxicity study on the selection of the UF for database insufficiency was also carefully considered. Organ weight and histopathology findings reported in the available subchronic studies for these degradates indicated that the reproductive organs were not targets for any of the degradates. In the absence of data on functional reproductive capacity for these degradates, the available studies on the parent chemicals were used to inform the potential impact of this data gap. This approach was considered, with the caveat that the modes of action for the parent chemicals are apparently not the same as for these degradates, and thus direct comparisons are somewhat limited. On the other hand, such

comparison might be considered as overestimating the impacts of missing studies, since the parent chemicals were considered more likely to be biologically available and reactive than the degradates. Comparing the effect levels for reproductive toxicity versus the most sensitive systemic effects for the parent chemicals, the NOAEL for reproductive toxicity in rats for alachlor was 30-fold higher than the chronic dog NOAEL (which was based on anemia and hemosiderosis in kidney/spleen of males), but no reliable NOAEL from subchronic rat study was available for comparison (U.S. EPA, 2007a). For acetochlor, the critical reproductive toxicity NOAEL of 65.6 mg/kg-day in male rats (U.S. EPA, 2006a) was 30-fold higher than the chronic dog NOAEL of 2 mg/kg-day (which was based on testicular, hepatic and renal effects as well as increased salivation and other effects) and was about 7-fold lower than the NOAEL of 10 mg/kg-day from the subchronic rat study (which was based on decreased body weight/weight gain in males and females) (U.S. EPA, 2006a). Thus, for acetochlor, reproductive effects in rats were not the critical effect. Reproductive organ effects in dogs were a co-critical effect for acetochlor and such effects remain a possibility for these degradates (in the absence of specific tests for this endpoint in dogs for the degradates). For a related acetanilide, metolachlor, reproductive or developmental toxicity was not the critical effect, and the available studies in rats and dogs for the alachlor and acetolachlor degradates did not suggest a concern for testicular effects.

Because alachlor produced ocular effects (U.S. EPA, 2007a), the panel considered the potential for the degradates to produce ocular effects as well. The panel did not judge the ocular and periocular effects (dark material and hair loss around the eyes) observed for alachlor ESA in the drinking water study (Siglin, 1993) to be treatment-related. The panel acknowledged that the specific ocular effect observed for alachlor would not have been detectable in the rat strain used in the alachlor ESA study. However, this data gap was not considered critical in light of the

likely differences in the mode of action between the parent chemicals and these degradates and the fact that uveal degeneration in rats induced by alachlor (U.S. EPA, 1998) were not critical effect for the alachlor RfD.

A second potential data gap was considered based on data indicating potential for neurotoxic effects in several acetochlor rat and dog studies (U.S. EPA, 2006a). However, there was no indication of a neurotoxic potential for acetochlor degradates (Lees, 2000a, b; Williams, 2000a, b). The panel concluded that since no such effects were observed even at limit doses in subchronic studies, these degradates are not likely to be neurotoxic.

Absence of a longer-duration systemic toxicity study in a second species was also considered in selecting the appropriate database uncertainty factor, since only rat studies were available for the degradates. The panel discussed whether a 1-year dog study was needed, since some data for the parent chemicals suggest that the dog is the more sensitive species and effects that occur at one year are not found in the 90-day studies (U.S. EPA, 2006a, 2007a). However, the U.S. EPA no longer requires a chronic (1-yr) dog study as part of the required data set for pesticide registration; rather a 13-week study is deemed sufficient. The U.S. EPA Science Advisory Panel (2005) recently analyzed the value added by conducting a 1-year study in the dog and found that the longer duration study in dogs does not add significantly to the ability to identify the critical adverse effect level. The European Food Safety Authority (EFSA) Panel on Plant Protection Products (PPR) reached a similar conclusion that extension of a dog toxicity study beyond a 13-week duration provides little additional information (EFSA, 2009).

The panel compared effect levels from studies of rats versus dogs and for studies of different durations for the parent chemicals to evaluate the importance of missing studies for the degradates. These comparisons were only used as a qualitative guide because of the potential

differences in mode of action for the degradates versus the parent chemicals. Similar data were also reviewed for the effect levels for various species and study durations for metolachlor, and its ESA degradate, for which a subchronic dog study was available (U.S. EPA, 2005). This analysis did not indicate that the subchronic dog study would warrant a greater UF_D factor than considered by the panel. In no case of the parent chemicals did reproductive or developmental toxicity drive the assessment, and in all three cases, dog and rat effect levels were roughly quantitatively similar, at the same study exposure length. Given that the four degradates are less reactive, absorbed to a lesser extent, and less metabolized when compared with the parent compounds, greater variability in toxicity among these degradates is not expected than observed with their parent compounds.

A combined value of 10-fold (for UF_S and UF_D) was recommended by the panel for uncertainties in both the lack of certain studies to determine the critical effect and the lack of a chronic study as a basis of the RfD. This latter factor was considered by the panel to be best judged as 10, although it could be as high as 30,[2] because the available toxicology data for the parent compound suggest only a modest, if any, change between subchronic and chronic NOAELs, and the available information suggests that neither developmental nor reproductive toxicity is the critical effect. Moreover, the panel concluded that the same UF can be used for developing a safe dose for each degradate, because the chemical structures and data bases are similar and all have a similar spectrum of toxicity based on the available array of studies.

[2] An argument could be made for a UF of 3000 (10_H x 10_A x $30_{S\&D}$), where the 30x UF represents the combined uncertainties in the duration extrapolation and database insufficiency because of 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.

Furthermore, no data were available that would suggest significant mode of action differences. Thus, the panel considered that a composite UF of 1000 (10_H x 10_A x $10_{S\&D}$) for each degradate was reasonable. A composite UF of less than 1000 was not considered appropriate.

3.6. Mode of action data to justify a cumulative risk assessment approach

The potential for a cumulative risk assessment for these degradates was evaluated based on potential commonalities in critical effects and their underlying modes of action. The panel concluded that the data are inadequate to identify the mode of action for any of the observed effects of the degradates, except perhaps for the proposed effect of irritation and 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.

4. **Conclusion**

Based on the above discussions of critical effects, appropriate effect levels, and uncertainty factors, separate RfDs, one for each degradate, were developed by the panel. For alachlor ESA, the high dose in the dietary study of 12,000 ppm (males – 788 mg/kg-day; females – 926 mg/kg-day) serves as the appropriate NOAEL for the RfD, since it is lower than that seen in the drinking water study. An uncertainty factor of 1000 is applied to the NOAEL for males of 788 mg/kg-day, the lower of both sexes, resulting in an RfD of 8 E-1 mg/kg-day.

Alachlor ESA was similar to alachlor OXA in that no statistically significant effects were judged to be adverse in the 90-day feeding study with alachlor OXA (Lemen et al., 2000). The high dose of 13,000 ppm (males – 834 mg/kg-day; females – 1008 mg/kg-day) serves as the appropriate NOAEL. Applying a composite UF of 1000 to the lower of the NOAELs results in an RfD of 8 E-1 mg/kg-day.

For acetochlor ESA, the NOAEL of 3000 ppm (males – 225 mg/kg-day; females – 259 mg/kg-day) in the 90-day feeding study (Lees, 2000b) was identified as the appropriate point of departure. Applying an uncertainty factor of 1000 to the lower NOAEL results in an RfD of 2 E-1 mg/kg-day.

For acetochlor OXA, the NOAEL of 3000 ppm (males – 230 mg/kg-day; females 268 mg/kg-day) in the 90-day feeding study (Williams, 2000b) was identified as the appropriate point of departure. Applying the composite UF of 1000 to the NOAEL in males of 230 mg/kg-day results in an RfD of 2 E-1 mg/kg-day. For each degradate, the confidence in the RfD is judged to be low to medium and 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, 2006a) and the Reregistration Eligibility Decision (RED) for alachlor (U.S. EPA, 1998) are 2 E-2 mg/kg-day for acetochlor based on clinical signs (excessive salivation) and microscopic findings in the liver, testes and kidney in dogs; and 1 E-2 mg/kg-day for alachlor, based on hemosiderosis and hemolytic anemia in dogs. These RfDs are 10- and 80-fold lower than those for the corresponding degradates, which agrees

with the consensus opinion that the toxicity of the degradates is significantly less than the toxicity of the parent chemicals.

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Figure captions

Figure 1. Structures of Alachlor and Acetochlor and their Degradates

Figure 2a. Effect Levels for Body Weight - Alachlor and Degradates. Differences in NOAEL and LOAEL values identified from dietary or drinking water (DW) studies in rats using alachlor and its ESA and OXA degradates. Values are means for both male and female animals. The references for alachlor 28-day, 90-day, and chronic toxicity studies are Hotz et al. (1993), Wolf (1966), and Daly et al. (1981), respectively; see Table 1 for references for the studies for the degradates.

Figure 2b. Effect Levels for Body Weight - Acetochlor and Degradates. Differences in NOAEL and LOAELs identified from dietary studies in rats using acetochlor and its ESA and OXA degradates. Values are means for both male and female animals except for the 28-day studies for acetochlor and acetochlor OXA and the 90-day studies for acetochlor ESA and OXA where values represent the lower values in males. The references for the 28-day, 90-day, and chronic toxicity studies for acetochlor are Broadmeadow (1985), Broadmeadow (1986), and Virgo and Broadmeadow (1988), respectively; see Table 1 for references for the studies for the degradates.

Figure 3a. Alachlor ESA – Male Body Weight versus Time. Rats (10 animals/dose group) were administered alachlor ESA in their drinking water at a concentration of 0, 200, 2000, or 10000 ppm (0, 16, 157, and 896 mg/kg-day) for 91 days (Siglin, 1993; Heydens et al., 1996). Graph represents group mean body weight versus time.

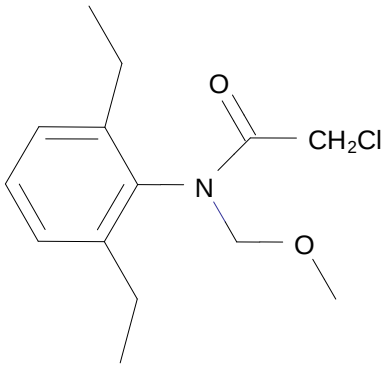
Figure 3b. Alachlor OXA – Male Body Weight versus Time. Rats (10 animals/dose group) were administered alachlor OXA in the diet at a concentration of 0, 400, 1300, 4000, or 13000 ppm (0, 24.9, 83.5, 261.1, and 834.6 mg/kg-day) for 90 days (Lemen et al., 2000). Graph represents group mean body weight versus time for the more sensitive sex.

Figure 3c. Acetochlor ESA – Male Body Weight versus Time. Rats (12 animals/dose group) were administered acetochlor ESA in the diet at a concentration of 0, 1000, 3000, or 12000 ppm (0, 75.0, 225.4 or 919.4 mg/kg-day) for 90 days (Lees, 2000b). Graph represents group mean body weight versus time for the more sensitive sex.

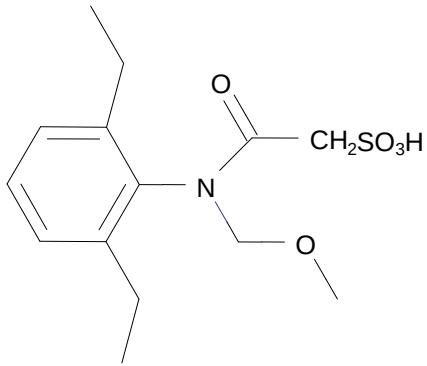
Figure 3d. Acetochlor OXA – Female Body Weight versus Time. Rats (12 animals/dose group) were administered acetochlor OXA in the diet at a concentration of 0, 1000, 3000, or 12000 ppm (0, 86.5, 268.0, and 1082.7 mg/kg-day) for 90 days (Williams, 2000b). Graph represents group mean body weight versus time for the more sensitive sex.

Figure 3e. Alachlor OXA – Male Body Weight versus Time. Rats (12 animals/dose group) were administered alachlor OXA in the diet at a concentration of 0, 3000, 6000, or 12000 parts per million (ppm) (0, 195, 389, and 788 mg/kg-day) (Kirkpatrick, 2002). Graph represents group mean body weight versus time for the more sensitive sex.

Figure 1



Alachlor



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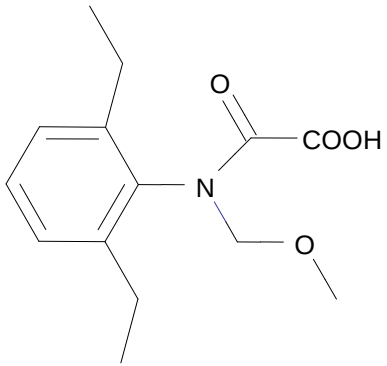

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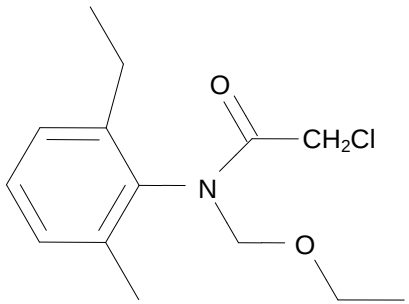

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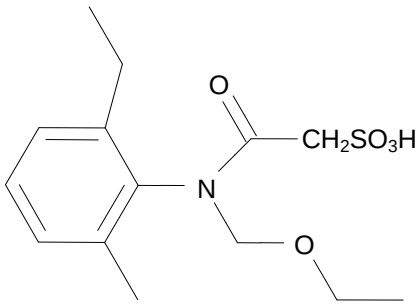

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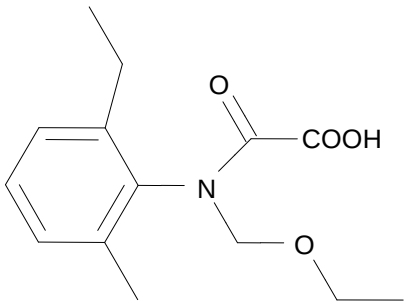

Acetochlor OXA

Figure 2a

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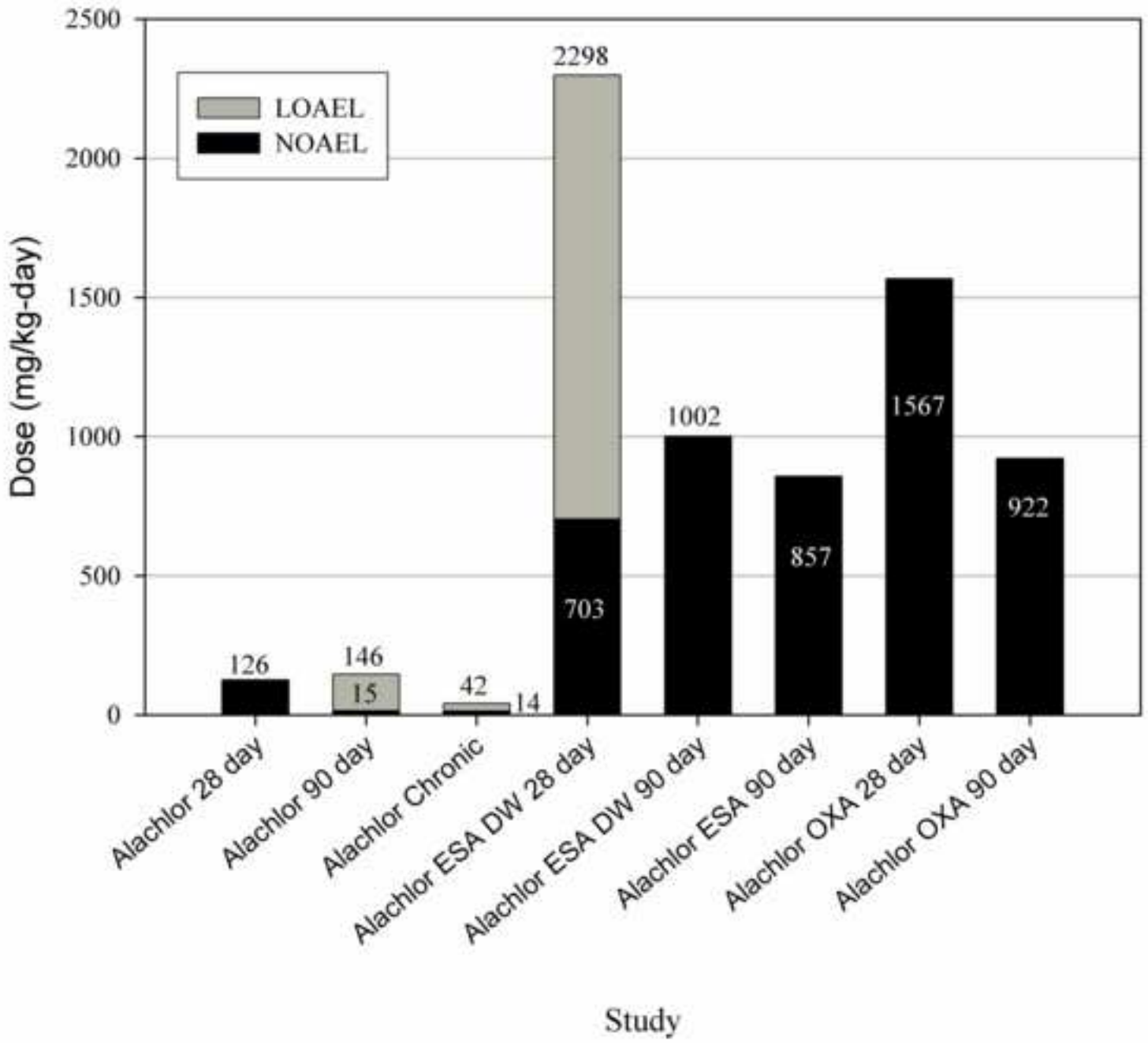

Figure 2b

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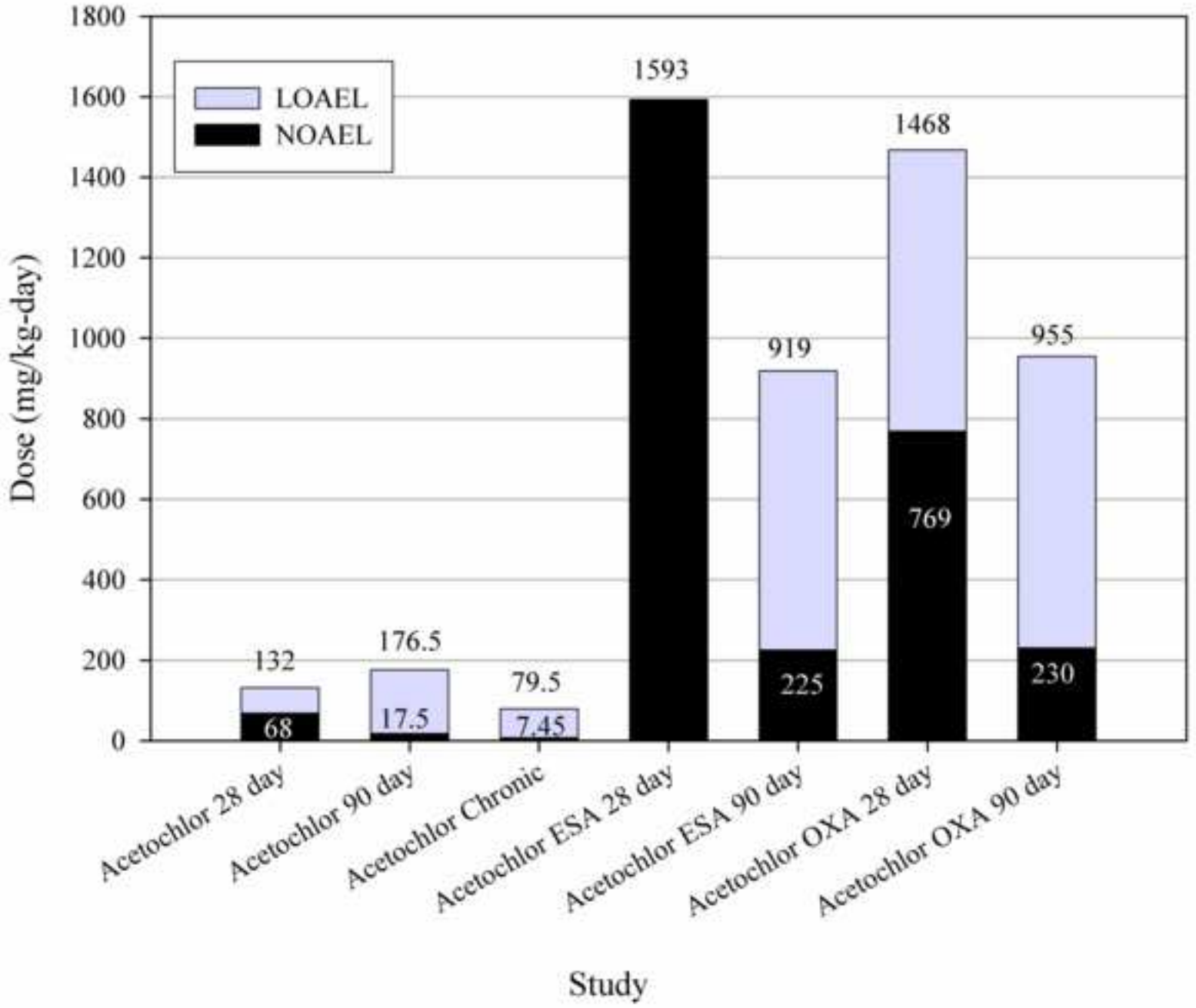

Figure 3a

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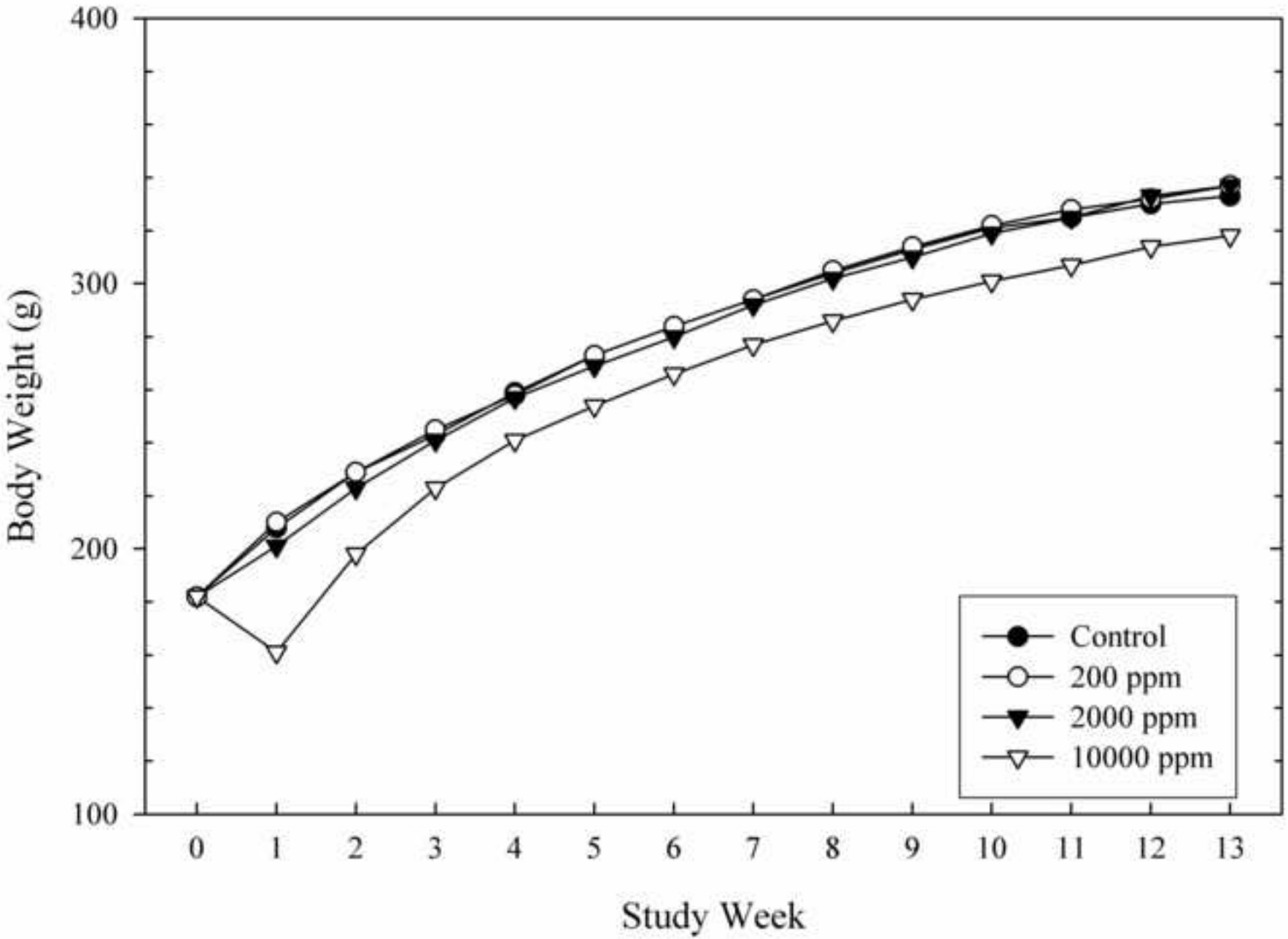

Figure 3b

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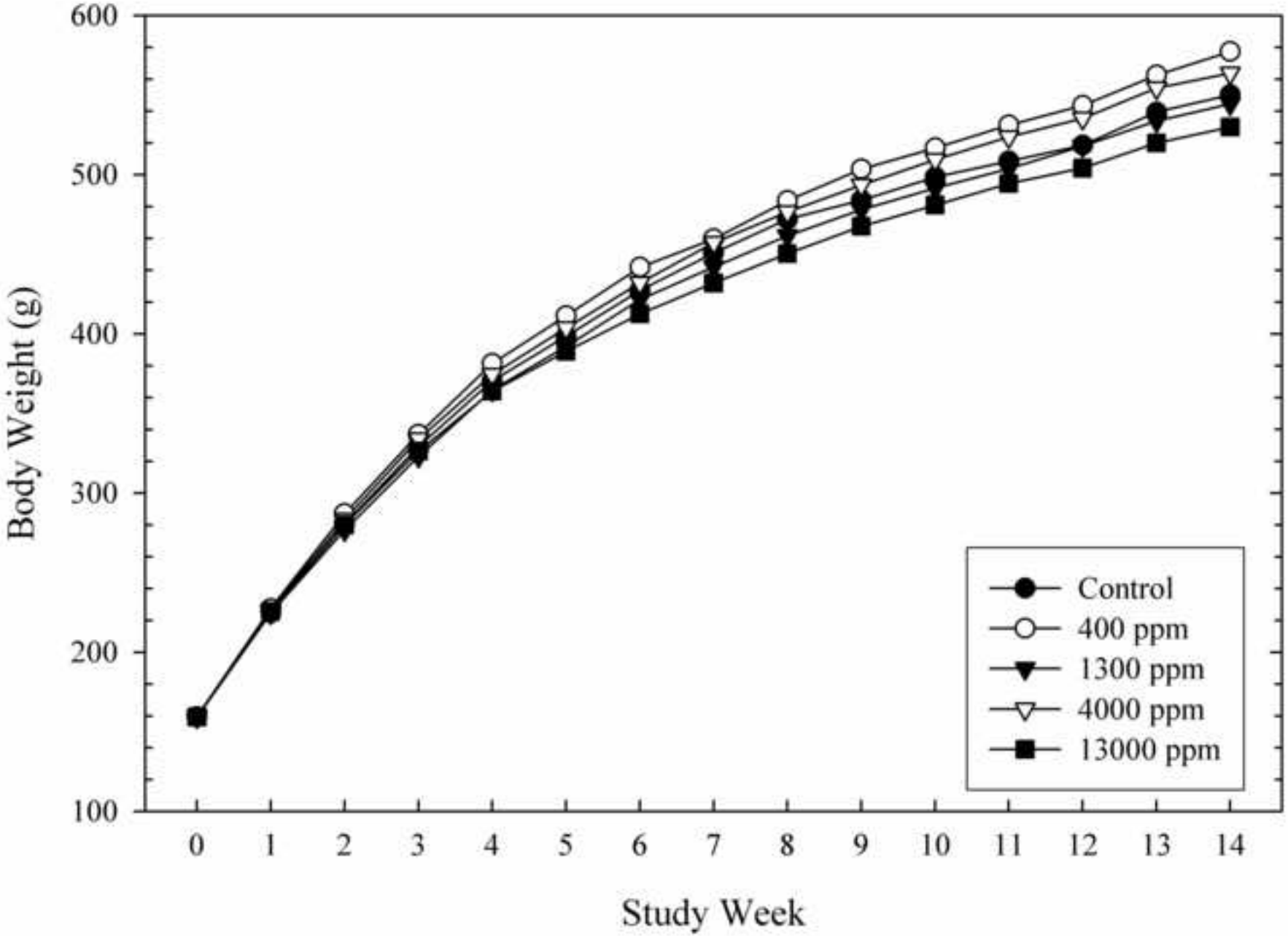

Figure 3c

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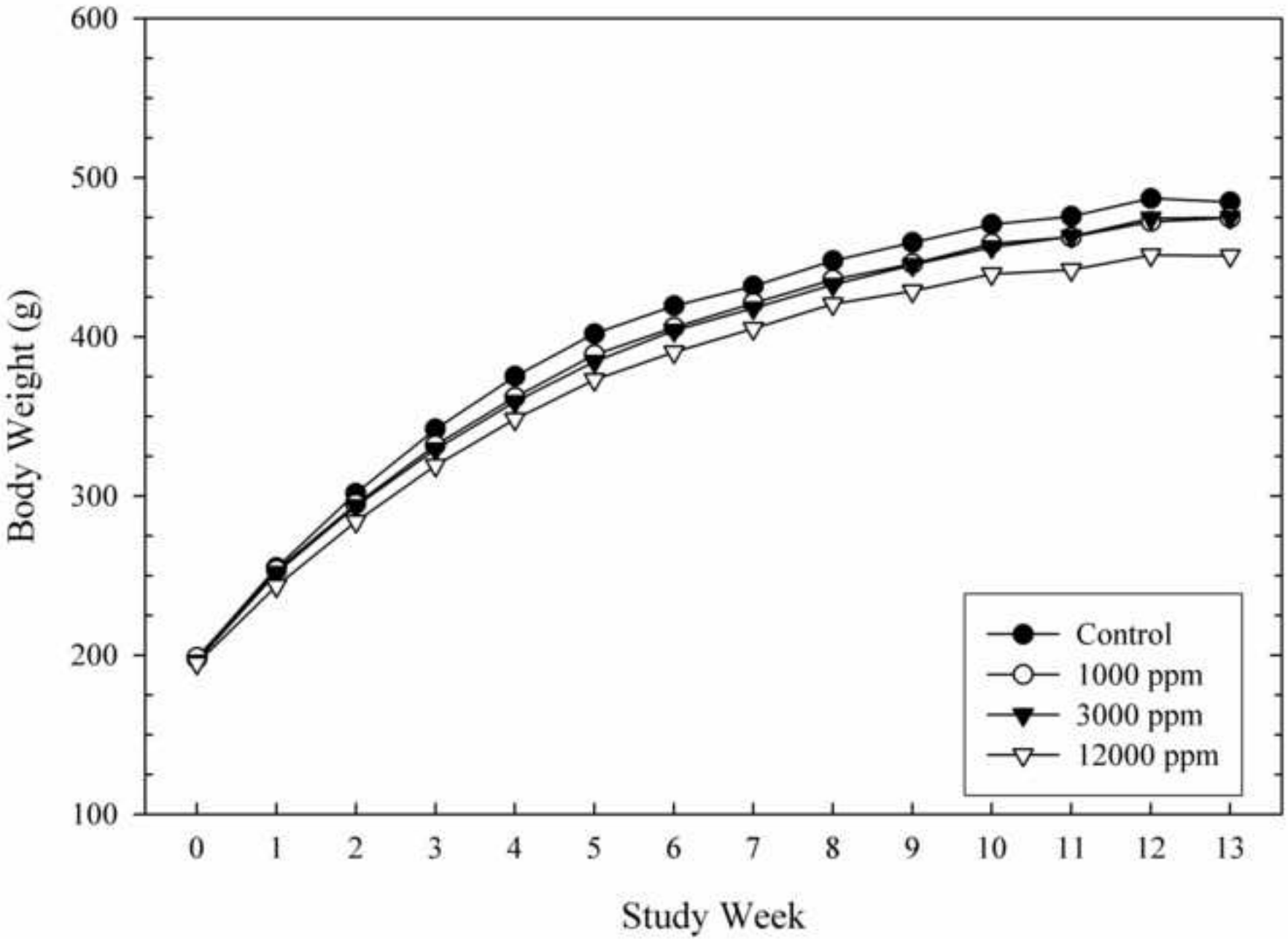

Figure 3d

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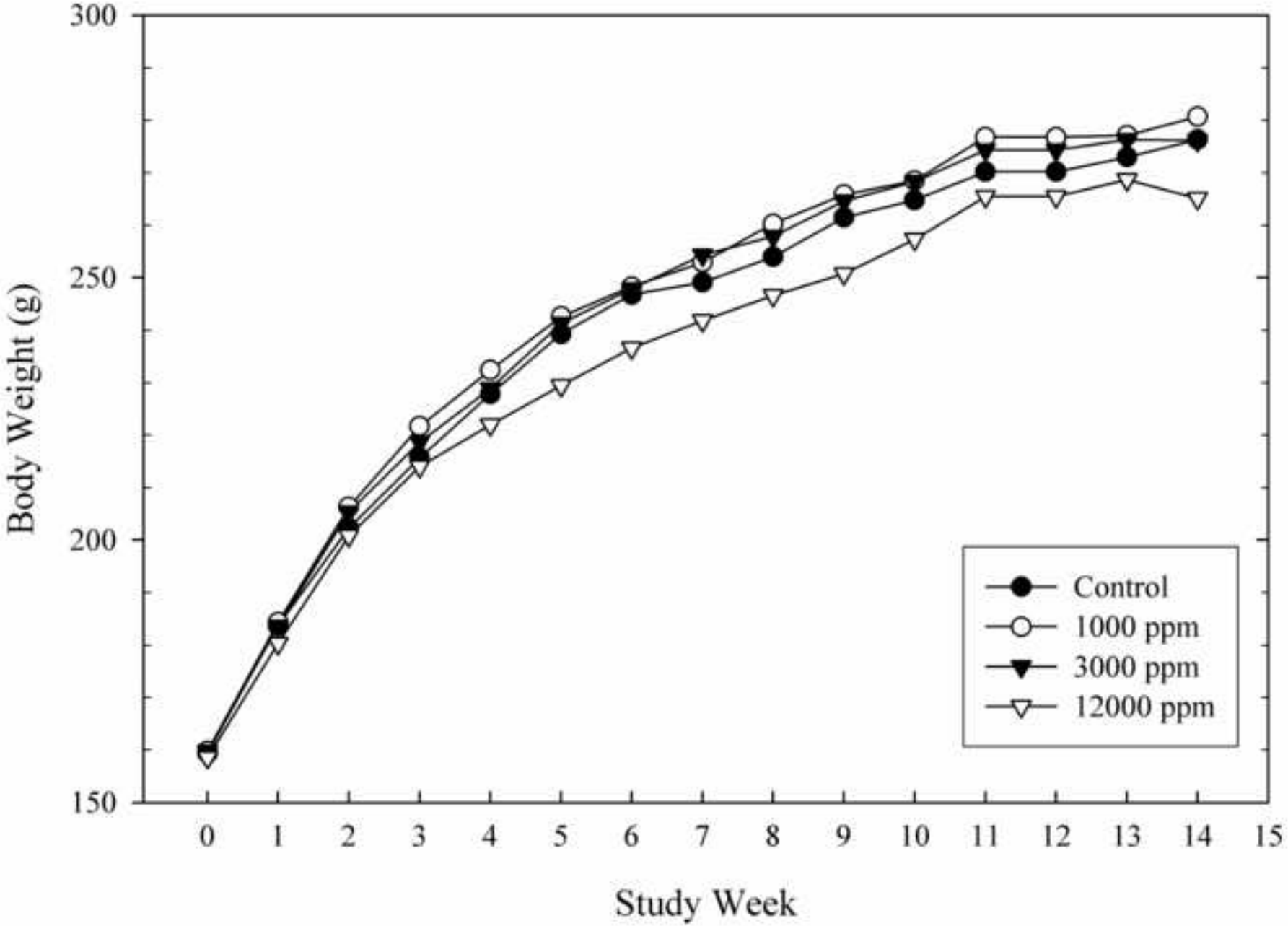

Figure 3e

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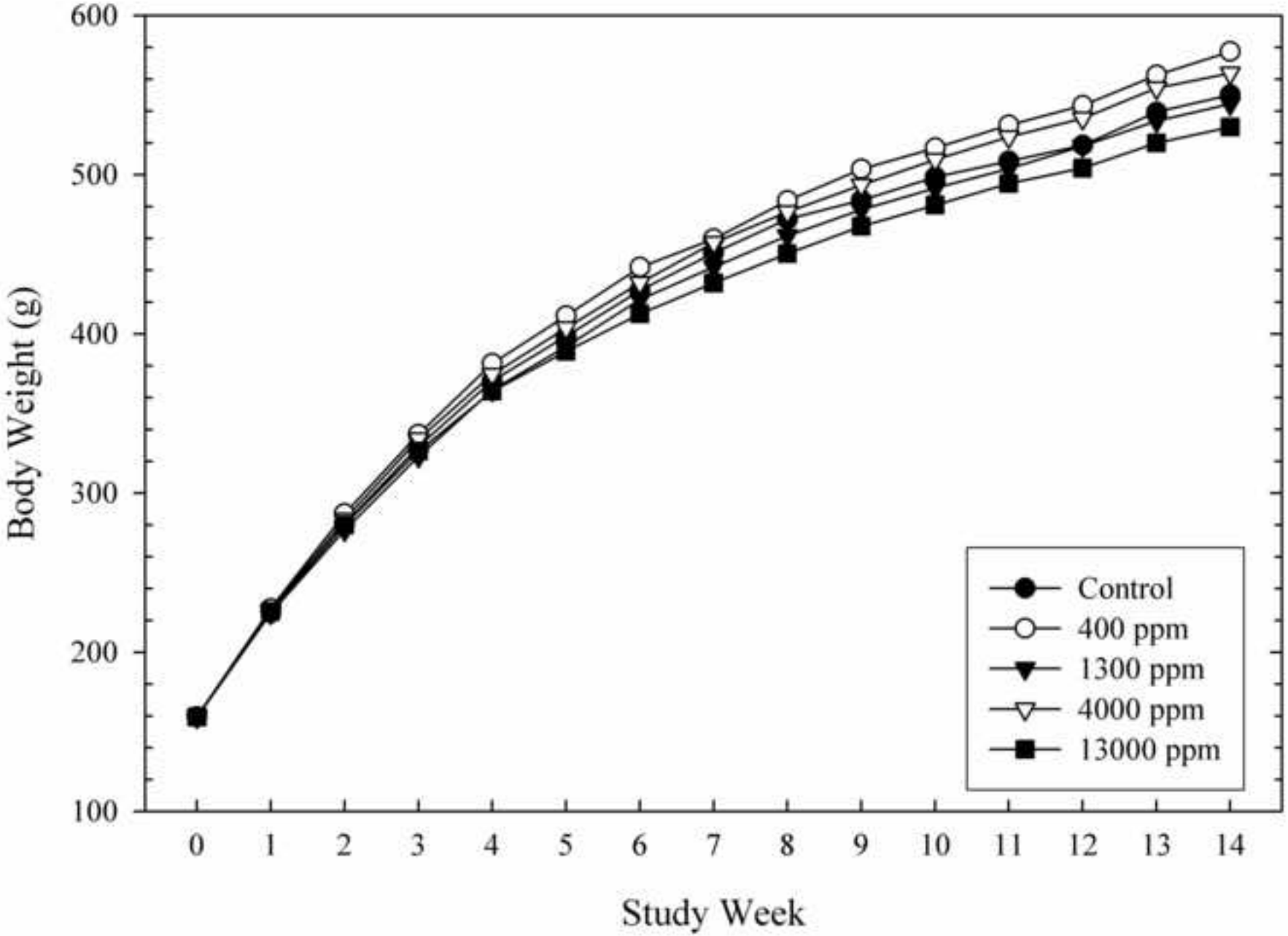

Table 1. Summary of the Subchronic Toxicity Studies Available for the Alachlor and Acetochlor Degradates					
Chemical	Duration of Study	Animal Model Species	Route of Administration	Dosing Regimen	Reference
Alachlor ESA	28 days	Rat	Diet	0, 700, 2000, 7000, and 20,000 ppm [Males – 0, 68, 183, 656, and 2217 mg/kg-day; Females – 0, 75, 205, 749, and 2378 mg/kg-day]	Siglin (1993)
	90 days	Rat	Diet	0, 3000, 6000, and 12,000 ppm [Males – 0, 195, 389, and 788 mg/kg-day;	Kirkpatrick (2002)

Table 1. Summary of the Subchronic Toxicity Studies Available for the Alachlor and Acetochlor Degradates					
				Females – 0, 222, 454, and 926 mg/kg-day]	
	91 days	Rat	Drinking water	0, 200, 2000, and 10,000 ppm [Males – 0, 16, 157, and 896 mg/kg-day; Females – 0, 23, 207, and r 1108 mg/kg-day]	Siglin (1993); Heydens et al. (1996)
	28 days	Rat	Diet	0, 1000, 10,000, and 20,000 ppm [Males – 0, 74.21, 754.26, and	Stout and Thake (2000)

Table 1. Summary of the Subchronic Toxicity Studies Available for the Alachlor and Acetochlor Degradates					
				1539.32 mg/kg-day; Females – 0, 83.35, 829.68, and 1595.26 mg/kg-day]	
Alachlor OXA	90 days	Rat	Diet	0, 400, 1300, 4000, and 13,000 ppm [Males – 24.9, 83.5, 261.1, and 834.6 mg/kg-day; Females – 0, 29.1, 95.4, 290.9, and 1008.3 mg/kg-day	Lemen et al. (2000)
	28 days	Rat	Diet	0, 3000, 6000, and 12,000 ppm	Lees (2000a)

Table 1. Summary of the Subchronic Toxicity Studies Available for the Alachlor and Acetochlor Degradates					
				[Males – 0, 370.3, 766.6, and 1578.7 mg/kg-day; Females – 0, 374.6, 762.3, and 1607.4 mg/kg-day]	
Acetochlor ESA	90 days	Rat	Diet	0, 1000, 3000, and 12,000 ppm [Males – 0, 75.0, 225.4, and 919.4 mg/kg-day; Females – 85.2, 259.1, and 1073.2 mg/kg-day]	Lees (2000b)
	28 days	Rat	Diet	0, 3000, 6000, and 12,000 ppm	Williams (2000a)

Table 1. Summary of the Subchronic Toxicity Studies Available for the Alachlor and Acetochlor Degradates					
				[Males – 0, 372.6, 768.5, and 1467.9 mg/kg-day; Females – 0, 367.2, 737.3, and 1506.5 mg/kg-day]	
Acetochlor OXA	90 days	Rat	Diet	0, 1000, 3000, and 12,000 ppm [Males – 0, 77.2, 230.2, and 955.2 mg/kg-day; Females – 86.5, 268.0, and 1082.7 mg/kg-day]	Williams (2000b)

Table 2a

Table 2a. Summary of Effects of Acetochlor ESA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Lees, 2000b)					
Parameter	**Sex**	**Dose – ppm (mg/kg-day, male / female)**			
		0	**3,000 (370.3 / 374.6)**	**6,000 (766.6 / 762.3)**	**12,000 (1578.7 / 1607.4)**
Plasma TSH (ng/ml)	M	9.81 ± 5.53	11.23 ± 4.64 (↑14.5%)	13.62 ± 4.54 (↑38.8%)	15.77 ±10.44 (↑60.8%)
	F	6.45 ± 2.99	4.16 ± 1.52 (↓35.5%)	6.26 ± 3.49 (↓2.9%)	4.02 ± 1.52 (↓37.7%)
Total Plasma T3 (mol/l)	M	1.27 ± 0.05	1.38 ± 0.19 (↑8.66%)	1.59[b] ± 0.17 (↑25.2%)	1.32 ± 0.18 (↑3.9%)
	F	1.06 ± 0.15	1.22 ± 0.22 (↑15.1%)	1.04 ± 0.12 (↓1.9%)	1.20 ± 0.16 (↑13.2%)
Free Plasma T3 (mol/l)	M	1.47 ± 0.37	1.50 ± 0.28 (↑2.0%)	1.70 ± 0.38 (↑15.6%)	1.88 ± 0.67 (↑27.9%)
	F	1.37 ± 0.34	1.41 ± 0.16 (↑2.9%)	1.24 ± 0.20 (↓9.5%)	1.27 ± 0.19 (↓7.3%)

Table 2a. Summary of Effects of Acetochlor ESA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Lees, 2000b)					
Total Plasma T4 (mol/l)	M	68.0 ± 9.5	64.9 ± 6.0 (↓4.6%)	71.6 ± 6.9 (↑5.3%)	78.0 ± 8.9 (↑14.7%)
	F	52.7 ± 9.6	52.8 ± 7.0 (↑0.2%)	67.3[a] ± 5.6 (↑27.7%)	44.4 ± 13.1 (↓15.7%)
Free Plasma T4 (mol/l)	M	14.54 ± 3.85	13.24 ± 2.15 (↓9.0%)	14.16 ± 0.83 (↓2.6%)	16.51 ± 2.59 (↑13.5%)
	F	7.95 ± 1.48	11.06 [a] ± 1.70 (↑39.0%)	12.02[b] ± 1.66 (↑51.2%)	8.53 ± 1.94 (↑7.3%)
T4-UDPGT activity – pmol/hour/g liver[c]	M	181.7 ± 55.9	190.0 ± 69.6 (↑4.6%)	179.2 ± 46.4 (↓1.4%)	245.4 ± 46.2 (↑35.1%)
	F	241.0 ± 67.0	207.3 ± 63.4 (↓14.0%)	244.0 ± 45.2 (↑1.2%)	373.1[b] ± 57.8 (↑54.8%)
T4-UDPGT activity – pmol/hour/ total liver[c]	M	2580 ± 922	2641 ± 674 (↑2.4%)	2233 ± 599 (↓13.4%)	3519[a] ± 600 (↑36.4%)
	F	2137 ± 707	1975 ± 779 (↓7.6%)	2114 ± 283 (↓1.1%)	3436[b] ± 250 (↑60.8%)

Table 2a. Summary of Effects of Acetochlor ESA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Lees, 2000b)

T4-UDPGT activity – pmol/hour/mg protein[c]	M	14.5 ± 4.6	13.6 ± 3.9 (↓6.2%)	15.5 ± 5.6 (↑6.9%)	18.5 ± 2.9 (↑27.6%)
	F	19.1 ± 6.3	19.4 ± 7.5 (↑1.6%)	18.7 ± 4.3 (↓2.1%)	33.4[b] ± 6.3 (↑74.9%)

M – male; F – female; ppm – parts per million; N = 5 animals/sex/group; TSH – thyroid stimulating hormone; T3 – iodothyronine; T4 – thyroxine; UDPGT –uridine diphosphate glucuronosyl transferase; [T4-UDPGT is the hepatic UDPGH-mediated clearance of the thyroid hormone thyroxine (T4)]; ppm – parts per million; [a]-Statistically significant difference from the control group mean at the 5% level (Student's t-test, two sided); [b]-Statistically significant difference from control group mean at the 1% level (Student's t-test, two sided); ± - (standard deviation); (%) – percent change from control; [c] – T4-UDPGT (hepatic T4-UDPGT) activity normalized to g liver, total liver, or mg protein.

Table 2b

Table 2b. Summary of Effects of Acetochlor OXA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Williams, 2000b)					
		Dose – ppm (mg/kg-day, male / female)			
Parameter	**Sex**	**0**	**3,000 (372.6 / 367.2)**	**6,000 (768.5 / 737.3)**	**12,000 (1467.9 / 1506.5)**
Plasma TSH (ng/ml)	M	15.90 ± 10.63	15.35 ± 7.11 (↓3.5%)	9.14 ± 5.25 (↓42.5%)	10.00 ± 7.37 (↓37.1%)
	F	7.63 ± 4.74	5.32 ± 3.34 (↓30.3%)	3.99 ± 1.99 (↓47.7%)	3.48 ± 1.49 (↓54.4%)
Total Plasma T3 (mol/l)	M	1.52 ± 0.14	1.32 ± 0.28 (↓13.2%)	1.30 ± 0.14 (↓14.5%)	1.15[b] ± 0.08 (↓24.3%)
	F	1.37 ± 0.23	1.37 ± 0.21 (0%)	1.21 ± 0.14 (↓11.7%)	1.20 ± 0.23 (↓12.4%)
Free Plasma T3 (mol/l)	M	2.01 ± 0.53	1.89 ± 0.42 (↓6.0%)	1.84 ± 0.68 (↓8.5%)	1.30[a] ± 0.61 (↓35.3%)
	F	1.81 ± 0.30	1.58 ± 0.46 (↓12.7%)	1.67 ± 0.18 (↓7.7%)	1.45 ± 0.70 (↓19.9%)

Table 2b. Summary of Effects of Acetochlor OXA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Williams, 2000b)					
Total Plasma T4 (mol/l)	M	74.4 ± 14.3	63.3 ± 15.8 (↓14.9%)	73.8 ± 8.1 (↓0.81%)	56.5 ± 5.4 (↓24.1%)
	F	54.4 ± 14.1	58.7 ± 13.0 (↑7.9%)	64.7 ± 12.0 (↑18.9%)	59.3 ± 23.6 (↑9.0%)
Free Plasma T4 (mol/l)	M	20.68 ± 2.59	17.41 ± 3.89 (↓15.8%)	22.66 ± 4.86 (↑9.6%)	16.31 ± 2.29 (↓21.1%)
	F	15.91 ± 4.80	16.04 ± 5.31 (↑0.82%)	16.02 ± 3.15 (↑0.69%)	16.35 ± 6.68 (↑2.7%)
T4-UDPGT activity – pmol/hour/g liver[c]	M	255.1 ± 62.0	212.3 ± 54.2 (↓16.8%)	252.4 ± 78.0 (↓1.1%)	293.7 ± 86.1 (↑15.1%)
	F	404.2 ± 58.8	321.1 ± 59.9 (↓20.6%)	374.5 ± 93.5 (↓7.3%)	253.1[b] ± 59.4 (↓37.4%)
T4-UDPGT activity – pmol/hour/ total liver[c]	M	4079 ± 781	3241 ± 807 (↓20.5%)	3899 ± 1173 (↓4.4%)	4203 ± 1021 (↑3.0%)
	F	3941 ± 554	3048 ± 644 (↓22.7%)	3510 ± 807 (↓10.9%)	2303[b] ± 50 (↓41.6%)

Table 2b. Summary of Effects of Acetochlor OXA on a Number of Biochemical Parameters Critical to Thyroid Homeostasis in Rats (28-day dietary study - Williams, 2000b)

T4-UDPGT activity – pmol/hour/mg protein[c]	M	16.5 ± 4.9	15.5 ± 5.7 (↓6.1%)	17.4 ± 7.9 (↑5.5%)	19.5 ± 4.8 (↑18.2%)
	F	31.7 ± 7.0	29.2 ± 4.3 (↓7.9%)	29.8 ± 8.3 (↓6.0%)	26.2 ± 10.8 (↓17.4%)

M – male; F – female; ppm – parts per million; N = 5 animals/sex/group; TSH – thyroid stimulating hormone; T3 – iodothyronine; T4 – thyroxine; UDPGT –uridine diphosphate glucuronosyl transferase; [T4-UDPGT is the hepatic UDPGH-mediated clearance of the thyroid hormone thyroxine (T4)]; ppm – parts per million; [a]-Statistically significant difference from the control group mean at the 5% level (Student's t-test, two sided); [b]-Statistically significant difference from control group mean at the 1% level (Student's t-test, two sided); ± - (standard deviation); (%) – percent change from control; [c] – T4-UDPGT (hepatic T4-UDPGT) activity normalized to g liver, total liver, or mg protein.

Table 3

Table 3. Hematological and Clinical Chemistry Parameters (mean ± SD)

Observed in a 91-day Drinking Water Toxicity Study in Rats with Alachlor ESA

(Siglin, 1993; Heydens et al., 1996)

Effect		**Sex**	**Drinking Water Concentration - ppm (mg/kg-day, male / female)**			
			0	**200** **(16 / 23)**	**2,000** **(157 / 207)**	**10,000** **(896 / 1108)**
Water intake (g/kg-day)	Week 1	M	115 ± 14.3	117 ± 9.6 (↑1.7%)	98 ± 22.3 (↓14.8%)	56[b] ± 45.4 (↓51.3%)
		F	144 ± 10.1	144 ± 10.6 (0%)	124[a] ± 8.4 (↓13.9%)	90[b] ± 31.5 (↓37.5%)
	Week 2	M	105 ± 6.2	105 ± 8.2 (0%)	102 ± 19.2 (↓2.9%)	142[a] ± 51.2 (↑35.2%)
		F	136 ± 15.2	128 ± 8.1 (↓5.9%)	116[a] ± 7.8 (↓14.7%)	148 ± 28.6 (↑8.8%)
	Week 13	M	62 ± 2.0	64 ± 1.6 (↑3.2%)	64 ± 2.8 (↑3.2%)	73[b] ± 3.9 (↑17.7%)
		F	93 ± 10.3	104 ± 11.2 (↑11.8%)	97 ± 9.6 (↑4.3%)	102 ± 9.9 (↑9.7%)

Table 3. Hematological and Clinical Chemistry Parameters (mean ± SD)

Observed in a 91-day Drinking Water Toxicity Study in Rats with Alachlor ESA

(Siglin, 1993; Heydens et al., 1996)

Effect	Sex	Drinking Water Concentration - ppm (mg/kg-day, male / female)			
		0	**200 (16 / 23)**	**2,000 (157 / 207)**	**10,000 (896 / 1108)**
Hemoglobin (g/dL)	M	16.45 ± 0.288	16.40 ± 0.216 (↓0.3%)	15.71 ± 0.957 (↓4.5%)	15.94 [b] ± 0.391 (↓3.1%)
	F	15.71 ± 0.857	16.23 ± 0.422 (↑3.3%)	15.98 ± 0.349 (↑1.7%)	16.02 ± 0.220 (↑2.0%)
Hematocrit (%)	M	46.01 ± 0.754	46.41 ± 1.523 (↑0.9%)	45.05 ± 1.678 (↓2.1%)	43.80 [a] ± 0.96 (↓4.8%)
	F	43.78 ± 2.974	45.37 ± 1.306 (↑3.6%)	43.55 ± 1.211 (↓0.53%)	44.14 ± 1.749 (↑0.82%)
Red Blood Cell Count (millions per µL)	M	9.520 ± 0.1033	9.60 ± 0.1633 (↑.84%)	9.22 [b] ± 0.2616 (↓3.2%)	9.022 [a] ± 0.1922 (↓5.23%)
	F	8.340 ± 0.5038	8.670 ± 0.2111 (↑4.0%)	8.360 ± 0.1646 (↑0.24%)	8.460 ± 0.2675 (↑1.4%)
Mean Cell Volume	M	48.34 ± 0.687	48.34 ± 1.054	48.85 ± 0.965	48.57 ± 0.714

Table 3. Hematological and Clinical Chemistry Parameters (mean ± SD)

Observed in a 91-day Drinking Water Toxicity Study in Rats with Alachlor ESA

(Siglin, 1993; Heydens et al., 1996)

Effect	Sex	Drinking Water Concentration - ppm (mg/kg-day, male / female)			
		0	**200 (16 / 23)**	**2,000 (157 / 207)**	**10,000 (896 / 1108)**
(femtoliters)			(0%)	(↑1.1%)	(↑0.5%)
	F	52.48 ± 0.547	52.33 ± 0.688 (↓0.29%)	52.10 ± 0.616 (↓0.72%)	52.15 ± 0.546 (↓0.63%)
Mean Cell Hemoglobin (pg)	M	17.28 ± 0.305	17.08 ± 0.326 (↓1.2%)	17.02 ± 0.830 (↓1.5%)	17.67 [b] ± 0.316 (↑2.3%)
	F	18.86 ± 0.455	18.72 ± 0.175 (↓0.74%)	19.12 ± 0.336 (↑1.4%)	18.94 ± 0.378 (↑0.42%)
Mean Cell Hemoglobin Concentration (g/dL)	M	35.76 ± 1.019	35.38 ± 1.181 (↓1.1%)	34.91 ± 2.269 (↓2.4%)	36.4 ± 0.738 (↑1.8%)
	F	35.92 ± 1.187	35.76 ± 0.729 (↓0.45%)	36.70 ± 1.010 (↑2.2%)	36.36 ± 0.999 (↑1.2%)

M – males; F – females; N=9-10 animals; (%) – percent change from control; ↑ - percent increase compared to control value; ↓ - percent decrease compared to control value; [a]-Statistically significant difference from the control group mean at the 5% level (Student's t-test, two sided); [b]-Statistically significant difference from control group mean at the 1% level (Student's t-test, two sided)

Table 4

Table 4. Potential Key Studies and Points of Departure (POD) for RfD Derivation for Acetanilide Degradates					
Degradate	**Critical Effect(s)**	**NOAEL**	**LOAEL**	**POD[a]**	**Reference**
		(mg/kg-day, males / females)		**mg/kg-day**	
Alachlor ESA	No adverse effects on body weight	788 / 926	NA	788	Kirkpatrick (2002)
Alachlor OXA	No adverse effects on body weight	834 / 1008	NA	834	Lemen et al., (2000)
Acetochlor ESA	Decreased body weight gain, decreased food consumption, and decrease food utilization	225 / 259	919 / 1073	225	Lees (2000b)

Table 4. Potential Key Studies and Points of Departure (POD) for RfD Derivation for Acetanilide Degradates					
Acetochlor OXA	Decreased body weight gain and decrease food utilization	230 / 268	955 / 1082	230	Williams (2000b)

[a]-Lower of the NOAEL for males and females; NA – not applicable