

October 15, 2004

Clyde Livingston  
Chemical Regulatory Compliance  
Monsanto Company  
800 North Lindbergh Blvd.  
St. Louis, MI 63167

Dear Mr. Livingston:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for 2-chloro-N-(chloromethyl)-N-(2,6-diethylphenyl)acetamide posted on the ChemRTK HPV Challenge Program Web site on February 11, 2004. I commend Monsanto Company for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that Monsanto advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following e-mail addresses: [oppt.ncic@epa.gov](mailto:oppt.ncic@epa.gov) and [chem.rtk@epa.gov](mailto:chem.rtk@epa.gov).

If you have any questions about this response, please contact Dr. Ralph Northrop of the HPV Chemicals Branch, at 202-564-7666. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at [tsc-hotline@epa.gov](mailto:tsc-hotline@epa.gov).

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

/S/

Oscar Hernandez, Director  
Risk Assessment Division

Enclosure

cc: W. Penberthy  
M. E. Weber

**EPA Comments on Chemical RTK HPV Challenge Submission:  
2-Chloro-N-(chloromethyl)-N-(2,6-diethylphenyl)acetamide (CMA)**

**Summary of EPA Comments**

The sponsor, Monsanto Company, submitted a test plan and robust summaries to EPA for 2-Chloro-N-(chloromethyl)-N-(2,6-diethylphenyl)acetamide (CMA, CAS No. 40164-69-0) and the proposed analog alachlor (CAS No. 15972-60-8) dated December 15, 2003. EPA posted the submission on the ChemRTK HPV Challenge Web site on February 11, 2004.

EPA has reviewed this submission and has reached the following conclusions:

1. Analog Justification. The submitter proposed the use of data for the pesticide alachlor to satisfy physicochemical, fate and alga endpoints for CMA, but has not supported the case.
2. Physicochemical Properties. The submitter needs to provide measured vapor pressure for CMA and consider the need for water solubility testing.
3. Environmental Fate. The submitter needs to provide a measured hydrolysis value for CMA, and identify its hydrolysis products. The submitter needs to provide measured ready biodegradation data for CMA itself or for its hydrolysis products. The submitter needs to provide photodegradation and fugacity data for CMA unless the submitter can show that the hydrolysis of CMA will proceed rapidly enough that calculating the photodegradation and fugacity of CMA would be irrelevant.
4. Health Effects. Data are adequate for developmental toxicity, acute toxicity, and gene mutation, if additional information describing these tests is provided. Data are inadequate for assessing chromosomal aberrations, repeated-dose toxicity and reproductive toxicity. Test data need to be provided for these endpoints.
5. Ecological Effects. EPA reserves judgement on data adequacy for the submitted fish and invertebrate tests until it can be determined if the nominal concentrations measured were maintained during the tests. The submitter also needs to determine the hydrolysis half-life of the chemical in order to help explain the ecological test results. The use of alachlor as an analog for the alga endpoint has not been justified.

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.

**EPA Comments on the 2-chloro-n-(Chloromethyl)-n-(2,6-diethylphenyl)acetamide Challenge Submission**

**Test Plan**

Analog Justification. The submitter proposed the use of data for the pesticide alachlor to satisfy physicochemical, fate and algal toxicity endpoints for CMA: "The chemical structures of CMA and alachlor...are the same except that CMA has the chloromethyl moiety bonded to the acetanilide nitrogen and alachlor has a methoxymethyl group at that position. In other words, the chemical structures would be identical except that alachlor has CH<sub>3</sub>O- replacing and substituting for the labile Cl- of CMA." However, the mere assertion of structural similarity is insufficient to establish adequacy of an analog. The statement that alachlor is "a well-characterized chemical "analog" for CMA because they are so closely related in chemical structure and because CMA is readily converted to alachlor" is a non-sequitur, as the ability to synthesize one chemical from another does not bear directly on their suitability for sharing data. Although CMA and alachlor are structurally similar in a superficial way, there are important differences in their

chemistry that are ignored in this aspect of the submission. In fact, the submitter's own words belie the relevance of alachlor, not only by the reference to "the labile Cl- of CMA" but also in stating that "Biodegradation of alachlor by microbial organisms is the main method of degradation in the environment with a half-life in most soils of about 2-3 weeks", while asserting that CMA is hydrolyzed "extremely rapidly." Further, because CMA reacts readily with methanol or water, similar reactions are expected to occur with amine, hydroxyl, and thiol functional groups in biological systems. The test plan does not address any of these issues. In EPA's judgement, the submitter has not made a case for alachlor as an analog except for partition coefficient.

#### Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

The data provided by the submitter for melting point and boiling point are adequate for the purposes of the HPV Challenge Program.

*Vapor pressure.* The vapor pressure values provided by the submitter are not adequate for the purposes of the HPV Challenge Program because they are semiquantitative, open-ended, and are above the OECD testing threshold of  $7.5 \times 10^{-8}$  mm Hg. According to HPV Challenge guidelines, if a chemical has an estimated value above this value, then its vapor pressure needs to be determined. The submitter needs to provide a measured vapor pressure for this chemical following OECD TG 104.

*Partition coefficient.* The submitter provided measured partition coefficient data ( $\text{Log } K_{ow} = 3.09$ ) for the proposed analog alachlor, which contains a  $\text{N-CH}_2\text{-OCH}_3$  in place of  $\text{N-CH}_2\text{-Cl}$ . EPA estimated a  $\text{Log } K_{ow}$  of 3.84 for the sponsored chemical and 3.56 from the alachlor value (experimental value adjusted) using the KOWWIN software. While it is not clear whether  $\text{Log } K_{ow}$  measurement is relevant owing to CMA's rapid hydrolysis, (see stability in water section below), the  $\text{Log } K_{ow}$  values estimated by EPA suggest that the data provided are adequate for an assessment of this endpoint.

*Water solubility.* In the robust summary, the submitter indicates that CMA is unstable in water, and that it reacts slowly to form formaldehyde and hydrogen chloride. However, in the stability in water robust summary, the submitter indicates that CMA hydrolyzes extremely rapidly in water. These statements appear contradictory. The submitter did not provide a hydrolysis half-life; without this value, it is impossible to tell whether hydrolysis will proceed rapidly enough to render a water solubility measurement irrelevant. If hydrolysis is rapid enough that water solubility is irrelevant, then the submitter needs to provide a hydrolysis half-life to support this.

#### Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

*Photodegradation.* Alachlor is not an adequate analog for this endpoint because the methoxymethyl group of alachlor will result in different chemical and physical behaviors when compared to the chloromethyl group of CMA. Thus, the submitter needs to provide photodegradation data for CMA.

*Stability in water.* The robust summary indicates that CMA hydrolyzes extremely rapidly in water. However, the solubility in water robust summary indicates that it reacts slowly. The submitter needs to clarify this apparent discrepancy. Furthermore, the submitter provides no data supporting the statement that hydrolysis is extremely rapid. The submitter needs to provide adequate supporting information on the hydrolysis rate of CMA or perform a hydrolysis test following OECD Guideline 111.

*Biodegradation.* The biodegradation information provided by the submitter is not adequate for the purposes of the HPV Challenge Program. The test plan states that CMA is expected to hydrolyze and therefore biodegradation data are not needed. However, the submitter did not provide hydrolysis rate or half-life data. Furthermore, the submitter provided conflicting water solubility and hydrolysis information. Under water solubility, the submitter indicates that CMA reacts slowly, and under hydrolysis, the submitter indicates that CMA reacts extremely rapidly. Without a hydrolysis half-life, it is not clear that this chemical

will hydrolyze before it will biodegrade as is suggested by the submitter. The submitter needs to test CMA for ready biodegradation, unless it provides data that show (i) in addition to the hydrolysis half-life, the identities of the major hydrolysis products; and (ii) their ready biodegradability. The submitter provided biodegradation data for alachlor. However, as indicated under Analog Justification, alachlor should not be used as an analog for CMA. The submitter also provided biodegradation data for an acetochlor intermediate described as structurally similar to the test compound, but data for this compound cannot be used without better substance identification.

*Fugacity.* The fugacity data provided by the submitter are inadequate for the purposes of the HPV Challenge Program. The submitter states in the test plan that CMA is expected to hydrolyze in the environment; therefore, fugacity data for this compound were not provided. The submitter did not provide hydrolysis rate and half-life data. Without a hydrolysis half-life, it is not clear that this compound will hydrolyze before transport/distribution can take place. The data for this endpoint are inadequate without a better indication as to how hydrolysis will affect the transport/distribution of CMA. The submitter did provide transport/distribution data for alachlor, although, as mentioned above, alachlor should not be used as an analog for CMA for environmental fate endpoints.

#### Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

Data are most likely adequate for acute toxicity; however, additional information is needed to determine adequacy. Data are adequate for developmental toxicity, but important details are missing from the robust summaries. Information on gene mutation may be adequate, if additional information about the test can be described.

*Repeated-dose and reproductive toxicity.* Data are inadequate. The repeated-dose study was only 14 days and lacked histopathological examinations. The reproductive toxicity endpoint was not addressed by the developmental study submitted. Therefore, EPA recommends that the submitter conduct a combined repeated-dose/reproductive/developmental screening test (OECD TG 422) to address the reproductive and repeated-dose endpoints.

*Chromosomal Aberration.* A DNA repair assay is not an adequate test for addressing chromosomal aberrations; therefore, a chromosomal aberrations assay is needed. The submitter may conduct an *in vitro* chromosomal assay (OECD TG 473) or include this phase in the combined screening test protocol.

#### Ecological Effects (fish, invertebrates, and algae)

*Fish and invertebrates.* The data appear inadequate for CMA acute toxicity to fish and invertebrates because it is not clear that test concentrations were maintained during the study. In addition, the sponsor provided no quantitative data on the hydrolysis rate of CMA. Without these data, it is not clear what CMA concentrations were at the end of the test study period, or even if any CMA remained. If the information is unavailable, the tests need to be performed using OECD guidance (see *Guidance on Aquatic Toxicity Testing of Difficult Substances and Mixtures* on the OECD Web site at [http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono\(2000\)6](http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jm-mono(2000)6) ).

*Algae.* Data were submitted only for alachlor. As stated earlier, alachlor's suitability as an analog is unsupported in the test plan. Without attention to the issues identified in EPA's comments on the analog proposal, the relevance of the algae data is in question. Submission of alachlor data for fish and invertebrates could shed light on the issue by providing a comparison with the corresponding CMA data. The submitter also needs to clarify whether the alachlor data were determined using measured or nominal concentrations.

## **Specific Comments on the Robust Summaries**

### **General comments**

The robust summaries did not provide enough details. The submitter should consult EPA guidance documents for the preparation of robust summaries (<http://www.epa.gov/opptintr/chemrtk/guidocs.htm>).

### **Health Effects**

#### *Acute Toxicity*

*Oral.* Missing study details included test guideline, dose levels, number of animals per sex per group, method of administration (e.g., gavage), whether or not body weight determinations were made, tissues examined at necropsy, duration of post-exposure observation, tabulation of signs of toxicity and/or mortality by dose level and sex, 95% confidence interval, and statistical methods.

*Dermal.* Missing study details included test guideline, dose levels, number of animals per sex per group, preparation of the skin prior to dosing, whether or not body weight determinations were made, tissues examined at necropsy, duration of post-exposure observation, tabulation of signs of toxicity and/or mortality by dose level and sex, 95% confidence interval, and statistical methods.

#### *Genetic Toxicity*

*Gene Mutation.* Missing study details noted in the summary for the bacterial/yeast gene mutation assay included test substance purity, test guideline, test concentrations, identification of strains used, identity of positive controls, number of replicates per test concentration, identification and source of metabolic activation, criteria for a positive response, whether or not both positive and negative controls gave the appropriate response, mean number of revertant colonies per plate for treated and control cultures, statistical methods and results, and whether or not cytotoxicity was observed.

#### *Developmental Toxicity*

Missing study details included number and percent of live offspring, full list of clinical chemistry and blood parameters evaluated, full list of parental organs and tissues that were weighed and histologically examined at necropsy, and tabulations of signs of maternal toxicity by dose level and sex.

### **Ecological Effects**

*Fish.* Missing study details noted for both summaries included mortality and signs of toxicity by test concentration and time point, mean fish loading, whether or not concentrations were monitored, temperature range during the study, water hardness, lighting conditions, total organic carbon (TOC), and control response.

*Invertebrates.* Missing study details included mortality and signs of toxicity by test concentration and time point, mean daphnia loading, whether or not concentrations were monitored, total organic carbon (TOC), hardness, lighting conditions, and control response.

*Algae.* Missing study details included results of test concentration measurements, whether or not the reported EC<sub>50</sub> was based on measured concentrations, control growth during the study, percent inhibition on cell density per concentration, lighting, pH, temperature, and control response.

### **Followup Activity**

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.