December 28, 2001

Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

Registration Number: [redacted]

Dear Administrator Whitman:

FMC Corporation actively supports the EPA HPV Challenge Program, AR-201 and is pleased to submit the proposed test plans and robust summaries for the two single chemicals:

- Phenol, 2-[(2-methyl-2-propenyl) oxy]; Methyallyloxyphenol -- CAS No. 4790-71-0

- Cyclopropanecarboxylic acid, 3(2,2-dichloroethenyl)-2,2-dimethyl-, methyl ester -- CAS No. 61898-95-1

In addition to the above referenced two single chemicals, FMC's HPV Challenge commitment to EPA for 2001 included the single chemical Orthoacetic acid, trimethyl ester (TMOA) – CAS No. 1445-45-0. Please note that FMC respectfully requested the withdrawal of Orthoacetic acid, trimethyl ester (TMOA) from the program in a correspondence dated September 13, 2001 to EPA (Ms. Linda M. Clark to Chemical RTK Program). It came to FMC's attention that our sole foreign supplier of TMOA, Degussa-Huls AG, committed to test this chemical in the ICCA chemical testing initiative regarding High Production Volume chemicals as stated by Degussa-Huls AG in their letter to EPA dated January 25, 2000. EPA assigned this letter the code AR201-12311. FMC, therefore, withdrew support of TMOA given the fact it is being tested in the ICCA HPV program.

FMC proposes Closed System Intermediate Status for the two single chemicals and information supporting exemption claims for Reduced SIDS Testing has been prepared. Please note for the single chemical, Phenol, 2-[(2-methyl-2-propenyl) oxy; Methyallyloxyphenol, the test plan for aquatic toxicity indicates testing for estuarine species and not freshwater species as the potential for exposure at the manufacturing site where the chemical undergoes reaction is to estuarine species. This rationale was discussed with Mr. Richard Hefter, Chief of the HPV Chemicals Branch with verbal approval given FMC on October 3, 2001. Additionally, FMC plans to submit documentation supporting Closed System Intermediate Status for Cyclopropanecarboxylic acid, 3(2,2-dichloroethenyl)-2,2-dimethyl-, methyl ester as soon as received from the FMC customer.

This submission includes one electronic copy of documents in *Microsoft Word 97* and IUCLID formats. FMC understands there will be a 120-day review period for the test plan and that all comments received by EPA will be forwarded to FMC for consideration. Please contact me if you have comments or questions regarding this submission.

Sincerely,

LuAnn Maloney
For FMC Corporation
Tel. 215.299.6680 Fax 215.299.6468

TEST PLAN

Phenol, 2-[(2-methyl-2-propenyl)oxy]; Methyallyloxyphenol (MOP) CAS No. 4790-71-0

STUDY	INFORMATION (Yes or No)	OECD STUDY (Y/N)	GLP (Y/N)	OTHER STUDY (Y/N)	ESTIMATION METHOD (Y/N)	ACCEPTABLE (Y/N)	SIDS TESTING REQUIRED (Y/N)
Physical/Chemical Properties							
Melting Point	No						No
Boiling Point	Yes	No	No	Yes	No	Yes	No
Vapor Pressure	Yes	No	No	Yes	No	Yes	No
Partition Coefficient	No						Yes
Water Solubility	No						Yes
Enviromental Fate and Pathways							
Photodegradation	Yes	No	No	No	Yes	Yes	No
Hydrolysis	No						Yes
Fugacity	Yes	No	No	No	Yes	Yes	No
Biodegradation	No						Yes
Aquatic Toxicity							
Acute Toxicity to Fish	Yes	No	Yes	Yes	No	Yes	No
Acute Toxicity to Daphnia	No						No
Acute Toxicity to Mysid Shrimp	Yes	No	Yes	Yes	No	Yes	No
Toxicity to Algae	Yes	No	Yes	Yes	No	Yes	No
Mammalian Toxicity							
Acute Inhalation	No						No
Acute Oral	Yes	No	Yes	Yes	No	Yes	No
Genotoxicity *In Vitro* (Bacterial Test)	Yes	Yes	Yes	No	No	Yes	No

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TEST PLAN (continued)

Phenol, 2-[(2-methyl-2-propenyl)oxy]; Methyallyloxyphenol (MOP) CAS No. 4790-71-0

STUDY	INFORMATION (Yes or No)	OECD STUDY (Y/N)	GLP (Y/N)	OTHER STUDY (Y/N)	ESTIMATION METHOD (Y/N)	ACCEPTABLE (Y/N)	SIDS TESTING REQUIRED (Y/N)
Mammalian Toxicity							
Genotoxicity *In Vitro* (Mammalian Cells)	Yes	Yes	Yes	No	No	Yes	No
Genotoxicity *In Vivo* (Chrom. Aberrations)	Yes	Yes	Yes	No	No	Yes	No
Repeated Dose	No						No*
Reproductive Toxicity	No						No*
Developmental Toxicity	No						Yes*

- Reduced testing requirements for Closed-System Intermediates. Actual initiation of Developmental Toxicity testing is deferred until 2003 as instructed by EPA guidance.

Methallyloxyphenol (MOP) is produced as an in-process intermediate in the 7-Hydroxy Process in the FMC Baltimore Plant.

7-Hydroxy Process Description: (also see process flow diagram)

STEP 1: MOP Reaction

A. MOP Reaction - Purpose is to produce crude MOP in a batch reaction at elevated temperatures and pressures.

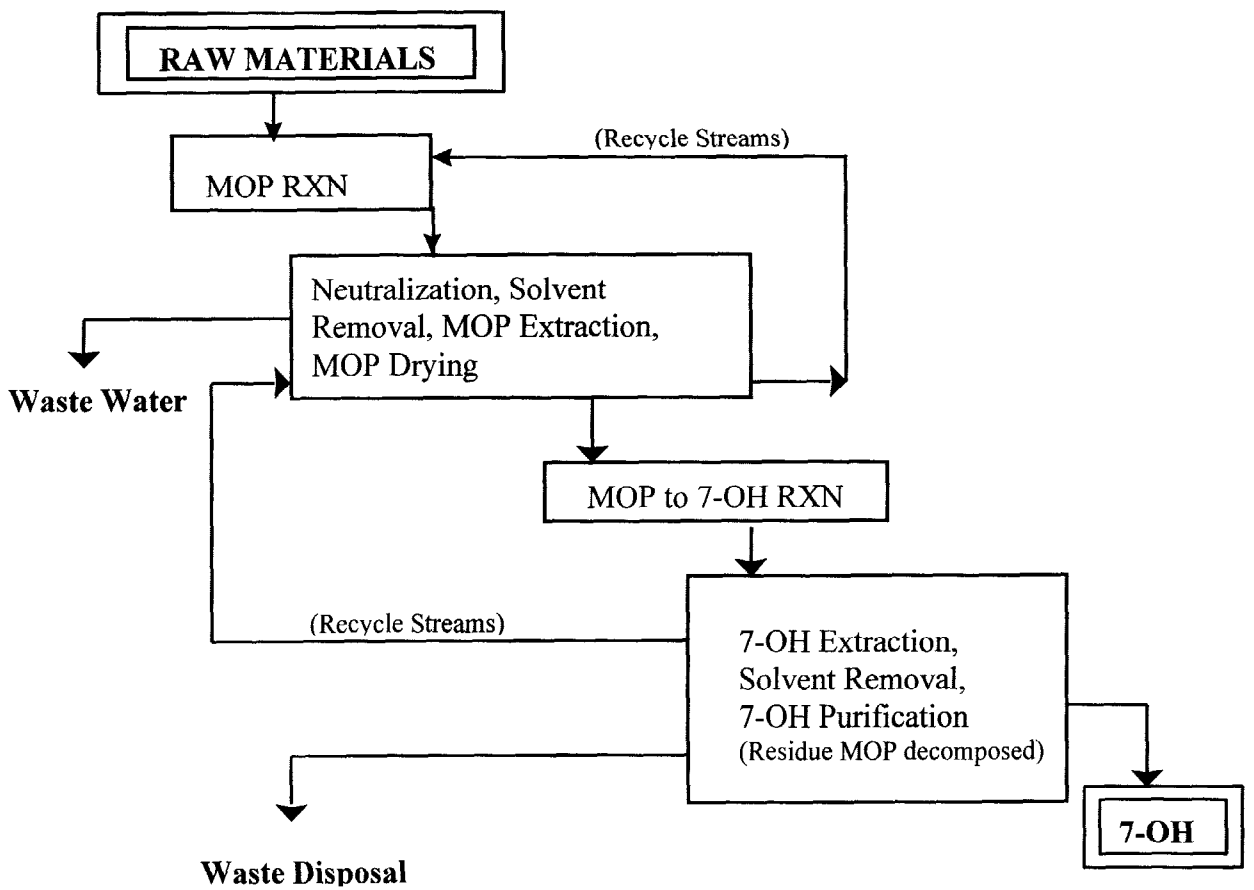
B. Neutralization, Solvent Removal, MOP Extraction, MOP Drying - Purpose is to prepare the MOP for reaction in Step 2.

STEP 2: 7-Hydroxy Reaction

A. MOP to 7-OH Reaction - Purpose is to react the MOP into 7-OH at elevated temperatures and pressures. Less than 0.3% MOP remains after reaction and that is degraded during purification.

B. 7-OH Extraction, Solvent Removal, 7-OH Purification - Purpose is to clean up the 7-OH for a final product assay of 98.7% or more.

Process Flow Diagram:



Monitoring Data:

Wastewater Monitoring-
All aqueous streams from the 7-Hydroxy process are directed through a wastewater stripper to remove solvents and then sent to on-site wastewater treatment prior to discharge to the Public Owned Treatment Works (POTW). On-site wastewater treatment consists of carbon beds through which MOP concentration and removal efficiency are monitored daily. The average MOP concentration into the carbon beds for 2001 year-to-date is 143 ppm. The average MOP concentration out of the carbon beds for 2001 year-to-date is 2 ppm. These numbers translate to an average MOP removal efficiency of 98.6% for 2001 year-to-date. This removal efficiency is well above the minimum limit of 93%.

IH Data-
Air and personnel exposure (sampling and normal job duties) monitoring for MOP in the 7-Hydroxy process area were performed in 1986, 1988 and 1990. This monitoring found the air and personnel exposure concentration of MOP at an average of 5.4 ppb. This is well below the 8-hour exposure limit for MOP of 4,900 ppb.

Presence in Distributed Product:

Presence of MOP in finished 7-Hydroxy is < 0.1%. 7-Hydroxy product chemistry demonstrates that the MOP is degraded during high temperature purification.

Also, 7-Hydroxy is an intermediate used to make Carbofuran. The Carbofuran Confidential Statement of Formula (CSF) does not list MOP due to the five-batch analysis demonstrating MOP levels at < 0.1% in the product.

Transport Data:

MOP is not transported outside of the process area.

Data Search:

To the best of FMC's knowledge and extensive experience with this particular intermediate, MOP is not contained in other end-use products and documentation supporting this claim will be submitted to EPA as an addendum.

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PHYSICAL AND CHEMICAL DATA

1. MELTING POINT

The material is a liquid at room temperature. See Boiling Point.

2. BOILING POINT

Test Substance:	2-methallyloxyphenol
Method:	Distillation
GLP:	No
Year:	1984
Results:	The material undergoes a Claisen rearrangement before reaching the boiling point at atmospheric pressure
Data Quality:	4b
References:	FMC Chemical Data Sheet No. 367, Revision 1, 1984

3. VAPOR PRESSURE

Test Substance:	2-methallyloxyphenol
Method:	Gas Saturation
GLP:	No
Year:	1982
Results:	0.02 mm Hg at 25 °C (mathematical extrapolation from higher temperature vapor pressure data)
Data Quality:	4b
References:	FMC Chemical Data Sheet No. 367, Revision 1, 1984

4. PARTITION COEFFICIENT

No data available

5. WATER SOLUBILITY

No data available

ENVIRONMENTAL FATE AND PATHWAY

6. PHOTODEGRADATION

Test Substance:	2-methallyloxyphenol
Method:	Estimated by the AOP program (v. 1.90) which estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2000
Results:	For reaction with hydroxyl radicals, the predicted half-life is 1.5 hours with a rate constant of 8.62×10^{-13} cm^3/molecule-sec.
Remarks:	The photodegradation calculation by an acceptable method is assigned a reliability code of 2f.
References:	AOPWIN version 1.90, Syracuse Research Corporation, Syracuse, NY

7. STABILITY IN WATER (HYDROLYSIS)

No data available

8. TRANSPORT/DISTRIBUTION (FUGACITY MODEL)

Test Substance:	2-methallyloxyphenol
Method:	Estimated by EPIWIN program (v. 3.05) Level III Fugacity Model
GLP:	No
Year:	2001
Results:	Distribution using Level III Fugacity Model

Air	0.423%
Water	40.4%
Soil	58.9%
Sediment	0.293%

Remarks:	Code 2f
References:	EPIWIN version 3.05, Syracuse Research Corporation, Syracuse, NY

9. BIODEGRADATION

No data available

ECOTOXICOLOGY

10. ACUTE TOXICITY TO FISH

Test Substance:	2-methallyloxyphenol (purity not defined)
Method:	American Society for Testing Materials, 1980, Standard Practice D729-80 A 96-hr flow-through acute toxicity test was conducted in the sheepshead minnow. Groups of 20 fish (10 per replicate) were exposed to 2.3, 4.4, 8.8, 18, or 38 mg/L (measured) delivered into seawater via an acetone stock solution, to 14 ul/l acetone in seawater (solvent control) or to seawater alone (control). Observations for mortality, morbidity and behavior were recorded every 24 hours throughout the test. Measurements of the test concentrations were made on samples from all the test chambers at 0, 48 and 96 hours of the test by HPLC. Water quality parameters (temperature, pH, dissolved oxygen) were measured at 24-hour intervals throughout the exposure.
Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Test Concentration:	0, 2.3, 4.4, 8.8, 18, 38 mg/L (measured)
Exposure Period:	96 hours
Analytical Monitoring:	Seawater samples were analyzed on the day they were collected using HPLC. Manual injection by constant-pressure nitrogen filling of the HPLC injection loop was employed to obtain the required accuracy over a wide concentration range; this allowed all samples to be analyzed without dilution.
GLP:	Yes
Year:	1986
Results:	Mortality of sheepshead minnows for 96 hours ranged from 5% of the fish exposed to 4.4 mg/L to 100% of those exposed to 18 and 38 mg/L. No control or solvent control mortality was observed. Water quality parameters remained within acceptable limits for the duration of the exposure. The test was maintained at 22 to 23°C. The salinity of the test water was 20 ppt. The dissolved oxygen concentrations remained $\geq$ 5.8 ppm or $\geq$ 77% of saturation in all test aquaria throughout the test. The pH ranged from 7.3 to 7.7 units in all test aquaria throughout the test. The 96-hr LC50 was equal to 9.8 mg/L (confidence interval = 7.9 – 12 mg/L).

Data Quality: 1b

References: "Acute toxicity of FMC 10233 technical to the sheepshead minnow (*Cyprinodon variegatus*)", ESE, Inc., March 17, 1986, FMC Study A1986-2014

11. ACUTE TOXICITY TO FISH

Test Substance: 2-methallyloxyphenol (97.9%)

Method: ASTM, 1980, Standard Practice D729-80, "Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians"

The acute toxicity of 2-methallyloxyphenol to the Atlantic Silverside, *Menidia menidia,* was conducted for 96 hours from January 8 to 12, 1986 at Environmental Science and Engineering, Inc., in Gainesville, Florida. The flow-through saltwater toxicity test was performed at 22 ± 1°C with 5 concentrations of test substance, a natural seawater control and an acetone solvent control. Salt water used for holding and testing was filtered (5-micrometer) natural seawater. The test water was collected at Marineland, Florida and diluted to 20 parts per thousand salinity with well water from Gainesville, Florida. Prior to delivery to the test system, the seawater was sterilized by ultraviolet light.

Juvenile Atlantic silversides (*Menidia menidia*) were obtained from a commercial supplier (S/P, Inc.) and maintained for 8 to 16 days until testing. During holding, fish were fed live brine shrimp nauplii daily. During the 48-hour period immediately prior to test initiation, salinity was 25 parts per thousand and water temperature was approximately 22°C. Test fish were 15 ± 2 millimeters standard length and 0.0256 ± 0.0085 grams wet weight.

Nominal concentrations of 2-methallyloxyphenol were 0.62, 1.25, 2.5, 5.0 and 10 ppm. Ten fish were impartially distributed to each of two replicates of each treatment. The test system was a solenoid-activated diluter with a dilution factor of 0.5. The test was performed in seven duplicate sets of glass aquaria designed to maintain 9 liters of test solution or dilution water. The flow rate to each duplicate aquarium was sufficient to provide approximately 7.5 daily volume turnovers. A 14-hour light and 10 hour dark photoperiod was automatically maintained. Water samples (approximately 50mL) were collected from all treatment replicates on days 0, 2 and 4 to monitor actual exposure concentrations by HPLC. LC50's and their 95 percent confidence limits were calculated by computer program (Stephan, 1982) which estimated the LC50's using the following statistical methods: moving average angle, probit, and non-linear interpolation. Confidence limits for LC50 values determined by non-linear interpolation were calculated by binomial probability.

	Observations for mortality, morbidity and behavior were recorded and reported for all test concentrations every 24 hours throughout the test. During the observation period, dead fish were removed from the aquaria. Water quality parameters such as temperature, pH and dissolved oxygen were measured every 24 hours throughout the test.
Species:	Atlantic Silverside, (*Menidia menidia*)
Test Concentration:	control, solvent control, 0.684, 1.42, 2.96, 6.16 and 11.6 ppm (measured)
Exposure Period:	96 hours
Analytical Monitoring:	Seawater samples were analyzed for 2-methallyloxyphenol following FMC Method Number ACG-111 as modified by Test Protocol Amendment 1 dated December 20, 1985. Manual injection by constant-pressure nitrogen filing of the HPLC injection loop was employed to obtain required accuracy over a wide concentration range, this allowed all samples to be analyzed without dilution.
GLP:	Yes
Year:	1986
Results:	The dissolved oxygen concentrations remained ≥ 6.5 ppm or ≥ 86 percent of saturation in all test aquaria throughout the test. The pH ranged from 7.5 to 7.8 units in all test aquaria throughout the test. The salinity of the test water was 20 to 22 parts per thousand. Measurement of the exposure concentrations at 0, 48 and 96 hours using HPLC showed that the desired test concentrations were maintained throughout the 96-hour exposure. Mean measured concentrations of 2-methallyloxyphenol were 0.684 to 11.6 ppm and from 110 to 123 percent of nominal concentrations. The average method recovery was 107 percent with a standard deviation of 5 percent. 2-methallyloxyphenol technical was acutely toxic to silversides in the mean measured concentration range of 1.42 to 11.6 ppm. After 96 hours of exposure, the mortality of silversides was 5 percent in test concentration 0.684 ppm, but since test concentration 1.42 ppm had no mortality throughout the test, the deaths were thought to be non-treatment related. The mortality was 100 percent in 11.6ppm treatment concentration. No mortality was observed in either control. The 96-hour LC50 was 4.55 ppm with 95 percent confidence limits of 2.96 to 6.16 ppm. The NOEC is 1.42 ppm. The 24-hour LC50 = 10.7 ppm, the 48-hour LC50 = 8.11 ppm, the 72-hour LC50 = 7.10 ppm. No observations of moribundity or behavioral changes were seen in this study.

Data Quality: 1b

References: Acute Toxicity of FMC 10233 Technical to the Atlantic Silverside (*Menidia menidia*). Environmental Science and Engineering, Inc.; ESE Study Number: 85-322-0800-2130, FMC Study Number A85-1920

12. ACUTE TOXICITY TO FISH

Test Substance: 2-methallyloxyphenol (68%)

Method: ASTM, 1980, Standard Practice D729-80, "Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians"

The acute toxicity of 2-methallyloxyphenol to the Marine Fish, Spot (*Leiostomus xanthurus*) was conducted for 96 hours from August 18 to 22, 1984 at Springborn Bionomics, Inc. located in Pensacola, Florida. Spot were collected from Santa Rosa Sound, FL and maintained at Springborn Bionomics, Inc. for 41 days in flowing seawater. During holding, fish were fed a commercial flake food and live brine shrimp (*Artemia salina*) nauplii daily. During the 4-day period immediately before the test, the salinity and temperature of the holding water were 21-24 parts per thousand and 21-24°C. No mortality was observed during the 48-hour period before the test. The seawater control fish we 36-45 millimeters standard length and 1.1-1.6 gram wet weight and appeared to be representative of the test population. Water used to maintain and test the fish was natural seawater obtained from Big Lagoon, a Gulf of Mexico estuary adjacent to Springborn Bionomics, Inc. The pump intake was located about 80 meters offshore at a depth of approximately 3 meters. The seawater was pumped by a 316 stainless steel pump through hard polyvinylchloride pipes, through sand-filled fiberglass filters, and through 10-micrometer porosity polypropylene core filters into an elevated fiberglass reservoir. Water was aerated in the reservoir and allowed to flow by gravity through PVC pipes into the laboratory. The water was additionally filtered through a 1-micrometer porosity polypropylene core filter before distribution into the test chambers. The dilution water was aerated in the test chambers for 24 hours prior to initiating the test.

The test was conducted in uncovered 85-liter glass aquaria, each of which contained a final liquid volume of 60 liters at a depth of 23 centimeters. Salinity was 21-23 parts per thousand and temperature was maintained at 21-22°C. Lighting was ambient room lighting supplemented with fluorescent lighting during daytime working hours. Five fish were tested per aquarium and all treatments duplicated, resulting in 10 fish per treatment. Loading was calculated to be 0.11g of fish tissue per liter of test solution. Because of problems maintaining dissolved oxygen concentrations at acceptable levels, the test containers were aerated on test days 2, 3 and 4 for a period of time sufficient to return dissolved oxygen concentrations to greater than 90% if saturation. Fish were not fed during the test.

Based on range-finding results, five concentrations, a natural seawater control and an acetone solvent control were used in this study. Nominal test concentrations were 0.065, 0.125, 0.25, 0.50 and 1.0 ppm. All LC50's and their 95 percent confidence limits were calculated using a computer program (Stephan, 1982) and values were estimated using the following statistical methods: moving average angle, probit, and binomial probability.

Observations for mortality, morbidity and behavior were recorded and reported for all test concentrations every 24 hours throughout the test. Water quality parameters such as temperature, pH and dissolved oxygen were measured every 24 hours throughout the test.

Species:	Marine Fish, Spot (*Leiostomus xanthurus*)
Test Concentration (nominal):	control, solvent control, 0.065, 0.125, 0.25, 0.50 and 1.0 ppm
Exposure Period:	96 hours
Analytical Monitoring:	No
GLP:	Yes
Year:	1984
Results:	2-methallyloxyphenol was acutely toxic to Spot. After 96 hours of exposure, mortality of spot was 10 percent in concentration 0.065. Since test concentrations > 0.065 to 0.50 ppm had no mortality throughout the test, the death was thought to be non-treatment related. Partial loss of equilibrium was noted in four out of ten fish in the 1.0 ppm test concentration at the 24-hour observation. There was 100 percent mortality in 1.0 ppm test concentration at the 48-hour observation. There was no mortality in the controls. The 96-hour LC50 was 0.71 ppm with 95 percent confidence limits of 0.50 – 1.0 ppm. The NOEC = 0.50 ppm. The 24-hour LC50 >1.0 ppm and the 48 and 72-hour LC50 = 0.71 ppm. The pH after 96 hours of exposure was 7.2 – 7.4 in all test containers.

Data Quality: 2c

References: Acute Toxicity of methallyloxyphenol to Marine Fish, Spot (*Leiostomus xanthurus*) in a Static Test System. Springborn Biomedics, Inc. Springborn Study Number: BP-84-10-102-R, FMC Study Number A84-1312

13. TOXICITY TO AQUATIC PLANTS

Test Substance: 2-methallyloxyphenol (99.5%)

Method: EPA Protocol No. EG-8, "Algal Acute Toxicity Test", August 1982

The toxicity of 2-methallyloxyphenol to algae, Skeletonema costatum, was investigated. The test was conducted from May 19 to 23, 1986 for FMC Corporation by Battelle New England Marine Research Laboratory in Duxbury, Massachusetts. An axenic culture of *Skeletonema costatum* was received from the Bigelow Lab for Ocean Sciences, West Boothbay Harbor, Maine. The culture had not been maintained in an enriched filtered seawater medium, therefore it was acclimated at Battelle for over two months to the artificial sea water (Rila) medium in which it would be tested.

On the day of test initiation, seven 500-mL flasks of freshly prepared Rila medium without EDTA were inoculated with enough 7-day-old algal culture to achieve an initial cell density of approximately 7.7×10^4 cells/mL. Nominal concentrations of 75.0, 37.5, 18.75, 9.4, and 4.7 mg 2-methallyloxyphenol /L diluent were prepared as primary spiking solutions by spiking a stock solution of 2-methallyloxyphenol dissolved in acetone into five of the seven flasks. The remaining two flasks were used for a solvent control and a Rila medium control. Each primary stock

solution was then dispensed into three pre-rinsed replicate 250-mL flasks. The assignment of primary stock solutions to 500-mL flasks and 250-mL replicate flasks, and the flask positions on the shaker tables were determined by random assignment. The test began when replicate flasks were placed on two shaker tables (60 $\pm$ 10 rpm) located in a controlled temperature (20 $\pm$ 2°C) and lighting (14:10 light:dark) environment. The salinity of Rila medium was verified at the start of the test. The pH and cell densities were determined at 0 and 96 hours. Absorbance in each test replicate was measured at 24, 48, 72 and 96 hours. Light intensity and shaker table rpms were measured at 0, 24, 48, 72 and 96 hours. Temperature was monitored and recorded hourly. At the end of 96 hours, direct counts of each replicate were made using a hemocytometer and the percent mean cell density from each concentration relative to the mean cell density of the control was calculated. Prior to any other statistical analyses, the mean cell density of the Rila medium control and the solvent control were compared using an F-test to determine if the test data met the assumptions necessary for application of a parametric test. Because these assumptions were met, a t-test was then used to determine if there was a statistical difference between control treatments.

Species: *Skeletonema costatum*

Test Concentration: 76.4, 37.64, 19.43, 10.59, 3.45 and 0 in the controls (measured)

Exposure Period: 96 hours

Analytical Monitoring: Concentrations of 2-methallyloxyphenol were analyzed in seawater by high performance liquid chromatography. Duplicate aliquots of test solution were collected from each test replicate at 0, 48, and 96 hours. Samples were centrifuged for ten minutes at 3000 rpm to sediment algal cells and the supernatant was collected for direct injection and analysis by HPLC. All samples were analyzed by 48 hours of collection. The external standard method was used for calibration. No attempt was made to verify the stability of the acetone carrier in the test system.

GLP: Yes

Year: 1986

Results: Throughout the test, the shaker table oscillations varied from 52 to 68 rpm, the light intensity from 350 to 400 foot candles, and the temperature from 18 to 23°C. The Rila medium used to prepare test solutions had a salinity of 30 o/oo and a pH of 8.07. After the addition of algal culture and test solution spike, the mean 0-hour pH was 6.72. The mean 96-hour pH was 7.29. The 0-hour mean cell density was 6.9 x 10^4 and the final cell densities ranged from 5.5 x 10^5 cells/mL in the Rila medium to 6.2 x 10^2 at a mean measured concentration of 76.40 mg 2-methallyloxyphenol /L. The mean cell densities of the control and solvent control treatments were not significantly different.

Analysis of the test solutions revealed that the mean measured concentrations ranged from 73.4 to 112.7 percent of the nominal concentrations. Mean measured concentrations were76.40, 37.64, 19.43, 10.59, and 3.45 mg/L. The calculated EC50 for *Skeletonema costatum* cell density based on the mean measured concentrations was 26.57 mg/L with 95% confidence limits (24.16-29.40). This value corresponds to the 96-hour absorbance measurement that place the EC50 between 19.43 and 37.48 mg/L. The extrapolated EC90 was 42.86 mg/L. The highest no observed effect concentration was 19.43 mg/L.

Examinations of the cells at 96 hours revealed no aberrant cell or chain formations in any concentration tested. Loss of color (chlorophyll) and general fading of cells (representing disintegration of the cell wall and death of the cell) was noted only at 37.64 mg/L concentration. The 74.6 mg/L concentration contained essentially no viable cell material. Cells in control treatments were actively dividing at 96 hours, with development of new cell membranes clearly evident.

At the end of 96 hours, a 0.5 mL aliquot of culture from the three 75.0 mg/L replicates was diluted with fresh medium to a concentration of 4.7 mg/L and grown under the test conditions for nine days. After nine days of incubation, no evidence of cell growth could be detected under microscopic examination.

Data Quality: 1a

References: Algal Toxicity Test with FMC 10233, Battelle New England Marine Research Laboratory; Battelle Study Number N 0950-4804. FMC Study Number A85-1918

14. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Daphnia magna – see Mysid Shrimp data below.

15. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: 2-methallyloxyphenol (98.1%)

Method: ASTM 1980, "Standard Practice for Conducting Acute Toxicity Testes with Fishes, Macroinvertebrates and Amphibians, E729-80; US EPA 797.1930

The test was performed under flow-through conditions with five concentrations of test material, a dilution water control and a solvent control. Mysids, which were less than 24-hr old at the beginning of the test, were produced at Enseco Inc., Marblehead.

The nominal concentrations of 2-methyallyloxyphenol as received were: 2040, 1260, 1020, 630, 390 and 0 ug/l (control and solvent control). The concentrations were based on results of static and flow-through range-finding tests. Twenty mysids were randomly distributed among two replicates (10 mysids per replicate) of each treatment. Within each exposure

vessel, the mysids were placed in a cage that consisted of a 9-cm diameter glass petri dish with a 9-cm high collar of Nitex screen. Culture vessels were randomly arranged in a water

bath that was placed in an environmental chamber. Natural, filtered seawater that was used as dilution water was collected daily from the Atlantic Ocean at Marblehead, Massachusetts. Dilution water was characterized for the following parameters: boron - 3.2 mg/l, residual chlorine – No Detection (detection limit 0.02 mg/l), fluoride – 0.63 mg/l, ammonia (as nitrogen) – No Detection (detection limit 0.10 mg/l), total organic carbon – 22.0 mg/l, total suspended solids – No Detection (detection limit 1.0 mg/l), organochlorine pesticides – No Detection (detection limit 0.1 ug/l) and total organic chlorine – No Detection (detection limit 1.0 ug/l).

Test media was formulated and delivered to each exposure vessel by proportional diluter. Mysids were fed with live Artemia salina nauplii at least once each day. The number of live mysids and the occurrence of abnormal behavior (inability to maintain positioning, uncoordinated swimming or cessations of swimming) was determined daily. At the conclusion of the test, the weight of surviving control and solvent control mysids was determined after the mysids were rinsed in deionized water. Control and solvent control mysid had an average weight of 0.08 mg. Aeration was not employed during the test and the photoperiod was adjusted to 16-hr light and 8-hr dark. Dissolved oxygen concentration, pH, salinity and temperature were measured at 24-hr intervals during the test.

Species:	Mysidopsis bahia
Test Concentration:	527, 660, 1080, 1363, 2307 ug/L (measured)
Exposure Period:	96 hours
Analytical Monitoring:	Analytical determination of test material concentrations was performed on composite samples that were prepared with well-mixed aliquots removed from each of two replicate test vessels at each concentration. Samples were collected from each test vessel after 0, 48 and 96-hr exposure. All samples were shipped to the analytical laboratory at Enseco in Cambridge, Massachusetts in coolers with ice, and passed through a 0.45micron filter prior to analysis. The analytical procedure utilized high performance liquid chromatography.
GLP:	Yes
Year:	1987
Results:	Both control and solvent control survivals were 95%. The highest and lowest measured salinity values were 20 and 21 o/oo. The difference in average temperatures between any two test chambers was always less than 1°C, and the temperature measured in any test chamber was always less than 2°C different from the average. All measured concentrations of dissolved oxygen were above 60% saturation (5.0 mg/l at 25°C).

Media in all test vessels that contained 2-methallyloxyphenol, and all control vessels, was initially clear and remained clear throughout the test. The mean, measured concentrations of test material agreed generally with the nominal concentrations, and the test material concentrations remained stable throughout the 96-hr exposure period. Mean, measured concentrations of 2-methallyloxyphenol were: No Detection (less than 5 ug/l, control and solvent control), 527, 660, 1080, 1363 and 2307 ug/l. No sample contained measured concentrations of 2-methallyloxyphenol that were less than 50% or more than 130% of the appropriate mean measure concentration.

The 24-, 48-, and 72-hr LC50 for mysids under flow-through conditions was above 2307 ug/l. The 96-hr LC50 for mysids was 1327 ug/l. The 95-percent confidence interval for this LC50 is 1080-2307 ug/l 2-methallyloxyphenol.

Data Quality: 1b

References: "Flow-Through, Acute Toxicity of 2-Methallyloxyphenol to Mysids, Mysidopsis Bahia", Enseco Incorporated, March 1988, FMC Study Number A1987-2520-01

16. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: 2-methallyloxyphenol (98.5%)

Method: ASTM, 1980, Standard Practice D729-80, "Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians"; US EPA, 1985, TSCA Guidelines, 797.1930, "Mysid Shrimp Acute Toxicity Test"

The test was performed under flow-through conditions with five concentrations of test material, a dilution water control and a solvent control (DMF). Mysids, which were less than 24-hr old at the beginning of the test, were produced at Enseco Inc., Marblehead.

The calculated nominal concentrations of 2-methyallyloxyphenol as received were: 2700, 1440, 990, 570, 300 and 0 ug/l (control and solvent control). The concentrations were based on results of static and flow-through range-finding tests. Twenty mysids were randomly distributed among two replicates (10 mysids per replicate) of each treatment. Within each exposure vessel, the mysids were placed in a cage that consisted of a 9-cm diameter glass petri dish with a 9-cm high collar of Nitex screen. Culture vessels were randomly arranged in a water bath that was placed in an environmental chamber. Natural, filtered seawater that was used as dilution water was collected daily from the Atlantic Ocean at Marblehead, Massachusetts. Dilution water was characterized for the following parameters: boron - 3.6 mg/l, residual chlorine – No Detection (detection limit 0.02 mgl), fluoride – 0.80 mg/l, ammonia (as nitrogen) – No Detection (detection limit 0.10 mg/l), total organic carbon – No

Detection (detection limit 0.5 mg/l), total suspended solids – No Detection (detection limit 1.0 mg/l), organochlorine pesticides – No Detection (detection limit 0.1 ug/l) and total organic chlorine – No Detection (detection limit 1.0 ug/l).

Test media was formulated and delivered to each exposure vessel by proportional diluter. Mysids were fed with live Artemia salina nauplii at least once each day. The number of live mysids and the occurrence of abnormal behavior (inability to maintain positioning, uncoordinated swimming or cessations of swimming) was determined daily. At the conclusion of the test, the weight of surviving control and solvent control mysids was determined after the mysids were rinsed in deionized water. Control and solvent control mystids had an average weight of 0.10 mg, resulting in a loading rate of 0.0001 g/l. Aeration was not employed during the test and the photoperiod was adjusted to 16-hr light and 8-hr dark.

Dissolved oxygen concentration, pH, salinity and temperature were measured at 24-hr intervals during the test.

Species: Mysidopsis bahia

Test Concentration: 255, 417, 643, 880 and 2255 ug/L collected at 0, 48 and 96 hrs
338, 548, 860, 1175 and 3000 ug/L collected at 0 and 96 hrs

Exposure Period: 96 hours

Analytical Monitoring: Analytical determination of test material concentrations was performed on composite samples that were prepared with well-mixed aliquots removed from each of two replicate test vessels at each concentration. Samples were collected from each test vessel after 0, 48 and 96-hr exposure. All samples were shipped to the analytical laboratory at Enseco in West Sacramento, California in coolers with ice, and passed through a 0.45micron filter prior to analysis. The analytical procedure utilized high performance liquid chromatography.

GLP: Yes

Year: 1988

Results: This 96-hr flow-through study was conducted to confirm results obtained in an earlier study (see study above). The dilution water in the earlier study had a dissolved organic carbon concentration which was unacceptably high (> 2 mg/l). The DOC in this confirmatory study was < 0.5 mg/l.

Both control and solvent control survivals were 95%. The highest and lowest measured salinity values were 20 and 21 o/oo. The difference in average temperatures between any two test chambers was always less than 1°C, and the temperature measured in any test chamber was always less than 2°C different from the average. All measured concentrations of dissolved oxygen were above 60% saturation (5.0 mg/l at 25°C).

Media in all test vessels that contained 2-methallyloxyphenol, and all control vessels, was initially clear and remained clear throughout the test. The mean, measured concentrations of test material agreed generally with the nominal concentrations, and the test material concentrations remained stable throughout the 96-hr exposure period. Mean, measured concentrations of 2-methallyloxyphenol when calculated using samples collected at 0, 48 and 96 hours after test initiation were: No Detection (less than 25 ug/l, control and solvent control), 255, 417, 643, 880, and 2255 ug/l. Analyses of samples collected at 48 hours after test initiation resulted in unexpectedly low concentrations of 2-methallyloxyphenol. it is believed that this was due to improper sample collection. Mean measured concentrations of 2-methallyloxyphenol using only the samples collected at 0 and 96 hours were: No Detection (control and solvent control), 338, 548, 860, 1175 and 3000 ug/l.

Samples collected at 48 hours resulted in lower concentrations than expected, which was believed to have been due to improper sample collection.

The 24-, 48-, and 72-hr LC50 for mysids under flow-through conditions were > 2255, 2015 and 1689 ug/l, respectively, from samples collected at 0, 48 and 96 hours. The 96-hr LC50 for mysids was 1130 ug/l. The 95-percent confidence interval for this LC50 is 900-1553 ug/l 2-methallyloxyphenol.

The 24-, 48-, and 72-hr LC50 for mysids under flow-through conditions were > 3000, 2682 and 2249 ug/l, respectively, from samples collected at 0 and 96 hours. The 96-hr LC50 for mysids was 1505 ug/l. The 95-percent confidence interval for this LC50 is 1198-2067 ug/l 2-methallyloxyphenol.

Data Quality: 1b

References: "Flow-Through, Acute Toxicity of 2-Methallyloxyphenol to Mysids, Mysidopsis Bahia", Enseco Incorporated, October 1988, FMC Study Number A1987-2520-03

17. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: 2-methallyloxyphenol (97.9%)

Method: ASTM, 1980, Standard Practice D729-80, "Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians"

The flow-through acute toxicity of 2-methallyloxyphenol to the mysid shrimp was conducted for FMC Corporation for 96 hours from January 6 to 10, 1986 at Environmental Science and Engineering, Inc. in Gainesville, Florida.

Mysid shrimp were collected from an in-house culture when they were ≤ 24 hours old and maintained under static conditions for 3 days prior to testing. Holding water possessed a salinity of 20 parts per thousand and was maintained at approximately 24°C. During holding, mysids were fed live brine shrimp (*Artemia salina*) daily. No deaths were observed during the 3-day holding period.

Salt water used for holding and testing was filtered (5-micrometer) natural seawater. The test water was collected at Marineland, Florida, and diluted to ca. 20 parts per thousand salinity with well water from Gainesville, Florida. Test water was sterilized by ultraviolet light prior to entering the test system. The test system was a solenoid diluter with a dilution factor of 0.5. The system consisted of seven duplicate sets of glass aquaria designed to maintain approximately 9 liters of test solution or dilution water. Five concentrations, a natural seawater control and an acetone solvent control were used in this study. Flow rate to each duplicate aquarium was sufficient to provide ca. 6.8 daily volume turnovers. Water and ambient air temperature were regulated to maintain temperature. A 14-hour light and 10-hour dark photoperiod was maintained.

Nominal test concentrations were 62.5, 125, 250, 500, and 1000 ppb 2-methallyloxyphenol. Test concentrations were prepared fresh with each diluter cycle. During each cycle, volumes of dilution water were mixed with volumes of test solutions from the mixing chamber for provide the five test concentrations utilized in the test. A solvent control was maintained through injection of 25uL of acetone into the solvent control cell with each cycle.

The test was initiated when 10 shrimp were impartially distributed to each test chamber providing 20 shrimp per treatment. During testing, shrimp were fed live brine shrimp daily. Water samples were collected from all treatment replicates on days 0, 2, and 4 to monitor actual exposure concentrations. The LC50's and their 95 percent confidence limits were calculated by computer program (Stephan, 1982) using the following statistical methods: moving average angle, probit, and non-linear interpolation. Confidence limits for LC50 values were calculated by binomial probability.

Observations for mortality, morbidity and behavior were recorded and reported for all test concentrations every 24 hours throughout the test. Water quality parameters such as temperature, pH and dissolved oxygen were measured every 24 hours throughout the test.

Species: Mysid shrimp (*Mysidopsis bahia*)

Test Concentration: control, solvent control, 67, 138, 257, 495 and 1,053 ppb (measured)

Exposure Period:	96 hours
Analytical Monitoring:	Seawater samples were analyzed for 2-methallyloxyphenol following FMC Method Number ACG-111 as modified by Test Protocol Amendment 1 dated December 20, 1985. Manual injection by constant-pressure nitrogen filling of the HPLC injection loop was employed to obtain the required accuracy over a wide concentration range; this allowed all samples to be analyzed without dilution.
GLP:	Yes
Year:	1986
Results:	Measurement of exposure concentration samples from 0, 48 and 96 hours using HPLC showed that test concentrations were maintained throughout the 96-hour exposure. Mean measured concentrations were 67, 138, 257, 495 and 1,053 ppb and were from 99 to 110 percent of nominal concentrations. The average method recovery was 107 percent with a standard deviation of 5 percent. Some contamination of controls was found on day 0, but none was detected on test days 2 and 4. An additional sample was collected of incoming seawater on day 0 as a check on the contamination. No 2-methallyloxyphenol was detected in the incoming seawater and the source of the contamination was not found. 2-methallyloxyphenol was acutely toxic to mysid shrimp in the mean measured concentration range of 67 to 1053 ppb. After 96 hours of exposure, mortality of mysid shrimp ranged from 10 percent in 67 ppb to 100 percent in 1,053 ppb 2-methallyloxyphenol. One observation of partial loss of equilibrium was observed in the 24-hour observation period of the 138 ppb test concentration. No mortality was observed in the seawater control. There was 6 percent in the solvent control where cannibalism was noted in the 72-hour observation. Due to a hole in one of the solvent control screen chambers, three mysids escaped during the first 24 hours and were eliminated from all calculations of mortality. The 96-hour LC50 was 201 ppb with 95 percent confidence limits of 149 to 265 ppb. The 24-hour LC50 > 1,053 ppb; the 48-hour LC50 = 520 ppb and the 72-hour LC50 = 208 ppb. An NOEC value was not determined. The test was maintained at 22 to 23°C. The salinity of the test water was 18 to 22 parts per thousand. The dissolved oxygen concentrations remained $\geq$ 6.5 ppm or $\geq$ 88 percent of saturation in all test aquaria throughout the test. The pH ranged from 7.5 to 7.8 units in all test aquaria throughout the test.
Data Quality:	1b

References: Acute Toxicity of FMC 10233 Technical to the Mysid Shrimp (*Mysidopsis bahia*) Under Flow-through Conditions. Environmental Science and Engineering, Inc.; ESE Study Number 85-322-0800-2130, FMC Study Number A85-1919

18. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test Substance: 2-methallyloxyphenol (68%)

Method: ASTM, 1980, Standard Practice D729-80, "Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians"

The static marine toxicity of 2-methallyloxyphenol to the mysid shrimp was conducted for FMC Corporation for 96 hours from September 18 to 22, 1984 at Springborn Bionomics, Inc. in Pensacola, Florida.

Mysid shrimp were collected from an in-house culture when they were $\leq$ 24 hours old and reared for an additional 3 days in flowing seawater. During holding, mysids were fed live brine shrimp (*Artemia salina*) nauplii daily. During the 4-day period immediately before the test, the salinity and temperature of the holding water were 23 parts per thousand and 21-23°C, respectively. Mysids appeared to be in good condition prior to initiation of the test. Water used to maintain and test the mysids was natural seawater that was obtained from Big Lagoon, a Gulf of Mexico estuary adjacent to Springborn Bionomics, Inc. The pump intake was located about 80 meters offshore at a depth of approximately 3 meters. The seawater was pumped by a 316 stainless steel pump through hard polyvinylchloride pipes, through sand-filled fiberglass filters, and through 10-micrometer porosity polypropylene core filters into an elevated fiberglass reservoir. Water was aerated in the reservoir and allowed to flow by gravity through PVC pipes into the laboratory. The water was additionally filtered through a 5-micrometer porosity polypropylene core filter before distribution into the test chambers.

The test was conducted in covered 1.6-liter glass culture dishes, each of which contained a final liquid volume of 1 liter at a salinity of 24 parts per thousand. Ten mysids were tested per dish and all treatments duplicated. The test was conducted under fluorescent lighting on a 14-hour light: 10-hour dark photoperiod and maintained 22-23°C. Mysids were fed on days 0, 2 and 3 during the test. Test solutions were not aerated.

	Based on range-finding results, five nominal concentrations, a natural seawater control and an acetone solvent control were used in this study. Nominal test concentrations were 0.23, 0.39, 0.65, 1.1, 1.8 and 3.0 ppm 2-methallyloxyphenol. The LC50's and their 95 percent confidence limits were calculated using a computer program (Stephan, 1982) which estimated the LC50 values using the following statistical methods: moving average angle, probit, and binomial probability. Observations for mortality, morbidity and behavior were recorded and reported for all test concentrations every 24 hours throughout the test at which time any dead mysids will be removed. Water quality parameters such as temperature, pH, salinity and dissolved oxygen were measured on days 0 and 4.
Species:	Mysid shrimp (*Mysidopsis bahia*)
Exposure Period:	96 hours
Analytical Monitoring:	No
GLP:	Yes
Year:	1984
Results:	2-methallyloxyphenol was acutely toxic to Mysid shrimp. After 96 hours of exposure, mortality of Mysids ranged from 10 percent in concentrations less than or equal to 0.39 ppm to 100 percent in concentrations greater or equal to 1.8 ppm. Mortality in the seawater control was 10 percent and the mortality in the solvent control 5 percent. The 96-hour LC50 was 0.81 ppm with 95 percent confidence limits of 0.39 – 1.8 ppm. There was no determination of an NOEC. The 24-hour LC50 = 1.4 ppm; the 48-hour LC50 = 1.3 ppm; the 72-hour LC50 = 0.81 ppm. This study was repeated.
Data Quality:	2c
References:	Acute Toxicity of methallyloxyphenol to the Mysid Shrimp (*Mysidopsis bahia*) In a Static Test. Springborn Bionomics, Inc. Springborn Study Number BP-84-10-100-R, FMC Study Number A84-1311

TOXICITY

19. ACUTE TOXICITY

A. ORAL

Test Substance: 2-Methallyloxyphenol (68%)

Method: An oral LD50 was conducted with test material administered undiluted by gavage beginning June 7, 1984 and terminated on June 13, 1984. Young adult Sprague-Dawley rats were received from Taconic Farms, Germantown, New York. The rats were randomized into their cages using a computer-generated table of random numbers. The condition of the animals was examined prior to formally being released for the study. Fresh tap water and Purina Laboratory Rat Cow 5001 were provided *ad libitum*. Room temperature was maintained from 65°F to 76°F during the study, while relative humidity ranged from 57% to 91%. The animals were individually housed in stainless steel suspended rat cages and maintained in a room with a 12-hour fluorescent light and 12-hour dark cycle.

Animals were fasted overnight prior to dosing. Immediately prior to dosing, male body weights ranged from 210 to 296 grams and female weights were between 201 and 236 grams. Doses of 4500, 4000, 3000, 2500 and 2000 mg/kg were run in males and doses of 4500, 3000, 2700, and 2500 mg/kg were run in females. The various doses of test material were administered as a single treatment via gavage directly into the stomach of each animal approximately four hours after initiation of the light cycle. Observations for toxicity and mortality were conducted at 0.5, 1, 2, 3, 4 and 6 hours on the day of dosing and twice daily for 13 days. On day 14 the animals were observed once. Body weights were recorded on days 0, 7 and 14 of study. Animals dying intercurrently were weighed upon discovery of death. Gross necropsies were performed on all animals which died during the study. Survivors were euthanized with CO2 gas on day 14 and submitted to gross necropsy.

LC50 calculations were performed using a TI-59 Logit-Linear Regression program (Texas Instruments Calculator Products Division, and modified for an Apple II+ computer).

Species/strain: Sprague-Dawley rats

Sex: Both

No. Animals/Group: 10/sex/group

Post dosing observation period: 14 days

GLP: Yes

Year:	1984
Results:	Predominant clinical signs included decreased locomotion, ataxia, recumbency, exophthalmos, hematuria, lacrimation, oral discharge and abdominogenital staining. Most signs of toxicity subsided by the 5th day of the study. All but one of the animals gained weight by day 14 of the study. The animal that lost weight was judged to have an middle-ear problem which was unrelated to treatment. Internal gross necropsy findings among decedents included red fluid in the intestines and bladder of several rats, a hemorrhagic stomach lining in one rat, and lung lesions accompanied by fluid in the pleural cavity of one rat. Animals that survived treatment and were euthanized on day 14 appeared normal when necropsied. The test material is classified as slightly toxic to adult rats under the conditions of this study. The oral LD50 in males is 3222 mg/kg (2724-3720, 95% confidence limits);oral LD50 in females is 2845 mg/kg (2563-3126 mg/kg, 95% confidence limits) and oral LD50 combined is 2943 mg/kg (2685- 3202, 95% confidence limits).
Data Quality:	1a
References:	Acute Oral Toxicity of FMC 10233 In Rats. FMC Toxicology Laboratory, FMC Study Number: A84-1280

B. INHALATION

No data available – see 19.A. *Acute Toxicity Oral* above.

20. GENETIC TOXICITY IN VIVO

Test Substance:	2-methallyloxyphenol
Method:	EPA Guideline: In vivo Chromosome Aberrations Groups of male rats (5/group) were dosed with test article and sacrificed at 6, 24 and 48 hours later for each dose and for a corn oil control group. A positive control group (5 rats/group) received 40 mg/kg cyclophosphamide and was sacrificed 24 hours later. Clinical observations were made twice daily or prior to sacrifice. Body weights were recorded once, prior to compound administration for the 6-hr sacrifice and twice, prior to compound administration and prior to colchicines administration for the 24-hr and 48-hr sacrifice groups. Two hours prior to sacrifice, animals were given a single intraperitoneal injection of 2.0 mg/kg colchicines to arrest cells in metaphase. Bone marrow cells were collected from both femurs. Two slides of freshly prepared bone marrow cells from each animal were stained and evaluated microscopically for the presence of cytogenetic abnormalities. At least 50 metaphase spreads were analyzed for each animal. Results were analyzed statistically using the Kruskal Wallis nonparametric analysis of variance and nonparametric pairwise group comparisons.
Species/strain:	Sprague-Dawley CD Rats
Sex:	Male
Route of Administration:	Oral gavage
Exposure Period:	6, 24 and 48 hours
Doses:	100, 300 and 1000 mg/kg
Vehicle:	Corn oil
GLP:	Yes
Year:	1984
Results:	The test material did not induce an increase in chromosome aberrations relative to the solvent control. No statistically significant differences were seen between the mean modal numbers and the mean mitotic indices of the test groups and the vehicle controls.

Data Quality: Code 1a

References: In Vivo Bone Marrow Chromosome Study in Rats. FMC Study A83-853. March 16, 1984

21. GENETIC TOXICITY IN VITRO

Test Substance: 2-methallyloxyphenol

Method: EPA Guideline, 1983. Mutagenicity Assay (Ames Test). Each treatment was run in triplicate with and without metabolic activation by rat liver microsomes. Induced with Aroclor 1254. Solubility and toxicity to bacterial were determined prior to the assay. Positive controls included sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene. The negative control was the vehicle, DMSO.

Type: *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)

System of Testing: *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538

Concentration: 20, 100, 500, 1,000 and 2,000 ug/plate with and without S9

Metabolic Activation: With and without S9

GLP: Yes

Year: 1983

Results: The test material was negative (not mutagenic) when tested in the presence of metabolic activation in all tester strains. The test material caused a positive increase in revertants/plate in tester strain TA1535 in the absence of metabolic activation. The test material also caused an increase in mutant frequency in tester strain TA100 in the absence of metabolic activation; however, this increase did not meet the criterion for a positive response due to the high background incidence in this strain. The test material is considered positive (mutagenic) in this assay. The positive and negative controls in this study were adequate for a valid test.

With Activation

Strain	Solvent DMSO	20 ug	100 ug	500 ug	1000 ug	2000 ug
	Revertants per plate					
TA98	28	29	32	31	25	26
TA100	134	135	135	117	111	84
TA1535	30	25	24	18	21	10
TA1537	9	7	14	6	8	4
TA1538	25	31	26	18	20	22

Without Activation

Strain	Solvent DMSO	20 ug	100 ug	500 ug	1000 ug	2000 ug
	Revertants per plate					
TA98	18	10	12	17	13	11
TA100	126	136	151	181	176	80
TA1535	44	50	71	128	142	127
TA1537	6	7	3	4	4	4
TA1538	9	11	7	8	8	7

Data Quality: Code 1a

References: "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames test)," Microbiological Associates, FMC Study Number A82-810. March 9, 1983; Mutation Research 48, 121-130, 1977.

22. GENETIC TOXICITY IN VITRO

Test Substance: 2-methallyloxyphenol

Method: EPA Guideline, 1983

Type: *Salmonella*/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test). Each treatment was run in triplicate with and without metabolic activation by rat liver microsomes. Induced with Aroclor 1254. Solubility and toxicity to bacterial were determined prior to the assay. Positive controls included methylnitronitrosaguanidine, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene. The negative control was the vehicle, DMSO.

System of Testing: *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538

Concentration: 61.63, 185.2, 555.5, 1666.7 and 5000 ug/plate with and without S9

Metabolic Activation: With and without S9

GLP: Yes

Year: 1984

Results: The test material was negative (not mutagenic) when tested in the presence of metabolic activation in all tester strains. The test material caused a positive increase in revertants/plate in tester strain TA1535 in the absence of metabolic activation. The test material also caused an increase in mutant frequency in tester strain TA100 in the absence of metabolic activation; however, this increase did not meet the criterion for a positive response due to the high background incidence in this strain. The test material is considered positive (mutagenic) in this assay. The positive and negative controls in this study were adequate for a valid test.

With Activation

Strain	Solvent DMSO	61.73 ug	185.2 ug	555.5 ug	1666.7 ug	5000 ug
	Revertants per plate					
TA98	31.3	38.3	30.0	38.3	20.7	Toxic
TA100	143.7	156.7	167.7	158.3	120.7	Toxic
TA1535	18.3	19.7	23.0	24.7	10.0	Toxic
TA1537	9.0	10.7	8.7	9.3	5.3	Toxic
TA1538	22.3	27.7	17.0	17.7	18.0	Toxic

Without Activation

Strain	Solvent DMSO	61.73 ug	185.2 ug	555.5 ug	1666.7 ug	5000 ug
	Revertants per plate					
TA98	15.7	15.7	15.7	17.0	8.0	Toxic
TA100	158.0	178.3	172.0	176.3	182.3	Toxic
TA1535	28.3	53.3	74.3	97.3	58.0	Toxic
TA1537	7.0	10.3	9.3	9.0	3.7	Toxic
TA1538	9	11	7	8	8	Toxic

Data Quality: Code 1a

References: "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames test)," Microbiological Associates, FMC Study Number A83-849. January 16, 1984; Mutation Research 48, 121-130, 1977

23. GENETIC TOXICITY IN VITRO

Test Substance: 2-methallyloxyphenol

Method: Modified Ames Test. One tester strain and no positive controls and single plates for each treatment.

Type: *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (ModifiedAmes Test)

System of Testing: *S. typhimurium* strain TA1535

Concentration: 2.5, 12.5, 62.5 , 312.5, 625, 1250, 2500, 5000, and 10,000ug/plate without S9

Metabolic Activation: Without S9

GLP: Yes

Year: 1983

Results: Eight samples of 2-methallyoxyphenol were evaluated in a modified Ames Test using TA1535 only in the absence of metabolic activation. All eight samples induced an increase in the revertants per plant. Four of the samples were considered "positive" or mutagenic based on the criterion set in the protocol, more than a two-fold increase in revertants/plate compared to the solvent control.

Summary of Maximum Fold-Increase in Revertants/plate compared to solvent control:

Sample	Fold Increase in Revertants/plate relative to DMSO Control
A	1.7
B	3.1
C	2.4
D	3.3
E	1.7
F	1.9
G	2.7
H	1.7

Data Quality: Code 2c

References: Modified Salmonella Plate Incorporation Mutagenicity Assay (Modieifed Ames Test), FMC StudiesA83-953, A83-594, A83-955, A83-956, A83-957, A83-958, A93-959, A83-960, October 25, 1983.

24. GENETIC TOXICITY IN VITRO

Test Substance: 2-methallyloxyphenol (64%)

Method: EPA Guideline. Cultured T5178Y cells were used in this study. The solvent was solubilized and diluted with DMSO. Test solutions were prepared under amber lights and kept in the dark during the exposure period. Cells were exposed to test article for 4 hours in the toxicity test and mutation assay. Following the initial exposure, cells were washed and incubated for two days at a cell density of 300,000 cells/ml. At the end of the expression period, cells were placed in cloning medium containing 2-4 ug/ml of trifluorothymidine (TFT) which allows only the TK -/- cells to grow. Two flasks per culture were cloned for each treatment. For each pair of flasks, one was labeled with TFT and the other was labeled for viable count. Six petri plates per test article concentration were prepared: three from the TFT labeled culture and three from the viable count culture. At the end of the cloning procedure mutant frequency was calculated by comparing the number of colonies on treated plates to those on the controls. Mutation frequency was calculated by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding viable count plates, normalizing for the number of cells.

Type:	L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay
System of Testing:	L5178Y cells
Concentration:	Activated: 0.0084, 0.0063, 0.0047, 0.0036, 0.0027, 0.0020, 0.0015, 0.0011, 0.0011, 0.0008, and 0.0006 ul/ml. Non-Activated: 0.0075, 0.0056, 0.0042, 0.0032, 0.0024, 0.0018 and 0.0013 ul/ml
Metabolic Activation:	With and without
GLP:	Yes
Year:	1984
Results:	In the non-activated assay, the total growth ranged from 13% to 99% in one assay and ranged from 21% to 108% in the other assay. In both on the non-activated assays the test material induced an increase in mutant frequency and was considered mutagenic in this assay. In the activated assay the total growth ranged from 7% to 95%. There was no increase in mutant frequency in the activated assay.
Data Quality:	Code 1a
References:	L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay, FMC Study A83-970. March 26, 1984; Mutation Research 31, 17-29 (1975)

25. GENETIC TOXICITY IN VITRO

Test Substance:	2-methallyloxyphenol
Method:	EPA Guideline
Type:	L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay
System of Testing:	L5178Y cells
Concentration:	Activated 3.3, 2.2, 1.5, 1.0, 0.7, 0.4, 0.3, 0.2 ug/ml Non-Activated: 330, 221, 148, 99.3, 66.5, 44.6, 29,9, 20.0 ug/ml
Metabolic Activation:	With and Without S9
GLP:	Yes
Year:	1984

Results:	The test article induced significant increases in the mutant frequency in both the presence and absence of metabolic activation in this assay at concentrations in which cell survival was greater than 10%. The test article was positive in this assay. The positive and negative controls met the criterion for a valid assay.
Data Quality:	Code 1a
References:	L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay, FMC Study A83-850

26. GENETIC TOXICITY IN VITRO

Test Substance:	2-methallyloxyphenol
Method:	EPA Guideline
Type:	Unscheduled DNA Synthesis in Rat Hepatocytes
System of Testing:	Rat primary hepatocytes
Concentration:	500, 100, 50, 10, 5, 1, 0.5 and 0.1 ug/ml
Metabolic Activation:	Not applicable
GLP:	Yes
Year:	1983
Results:	Rat hepatocytes were exposed to test article for approximately 24 hours. Nuclear grains were counted in 50 cells at each of five doses from 0.1 to 10 ug/ml. Cell survival ranged from 40% (high dose only) to 101%. Higher doses were cytotoxic. There was no net increase in the mean net nuclear grain count in the cell treated with the test article compared to the solvent control (100 cells counted). The positive controls showed the expected increase in net nuclear grain counts (50 cells counted). Both positive and negative controls met the criterion for a valid test. The test article is considered negative in this test.
Data Quality:	Code 1a
References:	Unscheduled DNA Synthesis Rat Hepatocyte Assay. FMC Study A83-851

27. GENETIC TOXICITY IN VITRO

Test Substance:	2-methallyloxyphenol
Method:	EPA Guideline

Type:	Cell Transformation Assay
System of Testing:	C3H/10T½ Mouse fibroblast cells
Concentration:	1, 3, 10, 30, 100, 300 ug/ml
Metabolic Activation:	No
GLP:	Yes
Year:	1983
Results:	There was no increase in the frequency of morphologically transformed Type III foci in mouse fibroblast cells in the presence of the test article at any concentration. There was no metabolic activation in this test. The positive and negative controls met the criteria for a valid study. The test material is considered negative in this assay in the absence of metabolic activation.
Data Quality:	Code 2b (due to no metabolic activation)
References:	Cell Transformation Assay without metabolic activation. FMC Report A83-852

28. REPEATED DOSE TOXICITY

No data available

29. REPRODUCTIVE TOXICITY

No data available

30. DEVELOPMENTAL TOXICITY

No data available

CRITERIA FOR RELIABILITY CODES

(Adapted from Klimisch et al 1997)

Code of Reliability	Category or reliability
1	**Reliable without restriction**
1a	GLP guideline study (OECD, EC, EPA, FDA, etc.)
1b	Comparable to guideline study
1c	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
2	**Reliable with restrictions**
2a	Guideline study without detailed documentation
2b	Guideline study with acceptable restrictions
2c	Comparable to guideline study with acceptable restrictions
2d	Test procedure in accordance with national standard methods with acceptable restrictions
2e	Study well documented, meets generally accepted scientific principles, acceptable for assessment
2f	Accepted calculation method
2g	Data from handbook or collection of data
3	**Not reliable**
3a	Documentation insufficient for assessment
3b	Significant methodological deficiencies
3c	Unsuitable test system
4	**Not assignable**
4a	Abstract
4b	Secondary literature
4c	Original reference not yet available
4d	Original reference not yet translated
4e	Documentation insufficient for assessment