APPENDIX A: Mixture Data for Alachlor

Several toxicities studies were identified in ECOTOX that dealt with mixtures involving alachlor. A brief overview of the most relevant studies is provided below. All of the ECOTOX mixture studies for alachlor can be found in **APPENDIX E**.

A 5-year 'field validation' study investigated the effects of three pesticides on estuaries in North Carolina from runoff from adjacent farm lands (MRID 44105503). Because the study involved investigating the runoff of three pesticides (alachlor, terbufos, and permethrin) that were applied on a working farm (44,186 acres; corn, winter wheat, soybean rotation) and their impact on the adjacent estuarine community, the potential effects of a single active ingredient cannot be separated out. Therefore, this study has been classified as 'supplemental' (it is scientifically valid but it cannot be used to assess risk of a single active ingredient). This study is used to help address issues related to environmental mixtures containing alachlor and other pesticides.

Alachlor applications during the study were to corn at an application rate of 1.4 lb a.i./acre. The peak alachlor concentration in the estuarine creeks during the study was 48 µg a.i./L (it occurred the day after an intense storm event two weeks after application). The peak concentration of alachlor in the farm ditches was 58 µg a.i./L following the same storm event. Alachlor did show seasonal carry-over at low concentrations. To explore abundance and biomass of the estuarine communities, biological sampling was conducted on nektonic and benthic communities. Based on comparisons with estuarine environments that received runoff from forested, non-agricultural areas to the estuarine areas that received agricultural runoff, the pesticides in the study did not have a measurable impact on the estuarine biological community adjacent to application sites.

In another study, Hayes *et al*. (2006) examined the effects of nine pesticides (alone and in mixtures) on larval growth, development, sex differentiation, and immune function in leopard frogs (*Rana pipiens*) (E85815). The nine pesticides (4 herbicides: atrazine, metolachlor, alachlor, and nicosulfuron; 3 insecticides: cyfluthrin, cyhalothrin, and tebupirimphos; and 2 fungicides: methalaxyl and propiconizole) were assessed individually (at 0.1 ppb) and in mixtures (at 0.1 ppb x each chemical and 10 ppb x each chemical). No single pesticide affected mortality or time to metamorphosis (except propiconizole) ($p > 0.05$). Alachlor alone had no impact on the measured endpoints. Animals exposed to pesticide mixtures at 0.1 ppb (of each chemical) had significantly longer larval periods. The mixtures, at 0.1 ppb, also impacted size at metamorphosis. All of the animals exposed to the 9-compound mixture at 10 ppb died after the first day of exposure. Based on the information in the study, it is not possible to determine which pesticides in the mixtures contributed to the observed effects or which pesticides (if any) were effectors, enhancers, or were neutral.

Pennington (1996, E106637) explored the acute toxicity of atrazine, alachlor, and a mixture of atrazine/alachlor to the estuarine phytoplankter *Pavlova* sp. (Prymnesiophyceae). A full review of this study has not been conducted at this time.

The author reports that the 96-hr EC_{50} values (based on growth rate) for atrazine and alachlor are 147 µg a.i./L and 5,661 µg a.i./L, respectively. The 96-hr EC_{50} values for the atrazine/alachlor mixture (1/33 ratio) were 63.97 µg a.i./L (atrazine) and 2,126 µg a.i./L (alachlor). The author concluded, therefore, that the atrazine/alachlor mixture showed additive toxicity with a tendency toward synergism.

Doherty (1997; E105925) studied the acute effects of five herbicides (alachlor, atrazine, fluridone, glyphosate, and imazapyr) singly and as mixtures on the estuarine diatom *Thalassiosira weisflogii*. A full review of this study has not been conducted at this time. Cultured *T. weisflogii* were used and all of the tests involved 48-hr static exposures and were conducted using technical grade active ingredients. The EC_{50} value for alachlor alone was 672 µg a.i./L based on a growth endpoint. In this study, the alachlor/atrazine and alachlor/glyphosate mixtures showed additive effects; the alachlor/fluridone mixture and a mixture containing all five herbicides showed synergistic effects; and the alachlor/imazapyr mixture showed antagonistic effects.