ROBUST SUMMARIES

201-14949

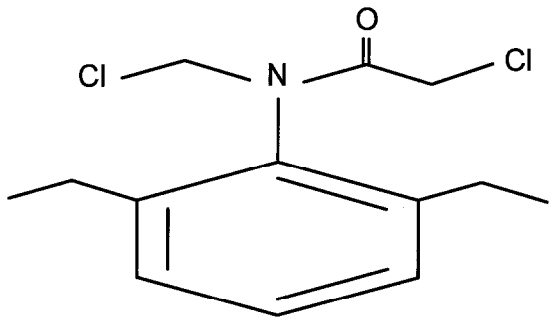
I. General Information

a. Substance Identification

CAS Registry Number: 40164-69-0

Chemical Name: Acetamide, 2-chloro-N-(chloromethyl)-N-(2,6-diethylphenyl)-

Structural Formula:



CMA

Other Names: CMA
2-Chloro-2',6'-diethylphenyl-N-(chloromethyl)acetanilide
Lasso Step II Intermediate
CP 57922
MON 39816

RECEIVED
OPPT CBIC
03 DEC 24 AM 9: 17

b. Substance Description

Chemical Formula:	C13H17Cl2N1O1
Molecular Weight:	M.W. = 274.19
Appearance:	Recrystallized (2X) product is white crystalline solid. Technical material is essentially clear, amber to orange-brown liquid at 40 C, yellow solid at ambient temperatures
Odor:	Chlorine-like odor
Flash point:	230 C (Method: Tag Closed Cup)
Stability and Reactivity:	CMA reacts with water giving off formaldehyde fumes and hydrogen chloride. Unstable at temperatures above 130 C. Certain conditions of storage followed by heating may result in the formation and release of bis(chloromethyl)ether and chloromethyl methyl ether.

Polymerization hazards:	None
Transportation hazards:	CMA is not hazardous under the applicable DOT, ICAO/IATA, or IMDG regulations.

c. Substance Used as Chemical Intermediate for the Manufacture of Acetanilide Herbicides

CMA can be used to manufacture either alachlor, [2-chloro-N-(2,6-diethylphenyl)-N-methoxymethylacetamide], or butachlor, [N-(butoxymethyl)-2-chloro-N-(2,6-diethylphenyl)acetamide], the active ingredients in several herbicide products. CMA is produced by Monsanto Company at a single manufacturing site within the United States of America located at Muscatine, Iowa.

CMA is only used as a process intermediate for the production of the final products used as herbicides known to be related to the acetanilide type of chemistry. During the final step in the manufacturing process of alachlor or butachlor, CMA can be converted to the desired pesticide active ingredient through a simple reaction with the appropriate alcohol, reaction with methanol will produce alachlor, or reaction with butanol will produce butachlor. The final products, as active pesticide ingredients, are thoroughly studied and characterized in risk assessment evaluations addressing toxicological endpoints identified by the SIDS endpoints. Within the United States, pesticide products are regulated by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and must be registered for use by the US EPA. Other countries also have similar regulations and an evaluation process in effect that must be completed before the pesticide can be approved for any specific application. Not only the active ingredient itself, but product formulations containing the active ingredient together with other inert ingredients and associated impurities must receive governmental registration approval. With considerable similarity in chemical structure between CMA and the more extensively evaluated active ingredient that is the final product, similar conclusions about the potential risks to human health or the environment already established for the active ingredient may also pertain to CMA.

Although CMA is only produced as a chemical intermediate for the manufacture of other acetanilide herbicide products, and is not the final pesticide chemical product that is distributed for commercial application, some CMA produced and stored in a small inventory warehouse at the Monsanto Company location at Muscatine, Iowa, can be shipped to other manufacturing plants located throughout the world for final conversion to either alachlor or butachlor. Thermal stability data show CMA to be stable up to 60 degrees C for prolonged periods of time, even better than alachlor. A number of standard drum linings as well as stainless steel and nickel are satisfactory container materials for CMA. Alachlor produced from storage stability samples of CMA was equivalent to, or better than, the alachlor control in terms of quality and yield. (D.E. Bissing and D.E. Baldus, "Shipping Feasibility Study of 2-Chloro-2',6'-Diethyl-N-(chloromethyl) Acetanilide (CMA)). CMA is stored and shipped in inert-lined containers, normally 55-gallon drums. Drum filling operations are performed with adequate ventilation and with containment controls such as a diked area in place to prevent exposures from accidental spills. The only potential for exposure to

CMA for the environment and the public in general is the remote possibility of a major accident during transportation.

The potential for human exposure is greatest for only a few workers at a single manufacturing facility located at Muscatine, Iowa. Perhaps a maximum of 50 workers total could have an assignment that might potentially expose them to CMA, and about 6 workers are assigned to the storage warehouse. Monitoring data by the industrial hygiene department (IH) of potential worker exposure to CMA is completed routinely on a bi-annual schedule (most recent was September, 2002) for all normal operations, and assessments are made for maintenance operations and other non-routine operations. No federal guidelines or exposure limits have been specified for CMA. The limit of detection for personal exposure monitoring has ranged between 10-15 ppb, and usual worker exposures have been below this limit of detection.

II. Physical-Chemical Data

a. Melting Point

Test Substance:	CMA (white crystalline solid, recrystallized 2X)
Result:	38 – 39.5 C
Method:	
Data Quality:	Data obtained from experimental measurements.
References:	Monsanto Company Material Safety Data, CP 57922, MSDS No. 040164690 (September, 2001).
Remarks:	Although recrystallized CMA has a melting point of 38 – 39.5 C, no difficulty is encountered keeping technical CMA liquid at temperatures above 30 C.
Other:	For comparison purposes, the melting point of alachlor is 39.5 – 41.5 C.

b. Boiling Point

Test Substance:	CMA
Result:	About 200 C at 760 mmHg Decomposes at temperatures greater than 110 C
Method:	
Data Quality:	Data obtained from experimental measurements.
References:	Monsanto Company Material Safety Data, CP 57922, MSDS No. 040164690 (September, 2001).
Remarks:	For comparison purposes, the boiling point of alachlor is 100 C at 0.02 mmHg, and 135 C at 0.3 mmHg. Alachlor also decomposes at temperatures greater than 105 C.
Other:	

c. Vapor Pressure

Test Substance:	CMA
Result:	<3 mmHg at 25 C <40 mmHg at 100 C
Method:	
Data Quality:	Data obtained from experimental measurements.
References:	Monsanto Company Material Safety Data, CP 57922, MSDS No. 040164690 (September, 2001).
Remarks:	For comparison purposes, the vapor pressure of alachlor is 2.2×10^{-5} mmHg at 25 C, and 0.02 mmHg at 100 C. The vapor pressure of alachlor was determined by extrapolation of the elevated boiling point data obtained under measured vacuum. Reference: Beestman, G.B., and Deming, J.M., Agronomy Journal, **66**, 308 (1974).
Other:	

d. Partition Coefficient

Test Substance:	Alachlor (pure active ingredient)
Result:	Alachlor K_{ow} = 1223
Method:	US EPA Chemical Fate Test Guidelines CG 1400, August 1982.
Data Quality:	Data obtained from experimental measurements.
References:	Dubelman, S. "Determination of the Octanol/Water Partition Coefficient of MAPC Products," Monsanto Company Report No. MSL-3219 (1983).
Remarks:	This result is an average of 12 replications of the US EPA partition method with a statistical coefficient of variation of 7.1%. This value was confirmed by determinations using an HPLC method with standards.
Other:	

e. Water Solubility

Test Substance:	CMA
Results:	Unstable in water (reacts slowly). CMA reacts with water to form formaldehyde and hydrogen chloride.
Method:	
Data Quality:	
References:	
Remarks:	Contact between water and CMA should be prevented. CMA is soluble in most organic solvents, such as benzene, toluene, xylene, chlorobenzene, acetone, or kerosene. For comparison purposes, the water solubility of alachlor is 240 ppm at 24 C.
Other:	

III. Environmental Fate Endpoints

a. Photodegradation

Test Substance:	[^{14}C]Alachlor (99.7% radiochemical purity)
Result:	Alachlor solution $t_{1/2}$: 239 days
Method:	US EPA Pesticide Assessment Guidelines, Subsection N-161-2; Photodegradation in Water.
Data Quality:	This is a well-documented US EPA guideline study conducted under GLP assurances.
References:	Kesterson, A.L., Lawrence, B.L., and Lawrence, L.J., "Solution Photolysis of [^{14}C]Alachlor in Natural Sunlight," Monsanto Company Report No. MSL-9916 (1990).
Remarks:	This study was designed and conducted according to US EPA guidelines to establish the significance of aqueous photolysis as a route of degradation for alachlor and to quantitate any degradation products formed. A buffered solution at pH 7 was selected since alachlor is known to be most stable to hydrolysis at this pH. [^{14}C]Alachlor in acetonitrile was aseptically prepared with sterile potassium dihydrogen phosphate – disodium hydrogen phosphate buffer (pH 7) to achieve a nominal concentration of 7.6 ppm. The final mixture was exposed to natural sunlight continuously for 30 days and maintained at 24.8 C (±0.3 C). One set of tubes was wrapped in aluminum foil to serve as dark controls. Samples were taken at 0, 5, 13, and 30 days. No degradation was seen in dark control samples. There was slight degradation in irradiated samples indicating the possibility of photodegradation. The half-life was 239 days for the irradiated samples. The major degradate was an unknown product which never accounted for greater than 0.9% of the applied radiocarbon. No other unknown product was >0.5% of the applied radiocarbon. The overall material balance for irradiated and dark control samples was 96.8% and 99.0%, respectively.
Other:	

b. Stability in Water (Hydrolysis)

Test Substance:	CMA
Result:	CMA is a chemical process intermediate that hydrolyzes extremely rapidly in water with the release of formaldehyde and hydrochloric acid. The hydrolysis of CMA leads to the creation of the corresponding acetanilide product, 2-chloro-N-(2,6-diethylphenyl)acetamide (CAS No.: 6967-29-9), that has also been identified as a minor metabolite of alachlor.
Method:	
Data Quality:	Data obtained from experimental measurements.
References:	Monsanto Company Material Safety Data, CP 57922, MSDS No. 040164690

	(September, 2001). Berk, H.C., Dietrich, D.K., and Kloek, J.A., "Research Data to Support TSCA PMN on MON-097 Intermediates – Biodegradation," Monsanto Company Report No. MSL-1909 (1981).
Remarks:	CMA is incompatible with water, amine base and alcohol materials.
Other:	

c. Biodegradation

Test Substances:	[^{14}C]Alachlor (98% radiochemical purity) Acetochlor process intermediate with a chemical structure very similar to CMA
Results:	Alachlor mean DT_{50} (time to 50% dissipation): 17 days Alachlor has been shown to degrade within days to more polar intermediates in soil and water both aerobically and anaerobically. The acetochlor process intermediate similar to CMA hydrolyzes, as expected with the release of formaldehyde and hydrochloric acid, to form the acetanilide product that was not significantly degraded further in both an activated sludge test and a shake flask test.
Method:	In order to assess the importance of microbial metabolism as a degradation pathway for alachlor in the environment and to identify and quantitate the metabolites formed, metabolism studies were conducted on soil under aerobic and anaerobic aquatic conditions. Other studies were performed to study the biotransformation of alachlor by activated sludge microorganisms. The biodegradation potential of several acetanilide herbicides including alachlor metabolites was estimated using commercially available computer modeling programs. And, research data was obtained on the biodegradability potential of the acetochlor process intermediate with a chemical structure very similar to CMA.
Data Quality:	Results obtained from various biodegradation studies (referenced below) conducted without GLP assurances to provide information to the US EPA for pesticide registration purposes.
References:	Suba, L.A., and Pearson, D.A., "The Environmental Studies of Alachlor," Monsanto Company Report No. MSL-0860 (1979). Dubelman, S., and Moran, S.J., "Estimation of Biodegradation and Adsorption Coefficients for Alachlor Metabolites Using Computer Models," Monsanto Company Report No. MSL-17502 (2001). Berk, H.C., Dietrich, D.K., and Kloek, J.A., "Research Data to Support TSCA PMN on MON-097 Intermediates – Biodegradation," Monsanto Company Report No. MSL-1909 (1981). Hahn, E.M., and Hallas, L.E., "Biotransformation of Alachlor by Activated Sludge Microorganisms From the Muscatine Waste Biosystem," Monsanto Company Report No. MSL-11601 (1991).
Remarks:	Soil metabolism studies of alachlor were conducted under aerobic conditions on three soils: a silt loam, a clay loam, and a sandy loam. Alachlor was found to

	biodegrade rapidly in each of these soils with a half-life of about 2 weeks. The rates of disappearance of alachlor, formation of CO_2, and formation and disappearance of metabolites were measured over a 25 week period. All significant metabolites have been unambiguously identified by spectral comparison with synthetically prepared materials. A variety of metabolites were identified. The most significant metabolites resulted from modification of the C-2 carbon to yield water soluble oxanilic and sulfonic acids. Under anaerobic aquatic conditions, alachlor displayed a half-life of 2-3 days. A study was undertaken in order to gain information on the biodegradability potential of the acetochlor process intermediate in an activated sludge waste treatment plant as part of the overall environmental fate evaluation of acetochlor production. CMA and the acetochlor process intermediate have nearly identical chemical structures and both intermediates hydrolyze extremely rapidly in water with the release of formaldehyde and hydrochloric acid. The hydrolysis of the acetochlor process intermediate created the corresponding acetanilide product that, in limited testing over 19 days, was not found to undergo further degradation in both an activated sludge test and a shake flask test.
Other:	

d. Transport between Environmental Compartments

Test Substance:	[^{14}C]Alachlor (98.68% radiochemical purity)
Result:	Alachlor Koc values for adsorption ranged from 131 to 192 with a mean of 158. The Koc values for desorption ranged from 196 to 313 with a mean of 230. According to published classification systems, alachlor has medium or moderate mobility in soils.
Method:	OECD Guideline 106: Adsorption/Desorption using a Batch Equilibrium Method; Janunary 21, 2000. Commission Directive 95/36/EC; July 14, 1995; amending Council Directive 91/414/EEC concerning plant protection products. Annex II, Part A: Chemical Substances, Section 7.1.2; Adsorption and Desorption, 1991.
Data Quality:	This is a well-documented OECD guideline study conducted under GLP assurances.
References:	"Adsorption/Desorption of [^{14}C]Alachlor on Soils," RCC Study Number 824927, RCC Ltd, Itingen, Switzerland (May, 2002). Hancock, J, and Orth, R.G., "Adsorption/Desorption Studies of Alachlor," Monsanto Company Report No. MSL-9886 (1990).
Remarks:	Adsorption/desorption studies are useful for generating essential information on the mobility of chemicals and their distribution in the soil, water and air compartments of our biosphere. They can be used in the prediction or estimation, for example, of the availability of a chemical for degradation, transformation and uptake by organisms; leaching through the soil profile; volatility from soil and run-off from land surfaces into natural waters. Adsorption data can also be used for comparative and modeling purposes. Adsorption and desorption isotherms were determined in an advanced test at five concentrations (1.981, 0.983, 0.193, 0.096 and 0.019 mg/L) covering two orders of magnitude and four soils. The soil/solution ratio of 1/1 was applied and the agitation time used was 48 hours. The equilibrium time was 24 hours for the

Other:	adsorption and for the desorption as well. The desorption isotherms were calculated from the values obtained after 48 hours of desorption. A mean adsorption Koc of 158 and mean desorption Koc of 230 was obtained. The 1/n values were in the range of 0.95 to 1.00 showing the linearity involved in the adsorption and desorption process. The calculated desorption Koc values were slightly higher than those obtained for the adsorption isotherms, indicating the partial irreversibility of the adsorption step.

IV. Ecotoxicity

a. Acute Toxicity to Fish

Test Substance:	CMA (97.1% sample purity, lot # QDL-0416)
Result:	24-hour LC_{50}: 27 mg/L (18-56 mg/L, 95% C.I.) 48-hour LC_{50}: 24 mg/L (18-32 mg/L, 95% C.I.) 96-hour LC_{50}: 15 mg/L (10-32 mg/L, 95% C.I.); slightly toxic 96-hour No-Effect Level: 10 mg/L, based on mortality and abnormal effects.
Method:	Static bioassay, acute toxicity Species: Rainbow trout Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.
Data Quality:	Reliable without restrictions. This is a well-documented US EPA guideline study conducted under GLP assurances. Study performed by ABC Laboratories, Columbia, MO (1983).
References:	"Acute Toxicity of Step II Intermediate (AB-83-126) to Rainbow Trout (*Salmo gairdneri*)," Static Bioassay Report 30635, Monsanto Study No. AB-83-126 (1983).
Remarks:	The CMA used as test material was received in good condition and was observed to be an amber crystalline solid that was stored at room temperature in the dark until needed for the study. Test concentrations were prepared based on the total compound. The test concentrations were obtained by transferring appropriate weights of the test compound directly to the test chambers. The solvent control received an aliquot (7.5 mL) of acetone equivalent to that used in all test concentrations. The acetone was used to facilitate the transfer of CMA into the test chambers. The working solution was prepared in nanograde acetone. Results were based on the nominal concentrations of 10, 18, 32, 56, and 100 mg/L with ten fish per concentration. The rainbow trout were challenged with a reference compound, Antimycin A, to verify that the fish were in good condition. The 96-hour LC_{50} for rainbow trout exposed to Antimycin A was 3.2×10^{-5} mg/L and was within the 95% confidence intervals reported in the literature. The no-effect concentration based on the lack of mortality and abnormal effects was 10 mg/L after 96 hours. The abnormal effects of mortality, surfacing, loss of equilibrium and fish on the bottom progressed from 100 mg/L initially to 18 mg/L after 96 hours. The dissolved oxygen concentrations, which ranged from 8.3 to 9.0 mg/L representing 77 and 83% saturation at 12 C, were considered adequate for testing. The pH values ranged from 7.0 to 7.2. The rainbow trout used for this experiment had a mean weight of 0.33 (±0.08) g and a mean standard length of 28 (±2.1) mm.
---	---
Test Substance:	CMA (97.1% sample purity, lot # QDL-0416)
Result:	24-hour LC_{50}: 24 mg/L (18-32 mg/L, 95% C.I.) 48-hour LC_{50}: 14 mg/L (10-18 mg/L, 95% C.I.) 96-hour LC_{50}: 13 mg/L (10-18 mg/L, 95% C.I.); slightly toxic

	96-hour No-Effect Level: 3.2 mg/L, based on mortality and abnormal effects.
Method:	Static bioassay, acute toxicity Species: Bluegill sunfish Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.
Data Quality:	Reliable without restrictions. This is a well-documented US EPA guideline study conducted under GLP assurances. Study performed by ABC Laboratories, Columbia, MO (1983).
References:	"Acute Toxicity of Step II Intermediate (AB-83-127) to Bluegill Sunfish (*Lepomis* macrochirus)," Static Bioassay Report 30634, Monsanto Study No. AB-83-127 (1983).
Remarks:	The CMA used as test material was received in good condition and was observed to be an amber crystalline solid that was stored at room temperature in the dark until needed for the study. Test concentrations were prepared based on the total compound. The test concentrations were obtained by transferring appropriate weights of the test compound directly to the test chambers. The solvent control received an aliquot (7.5 mL) of acetone equivalent to that used in all test concentrations. The acetone was used to facilitate the transfer of CMA into the test chambers. The working solution was prepared in nanograde acetone. Results were based on the nominal concentrations of 3.2, 5.6, 10, 18, and 32 mg/L with ten fish per concentration. The bluegill sunfish were challenged with a reference compound, Antimycin A, to verify that the fish were in good condition. The 96-hour LC_{50} for bluegill sunfish exposed to Antimycin A was 7.0×10^{-5} mg/L and was within the 95% confidence intervals reported in the literature. The no-effect concentration based on the lack of mortality and abnormal effects was 3.2 mg/L after 96 hours. The abnormal effects of mortality, surfacing, dark discoloration and fish on the bottom progressed from 32 mg/L initially to 5.6 mg/L after 96 hours. The dissolved oxygen concentrations, which ranged from 4.2 to 8.3 mg/L representing 48 and 94% saturation at 22 C, were considered adequate for testing. The pH values ranged from 6.7 to 7.5. The bluegill sunfish used for this experiment had a mean weight of 0.09 (±0.03) g and a mean standard length of 17 (±1.6) mm.
Other:	

b. Acute Toxicity to Aquatic Invertebrates

Test Substance:	CMA (97.1% sample purity, lot # QDL-0416)
Result:	24-hour EC_{50} *Daphnia magna*: 25 mg/L (18-32 mg/L, 95% C.I.) 48-hour EC_{50} *Daphnia magna*: 23 mg/L (18-32 mg/L, 95% C.I.), slightly toxic 48-hour No-Effect Level: 10 mg/L, based on the lack of mortality and abnormal effects.
Method:	Static bioassay, acute toxicity Species: *Daphnia magna* Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Stephan, C.E., Chairman. 1975. Committee on Methods for Toxicity Tests with Aquatic Organisms. US EPA, Ecological Research Series EPA-660/3-75-009, April, 1975.

Data Quality:	Reliable without restrictions. This is a well-documented US EPA guideline study conducted under GLP assurances. Study performed by ABC Laboratories, Columbia, MO (1983).
References:	"Acute Toxicity of Step II Intermediate (AB-83-128) to *Daphnia magna*," Static Acute Toxicity Bioassay Report No. 30636, Monsanto Study No. AB-83-128 (1983).
Remarks:	The CMA used as test material was received in good condition and was observed to be an amber crystalline solid that was stored at room temperature until needed for the study. Test concentrations were prepared based on total compound. Nanograde acetone was used in the preparation of all working stock solutions. The solvent control received an aliquot of 0.10 mL of acetone equivalent to that of the highest test concentration. The no-effect level was based on the absence of mortality and abnormal clinical signs, i.e., surfacing, clumping of the *Daphnia* together and daphnids laying on the bottom of the test chambers. The abnormal effects of mortality and daphnids lying on the bottom progressed from 100 mg/L initially to 18 mg/L after 48 hours. Results were based on the nominal concentrations of 10, 18, 32, 56 and 100 mg/L with ten *Daphnia* (first instar less than 24 hours old) per beaker selected for their respective bioassay. The dissolved oxygen concentrations, which ranged between 6.9 and 9.2 mg/L representing 77 and 102% saturation at 21 C respectively, were considered adequate for testing. The pH values of the treated chambers were consistent with the control and ranged from 8.3 to 8.4. The bioassay was conducted at 20 C (±1.0).
Other:	

c. Toxicity to Aquatic Plants

Test Substance:	Alachlor (94.9% sample purity, lot no. GLP-9905-9654-T)
Result:	Alachlor 96-hour EC_{50} (*Selenastrum*): 0.0029 mg/L (0.0028 – 0.0030 mg/L; 95% confidence limits); based on cell density. The 96-hour no-observable adverse effect concentration (NOAEC): 0.001 mg/L; based on cell density, area under the growth curve and growth rate. Based on the growth observed during the recovery phase, alachlor was considered to be algistatic, rather than algicidal, at the concentrations tested.
Method:	Static, acute toxicity Species: Freshwater green alga, *Selenastrum capricornutum* Length of test: 96-hour exposure, 10 day recovery phase The study protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines, OPPTS Number 850.5400: *Algal Toxicity Tiers I and II* (1996). The objective of this study was to determine the toxicity of alachlor to the freshwater green alga, *Selenastrum capricornutum*, during a 96-hour exposure period and a recovery period. The freshwater green alga, *Selenastrum capricornutum*, was exposed to a geometric series of five test concentrations, a negative (culture medium) control and

	a solvent (0.1 mL DMF/L) control under static conditions for 96 hours. Three "biological" replicate test chambers were maintained in each treatment and control group. One additional "analytical" replicate was maintained in each control and treatment group to provide test solution for verification of test concentrations at 72 hours. Two additional abiotic replicates at the highest test concentration were included in the experimental design for concentration verification at 72 and 96 hours. These replicates were used to monitor the effects of the experimental conditions on the stability of the test substance over the exposure period. Nominal test concentrations selected were 0.5, 1.0, 2.0, 4.0 and 8.0 µg active ingredient (a.i.)/L. Mean measured test concentrations were determined from samples of test medium collected from each treatment and control group at the beginning of the test, after approximately 72 hours, and at test termination. Prior to test initiation, an inoculum of the algal cells was prepared from the stock culture at a concentration of approximately 1.0×10^6 cells/mL. The concentration of algal cells was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately 1.0×10^4 cells/mL at test initiation. Samples were collected from each replicate test chamber at approximately 24-hour intervals during the test to determine cell densities, which were subsequently used to calculate areas under the growth curve and growth rates. Cell densities, areas under the growth curve and growth rates were used to calculate percent inhibition values relative to the control over the 96-hour exposure period. EC_{50}, E_bC_{50} and E_rC_{50} values were calculated, when possible, based upon cell density, area under the growth curve and growth rate, respectively, for each 24-hour interval of the exposure period. No-observed-adverse-effect-concentrations (NOAEC) were determined at 72 and 96 hours through statistical evaluation of the cell densities, areas under the growth curve and growth rates, as well as examination of the concentration-response pattern. At the end of the 96-hour exposure period, algistatic effects were differentiated from algicidal effects in two selected test concentrations.
Data Quality:	Reliable without restrictions. This is a well-documented guideline study conducted under GLP assurances. Study performed by Wildlife International, Ltd., Easton, MD (2001).
References:	"Alachlor: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*) that includes a Recovery Phase," Wildlife International Ltd. Project Number 139A-243, Monsanto Study Number WL-1999-194 (2001).
Remarks:	Daily growth rate recovers to a rate greater than or equal to the control value within 3 days after transfer to fresh medium not containing alachlor.
Other:	

V. Toxicological Data

a. Acute Toxicity

Test Substance:	CMA (96.4% sample purity)
Result:	Oral, rat LD_{50}: 1,650 mg/kg; slightly toxic (EPA pesticide category III) Dermal, rabbit LD_{50}: 5,400 mg/kg; practically nontoxic (EPA pesticide category IV) Eye irritation, rabbit: moderately irritating (EPA pesticide category II) Skin irritation, rabbit 4-hour exposure: severely irritating (EPA pesticide category II); corrosive (DOT regulation)
Method:	Methods are described in the following section for remarks.
Data Quality:	Reliable without restrictions. These were well-documented US EPA guideline studies conducted under GLP assurances. Studies performed by Bio/dynamics, Inc., East Millstone, NJ (1987).
References:	"Acute Oral Toxicity Study in Rats, Test Material: Step II Intermediate (Lasso)," Bio/dynamics Project No. 6922-86, Monsanto Company Report No. BD-86-359 (1987). "Acute Dermal Toxicity Study in Rabbits, Test Material: Step II Intermediate (Lasso)," Bio/dynamics Project No. 6923-86, Monsanto Company Report No. BD-86-359 (1987). "Primary Dermal Irritation Study in Rabbits (4- and 24-Hour Exposure), Test Material: Step II Intermediate (Lasso)," Bio/dynamics Project No. 6924-86, Monsanto Company Report No. BD-86-359 (1987). "Eye Irritation Study in Rabbits, Test Material: Step II Intermediate (Lasso)," Bio/dynamics Project No. 6925-86, Monsanto Company Report No. BD-86-359 (1987). "Skin Corrosivity Evaluation in Rabbits – DOT, Test Material: Step II Intermediate (Lasso)," Bio/dynamics Project No. 4487-87, Monsanto Company Report No. BD-87-217 (1987).
Remarks:	When undiluted CMA was administered to fasted albino rats of both sexes, the acute oral LD_{50} was calculated to be 1650 mg/kg. The dermal LD_{50} was calculated to be 5400 mg/kg when albino rabbits received a continuous 24-hour application of undiluted test material on intact skin. Thus, CMA is considered to be slightly toxic by ingestion in single doses and practically nontoxic by single dermal applications. When 0.1 mL of undiluted CMA was placed into the conjunctival sac of the rabbit eye, moderate irritation resulted. Four of the six rabbits' eyes had cleared by post-exposure day 7. The remaining rabbits had cleared by post-exposure day 14. Severe but generally reversible irritation resulted when 0.5 mL of undiluted CMA was held in continuous 4-hour semi-occlusive contact with rabbit skin. When 0.5 mL of undiluted CMA was held in continuous 4-hour contact with rabbit skin, all animals exhibited slight erythema at 4 hour observation. However, at 48 hours, three animals exhibited necrosis and the remaining three animals exhibited moderate to severe erythema. Thus, CMA would be considered corrosive as defined by the DOT regulation guideline 173.1300, Appendix A dated October 8,

	1981, effective July 1, 1982. Because CMA may be corrosive to skin, extreme care, including the use of protective equipment, must be taken to prevent skin contact with CMA. In case of contact, remove contaminated clothing and shoes, and immediately wash exposed areas thoroughly with soap and water. If irritation persists, call a physician. Contaminated clothing should be laundered before reuse. Because it is moderately irritating to eyes, care should be taken to avoid eye contact with CMA. In case of eye contact, flush immediately with large volumes of water. If irritation persists, call a physician.
Other:	

b. Repeated Dose Toxicity

Test Substance:	CMA (97.1% sample purity, lot 12-36)
Result:	Inhalation exposure in concentrations of 0.007 mg/L and above resulted in clinical signs of toxicity, lower body weights, hematological changes, and organ weight changes. A no-observable adverse effect level could not be determined for this study.
Method:	Four groups of 15 male and 15 female Charles River CD® (SD) BR rats were exposed to target concentrations of 0, 0.007, 0.02 and 0.07 mg/L CMA for 6 hours/day, 5 days/week, for 2 weeks. Nominal and analytical concentrations were determined once and four times daily, respectively. Distribution of the test material in each chamber was measured once during the study. Formaldehyde and hydrochloric acid vapors were also monitored periodically during the study. Animals were examined for gross signs of toxicity before, during and after each exposure period. Each animal was examined and weighed weekly. Routine hematology and blood chemistry examinations were conducted on all surviving animals at study termination. All animals were subjected to a complete gross necropsy.
Data Quality:	Reliable with restrictions. This is a well-documented study following standard testing procedures conducted at Monsanto Company's Environmental Health Laboratory intended to be a pilot study for a subsequent 90-day study.
References:	"Lasso® Step II Intermediate: Two Week Inhalation Pilot Toxicity Study in Rats," EHL No. 830003, Monsanto Company Report No. ML-83-002 (1986).
Remarks:	Mean analytical concentrations (± S.D.) of CMA for the 3 exposure levels were 0.007 (± 0.003), 0.019 (± 0.003) and 0.064 (± 0.009) mg/L. Mean nominal analytical concentration ratios ranged from 3.8 to 9.4, indicating that a large aerosol component was present. The test material appeared to be uniformly distributed within each chamber. Airflow, temperature and humidity were at acceptable levels throughout the study. Formaldehyde and hydrochloric acid were detected in the high exposure level chamber, apparently as a result of impurities in the test material and/or decomposition of CMA as a result of the high temperatures needed to produce measurable atmospheres. One high exposure level male died during the study. An increased incidence of hypoactivity and signs of ocular, nasal and/or respiratory irritation were noted in all exposed groups. Dose-related decreases in mean body weights (5 to 28% lower

	than controls) were also noted at all exposure levels but were greater in males than in females. Increases in red blood cell count, hematocrit and hemoglobin were noted at all exposure levels but were again generally more prominent in males than in females. Decreased white blood cell counts (primarily lymphocytes) were observed in all 3 exposed male groups and in high level females. Serum glucose levels were slightly increased in all exposed groups but there was not a clear dose-related trend. Absolute and/or relative organ weight changes were noted at all exposure levels. Organs affected included the spleen, liver, kidneys, brain and testes. With the exception of the spleen, absolute organ weights were decreased while organ-to-body weight ratios were increased. However, decreased absolute spleen weights were generally accompanied by decreased relative spleen weights. Thus, it appears that the spleen effects may be a direct toxic effect of the test material while the other organ weight changes may be secondary to general body weight loss. The splenic weight changes may also be associated with the reduced lymphocyte counts. However, in the absence of histopathological evaluation, interpretation of the organ weight changes remains speculative.
Other:	Dermal Sensitization Study in Guinea Pigs The purpose of this study is to determine the potential of CMA to produce delayed hypersensitivity (allergic reactions) subsequent to dermal exposure. This is accomplished by administering the compound on a repetitive dosing schedule for a defined period of time (induction phase), allowing an appropriate rest period, and then testing for hypersensitivity by the application of a challenge dose. Test Substance: CMA (97.1% sample purity, lot QDL 416) Result: All animals survived until termination of the study. Body weight gains in treated animals were comparable to or greater than those in control animals. No dermal irritation was seen in the negative (acetone-treated) controls during either the induction or challenge phases of the study. However, all animals treated with DNCB, a positive control, exhibited severe dermal irritation after two or four induction doses. All animals exhibited dermal irritation in response to the nonirritating challenge dose, indicating that DNCB was a dermal sensitizing agent. These results confirmed that the methods used in the study were effective in identifying delayed hypersensitivity when a known skin sensitizer was used. Minimal dermal irritation was observed after the first induction exposure to CMA. Beginning with the second or third induction exposure, mild to moderate irritation was apparent in most animals. Irritation continued throughout the induction period with some animals developing severe irritation. These data suggested some cumulative irritation or sensitization potential. Following challenge with a non-irritating dose of CMA two animals exhibited clear dermal responses with the remaining eight animals exhibiting either equivocal or no dermal responses. In an attempt to clarify these results, a re-challenge was performed 7 days later. The results were essentially the same. Method: For the induction phase, 0.2 mL of a 25% solution of CMA in acetone was applied dermally, using the closed patch technique, to the shaved backs of five male and five female Hartley albino guinea pigs. Applications were for six hours per day, three days per week for three weeks. Two weeks after the final dose, 0.2 mL

	doses of a 5% solution of test material in acetone (challenge dose) were applied to previously untreated areas of those guinea pigs which had received the induction doses and to three male and three female naïve animals. The latter animals served as an irritation control group. Two additional groups of guinea pigs were treated with 1-chloro-2,4-dinitrobenzene (DNCB), a positive control, in the same manner as that used with the test material. A fifth group of five male and five female guinea pigs was treated with acetone for the induction and challenge doses. These animals served as the negative control group. A re-challenge with the 25% solution of CMA in acetone was performed one week later employing similar techniques. Dermal irritation was scored 24 and 48 hours after each of the induction, challenge, and re-challenge applications. Throughout the study, all animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Body weights were recorded pretest and immediately prior to challenge. Data Quality: GLP/QA reviewed. Reference: "Lasso® Step II Intermediate: Dermal Sensitization Study in Guinea Pigs," Bio/dynamics Project No. 4992-84, Monsanto Company Report No. BD-84-049 (1985). Remarks: Under the conditions of this study, CMA exhibited slight potential to produce dermal sensitization in guinea pigs.

c. Genetic Toxicity

Test Substance:	CMA (98.0% sample purity, lot no. 5-30-85A)
Result:	CMA was not considered to be a genotoxic agent in the in vitro hepatocyte DNA repair assay.
Method:	The purpose of this study was to assess the potential of CMA to produce DNA damage in mammalian cells. This was accomplished by determining the extent of unscheduled DNA synthesis (assumed to represent DNA repair) by measuring ^{3}H-thymidine uptake following in vitro exposure of primary rat hepatocytes to the test material. Two separate experiments were conducted. In each, primary hepatocytes were isolated from the liver of an adult male Fischer-344 rat and allowed to attach to glass cover slips. The hepatocyte cultures were then exposed in triplicate for 19 to 20 hours to ^{3}H-thymidine and 8 to 10 concentrations of CMA up to 5000 µg/mL. Each experiment also included concurrent untreated medium controls, 1% acetone vehicle controls and 2-acetylaminofluorene (2-AAF) positive controls. After exposure, the cells were washed, fixed and mounted on slides. The slides were then dipped into Kodak NTB-2 radiographic emulsion, exposed at –20 C for 7 days and stained with methyl-green Pyronin Y. Fifty cells from each slide were evaluated. Thymidine uptake was assayed by counting the nuclear and cytoplasmic silver grains on the radiographic film. The net nuclear grain count was determined by subtracting the highest counts from 2 nuclear-sized areas of the cytoplasm from the nuclear count. A cell was considered to be undergoing DNA repair if the net nuclear grain count was 5 or greater. A test article is considered positive if the mean net nuclear grain count and the percentage of cells in repair were markedly elevated above the negative control values.

Data Quality:	Reliable without restrictions. This is a well-documented study conducted under GLP assurances. Study performed by SRI International, Menlo Park, CA (1986).
References:	"Evaluation of the Potential of Lasso® Step II Intermediate to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures," Monsanto Company Report No. SR-85-151 (SRI #LSC-8747-1).
Remarks:	Cytotoxicity was observed in both assays at 1000 and 5000 µg/mL. Unscheduled DNA synthesis (UDS) was therefore measured at concentrations ranging from 1 to 500 µg/mL. As expected, the 2-AAF positive controls elicited a strong positive response (net grain counts of 18.7 and 32.6; 77 and 90 percent of cells in repair). Although a dose-related increase in net grain counts were observed in cells treated with CMA, the net grain counts were always negative. These increased net counts were a result of a dose-related decrease in cytoplasmic background which may have been due to slight cytotoxicity. An increase in the percentage of cells in repair was noted at 5 and 10 µg/mL but not at other concentrations. It appeared that a genotoxic response was induced in a small number of hepatocytes. However, since there was no clear dose-response effect and since the net grain counts never exceeded zero, the observed response was not considered to represent a significant genotoxic effect.
Test Substance:	CMA (pure compound, viscous amber liquid)
Result:	Microbial mutagenic assay (Ames): No genetic activity was observed in any of the *Salmonella* bacterial strains nor in the *Saccharomyces* yeast strain, with or without metabolic activation. It is concluded that CMA was not mutagenic in the microbial assays.
Method:	Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 mL of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 mL containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37 C, and scored for the number of colonies growing on each plate. The concentrations of CMA tested ranged from 0.001 to 5.00 µL per plate. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.
Data Quality:	Reliable with restrictions. This is a well-documented study following standard testing procedures (1977) conducted without GLP assurances.
References:	"Microbial Mutagenic Assay with: Lasso Step II Intermediate (CP 57922)," Monsanto Company Report No. BIO-77-201, LBI Project No. 20838 (Litton Bionetics, Inc., Kensington, MD), September 1977. Ames *et al.*, Mutation Research, **31**, 347 (1975).

Remarks: Other:	The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. CMA was evaluated at 5 dose levels in each of 5 *Salmonella* bacterial strains (TA-1535, TA-1537, TA-1538, TA-98, and TA-100) and one strain of *Saccharomyces* yeast (D4). Assays were conducted with and without incorporation of a microsomal enzyme activation system. Appropriate positive and negative controls were employed in the study.

d. Reproductive/Developmental Toxicity

Test Substance:	CMA (97.1% purity, lot no. QDL-416)
Result:	Slight maternal toxicity but no embryo toxicity, fetotoxicity or teratogenicity was noted following the oral administration of CMA to pregnant female rats at dose levels up to 300 mg/kg/day during gestation days 6-15.
Method:	CMA was dissolved in corn oil and administered by gavage to 4 groups of 25 mated female Charles River CD® rats at dose levels of 0, 30, 100 and 300 mg/kg/day. Dosages were administered daily during gestation days 6-15. Fresh dosing solutions were prepared daily. Food and water were provided ad libitum. The animals were observed twice daily for mortality or overt signs of toxicity. Detailed physical examinations were conducted daily during gestation days 6 through 15. Body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20. All dams were sacrificed on day 20 of gestation and gross postmortem examinations were performed. The uteri and ovaries were removed and the numbers of viable and nonviable fetuses, early and late resorptions, implantation sites and corpora lutea were recorded. Fetuses were weighed, sexed and examined for external malformations. Half of the fetuses were then examined for soft tissue malformations using the Wilson sectioning technique while the remaining fetuses were stained with Alizarin Red S and examined for skeletal defects.
Data Quality:	Reliable without restrictions. This is a well-documented guideline study conducted under GLP assurances. Study performed by International Research and Development Corporation, Mattawan, MI (1985).
References:	"Lasso® Step II Intermediate: Teratology Study in Rats," Monsanto Company Report No. IR-84-045 (IRDC #401-273).
Remarks:	All animals survived for the duration of the study. Slight maternal toxicity, as indicated by excessive salivation, a slight increased incidence of alopecia, and slightly decreased weight gain was noted in high-dose dams. No abortions or premature deliveries were noted. There were no biologically meaningful differences in pregnancy rates, corpora lutea, implantation data, fetal weight or fetal sex ratios. Although there was a slight but statistically significant decrease in mean body weights in the high-dose fetuses, this was attributed to the slightly increased litter sizes in those dams and the resultant intrauterine competition. No malformations were noted in the control, low and high-dose groups, but 2 litters from the mid-dose group contained malformed fetuses. However, since no malformations were evident in the high-dose group and the defects found are not

Other:	uncommon in control animals, they were not considered to be related to treatment. The incidence of developmental variations in the treated groups was comparable to that of the controls.