US EPA ARCHIVE DOCUMENT

9-9-83

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003239

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Richard Mountfort, PM 23
Herbicide-Fungicide Branch
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

RBJ 9-9-83

SUBJECT: Anti-Foulant C-9211M
EPA Reg. No. 707-RTL
CASWELL Nos. 314B and 202C

Applicant:

Rohm & Haas Company
Independence Mall West
Philadelphia, PA 19105

Requested Action:

Request for use in formulating antifouling paints.

Recommendation(s):

The acute oral LD_{50} and dermal LD_{50} were performed using only male animals. Both sexes of animals must be tested.

The mutagenic evaluations (in vitro microbial assays) are not acceptable as submitted. The evaluation must employ strain specific positive controls in the assay without S-9 metabolic activation.

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A. Formulation of Anti-Foulant C-9211M

Active Ingredients:

4,5- Dichloro-2-n-octyl-3(2H)-isothiazolone----------35.00%
4-chloro-2-n-octyl-3(2H)-isothiazolone----------5.00%

Inert Ingrdients:----------60.00%
Total 100.00%

B. Chemical structure of isothiazolones:

C. Toxicology Data Submitted: Accession No. 237355

1. Acute Oral LD50 - Marine Antifoulant C-9211M (40% a.i.), (Rohm & Haas Co., TD No. 80-152, Report No. 80R-256, 1981).

10 male Charles River CD rats, weighing 211 to 218 grams were used in each of six dose levels of 0.69, 0.96, 1.33, 1.85, 2.57 and 3.57 g/kg. Rats were fasted overnight and gavaged with the undiluted test subtance. Animals were observed daily for 14 days for mortalities and signs of intoxication.

Results:

Acute Oral LD50 (male) = 1.89 (1.46-2,61) g/kg.

Necropsy of survivors revealed no remarkable gross lesions.

Toxic Signs

Diarrhea, salivation, ataxia, and tremors.

Classification: Core - Minimum
(1) Only male rats were tested.

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2. Acute Dermal LD50-Marine Antifoulant C-9211M (40% a.i.), (Rohm & Haas Co., TD No. 80-152, 1981)

6 male New Zealand White rabbits were used in each of six dose levels of 0.079, 0.50, 0.79, 1.25, 1.98 and 3.14 g/kg to study the percutaneous systemic toxicity of the material. The hair was removed and the undiluted material was held under an impervious cuff in continous 24 hour contact with the closely clipped skin. After exposure, the dressings were removed and the site of application was gently wiped to remove residual test substance.

Results:

Severe erythema and edema persisted through the 7 day; after 12 days the site of application showed flaking and peeling.

Gross necropsy observations included multiple red foci on gastric mucosa, liver mottled with pinpoint white foci, whitened small intestine.

No remarkable lesions were observed in the survivors.

Acute Dermal LD50 (Male) = 1.7 (1.0-4.5) g/kg

Classification: Core - Minimum
(1) only male rabbits used

Tox. Category: II

3. Primary Skin Irritation - Marine Antifoulant C-9211M (40% a.i.), (Rohm & Haas Co., TD No. 80-152, 1981)

One half (0.5 ml) of the undiluted material was applied to the intact and abraded skin of 6 male New Zealand White rabbits. Each test site was covered with an impervious patch in continuous 24 hr. contact with the intact and abraded skin. The sites were examined at 24, 72 hours and 7 days.

Results:

PII = 7.8/8.0 Tox. Category I

Severe irritant to both intact and abraded skin producing eschar, blanching, pocketing of edema, eschar peeling from test site and desication.

Classification: Core-Minimum

4. Primary Eye Irritation - Marine Antifoulant C-9211M (40% a.i.), Rohm & Hass Co., TD No. 80-152, Report No. 80R-256).

One-tenth (0.1 ml) of the undiluted test material was instilled into the conjunctival sac of 4 male New Zealand White rabbits. The eyes of 2 treated rabbits were washed approximately 20-30 seconds after dosing. Ocular reactions were recorded at 24, 48, 72, and 96 hours and then at 7, 14, 21 and 28 days. Ocular reactions based on the Draize Scoring Method were used.

Results:

Corneal opacity persisted beyond 21 days after dosing. Iris and cornea unscorable due to extreme swelling of lids. Iris could not be observed due to the density of the corneal opacity.

Irrigation of eyes 20-30 seconds after dosing reduced both the intensity and duration of the ocular effects.

Classification: Core-Minimum
(Although only 4 rabbits were used, sufficient information is obtained to determine the toxicity via eye contact).

Tox. Category: I

5. Acute Inhalation Study in Rats with Marine Antifoulant C-9211M (Rohm & Haas Co., Report No. 82R-19, March 11, 1982).

Charles River CD rats weight ranged from 178-230g were exposed to a single 4-hour whole body inhalation exposure to an aerosol of the test material. Ten male and ten female rats were used in each of seven dose levels of 0.21, 0.37, 0.53, 1.44, 2.12, 5.18 and 13.31 mg of product/L (analytical concentrations). Particle size ranged from a mean mass mediam diameter of 1.8 to 2.6 um and a geometric standard deviation of 1.19 to 2.0. An additional group consisting of 10 male and 10 female rats served as the vehicle control and was exposed to xylene vapors at an analytical concentration of 0.50 mg/L. Following exposure all animals were observed for phamacotoxic signs. Surviving animals in each group were necropsied on Day 14.

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The following organs were examined for macroscopic abnormalities: cervical lymph nodes, salivary glands, thyroids, trachea, lungs, heart/aorta, thymus, liver stomach, pancreas, spleen, intestines, kidneys, adrenals, bladder, gonads, uterus, eyes.

Results:

LC_{50} (M/F) = 0.72 (0.57-0.92) mg/L.

Pharmacotoxic Signs

Dose levels of 13.3 and 5.18 mg/L produced 100% mortality immediately following exposure.

At 2.12, 1.44, 0.53 and 0.37 mg/L most mortalities occurred within 24 hr. exposure except 2 males in the 1.44 mg/L level that were found dead on Day 2.

No mortalities occurred in the 0.21 mg/L dose level. These animals exhibited salivation, dyspnea and lacrimation.

Rats exposed to 0.53 mg/L or higher exhibited severe dyspnea, gasping, cyanosis, ataxia decreased activity, severe sensory irritation, salivation, lacrimation, eye squint, bradypnea, prostration and asphyxial convulsions. At 0.37 mg/L or greater severe symptoms of irritation were evident to mucous membranes, including the upper airway. Animals in the vehicle control group exhibited slight dyspnea.

Necropsy Observations

Red-mottled lungs and livers, foamy fluid in the trachea and lungs, gas filled gastrointestinal tract, dilation of the kidney medulla and corneal opacities.

Classification: Core-Minimum Study

Tox. Category: II

6. Delayed Contact Hypersensitivity Study in Guinea Pigs - Marine Antifoulant C-9211M, (Rohm & Haas Co., Report #81R-146, 4-22-82).

5 male and 5 female outbred Hartley-strain guinea pigs received the test substance, containing 78.63% active ingredient. The test subtance was applied as a 0.125% (w/v) solution in 80% (v/v) aqueous ethanol to the closely clipped backs once a week for three weeks (induction phase). The procedure consisted of applying a patch containing 0.4 ml of the test subtance for 6 hours/day. A control group of 5 male and 5 female guinea pigs and the treated group of guinea pigs were challenged with 0.0313% of the subtance dissolved in acetone. (Approx. 2 weeks after the last induction treatment). (Method used: Modified Buehler Techinque).

Results:

The challange concentration produced erythema reactions of grade 1 or greater in 8 of the 10 treated animals. No erythema reaction was observed in any of the control group guinea pigs. The severity of erythema was calculated to be 1.4 for the 24 hr. reading and 1.0 for the 48 hr. reading.

Under conditions of this study a grade 1 erythema reaction was judged to be the minimum response indicative of delayed contact hypersensitivity.

Conclusion:

C-9211M produced delayed contact hypersensitivity in guinea pigs under the conditions of this test.

Classification: Core-Minimum.

7. Mutagenicity Evaluation of Marine Antifoulant C-9211M, (Rohm & Hass Co., Report No. 81R-159, Dec. 11, 1981)

Procedure:

The test material (Marine Antifoulant C-9211M) was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation (liver extract from Aroclor 1254 pre-induced rats) as well as a saline buffer control mixture with each strain.

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A series of in vitro microbial assays employing Salmonella typhimurium at the following concentrations were used:

Strain TA 1535 at 0.00001-0.1 ul/plate
Strain TA 1537 at 0.00001-5.0 ul/plate
Strain TA 98 at 0.00001-5.0 ul/plate
Strain TA 100 at 0.00001-5.0 ul/plate

Positive controls used in the assay were:

2-Anthramine
2-Acetamidoflurene

Control diluent used, dimethylsulfoxide (DMSO), was previously shown to be nonmutagenic.

Results:

Strain TA1537 showed inhibition of growth at 1.0 ul/plate and above with activation and 0.001 ul/plate without activation.

Strain TA98 showed inhibition of growth at 0.1 ul/plate and above with activation and 0.01 ul/plate and above without activation.

Strain TA 100 showed inhibition of growth at 1.0 ul/plate and above with activation and 0.1 ul/plate and above without activation.

Strains, TA1537, TA98 and TA100 tested at 0.0001-0.005 ul/plate gave similar results.

Strain TA1535 showed inhibition at 0.1 ul/plate with activation and 0.01 ul/plate and above without activation.

The positive control gave expected positive response.

Conclusion:

Marine Antifoulant C-9211 M did not demostrate mutagenic acitivity in this assay as performed, however the strain specific positive controls under nonactivation system were not used and should be included in the report.

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The mutagenic activity of the test compound can not be evaluated without the required procedure, the study with specific positive control must be submitted.

Examples of specific positive controls are as follows:

Strain	Specific positive control
TA 1537	Sodium azide
TA 100	Sodium azide
TA 98	2-nitrofluorene
TA 1537	9-aminocridine

Conclusion: Study not acceptable as submitted.

8. Microbial Mutagen Test of Marine Antifoulant C-9211M (Rohm & Haas, Report No. 82R-45, August 27, 1982).

Procedure:

The test material Marine Antifoulant C-9211M, was tested for genetic activity with Salmonella typhimurium strains Ta 1535, TA1537 and TA98 at concentrations of 0.001-5.0 ul/plate and strain TA100 at concentration of 0.0001-1.0 ul/plate. Liver extract from Aroclor 1254 pre-induced rats was used.

Positive controls used in the assay were:

2-Anthramine
2-Acetamidofluorene

Control diluent used dimethylsulfoxide (DMSO) previously shown to tbe nonmutagenic.

Results:

Inhibition of growth was observed in all strains in concentrations of 1.0 ul/plate and above with activation and 0.01 ul/plate without activation.

Conclusion:

Marine Antifoulant C-9211 M did not demonstrate mutagenic activity in this assay, however the strain specific positive controls under nonactivation system were not used and should be included in the report.

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Examples of specific positive controls are as follows: Strain TA1535, TA100, sodium azide; strain TA98, 2-nitro-fluorene, TA1537, 9-aminocridine.

Conclusion: Study not acceptable as submitted.

9. Mammalian Cell Transformation Test of Marine Antifoulant C-9211M (Rohm & Haas Company, Report No. 81R-128, March 6, 1983).

Range Finding Test

This test employs the C3H 10T 1/2 clone 8 cells which are a fibroblastic cell line established from C3H mouse embryos by Reznekoff, et. al (1973).

Cultures of the cells were exposed to the test compound dissolved in an appropriate solvent over at least a 4 log dose range. The upper dose limit was determined by solubility or toxicity. After exposure to the test compound for 24 hours and subsequent incubation for 9 or 10 days, the plates were fixed, stained and the number of colonies counted. To determine the toxicity of the test compound, three plates were tested at each concentration and compared to the untreated solvent control group.

Cell Transformation Test
(Using C3H 10 T 1/2 Mouse Embryos Fibroblastic Cells)

Four doses were selected from the preliminary cytotoxicity test: the low dose to yield a survival greater than 90% relative to control; two does selected between 50 and 90% survival; and the high dose 10 and 50% survival. The concentrations tested were 0.01, 0.05, 0.075 and 0.12 nL of sample per ml.

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Plates for the determination of transformation and plating efficiency were as follows:

	Transformation		Plating Efficiency		
Controls	No. of Plates	No. of Cells/ plate	No. of Plates	No. of Cell plate	Expected % survival relative to control
Untreated	≥ 20	2000	≥ 3	200	100
Solvent (DMSO)	≥ 20	2000	≥ 3	200	100
Positive (DMBA 0.5 mg/ml)	≥ 20	2000	≥ 3	200	80-100
C-9211M*					
low conc.	≥20	2000	3	200	>90
low-mid-conc.	≥20	2000	3	200	50-90
high-mid-conc.	≥20	2000	3	200	50-90
high-conc.	≥20	2000	3	200	10-50

*C-9211M stripped of xylene because of possible interference of the xylene solvent with the test.

Cells were exposed to the test compound dissolved in dimethylsulfoxide for 24 hrs. at 37°C in Eagle Basal Medium supplemented with 10% fetal calf serum. After 6 weeks of incubation, the plates were stained with 10% Giemsa and scored for transformation foci. Cultures of the cells were exposed to the controls (untreated, solvent, positive) in the same manner and scored for transformation foci.

Results: (Scoring of transformation foci)

	Total Foci		
	Type* I	Type** II	Type*** III
Untreated Control	7	2	1
Solvent Control (DMSO)	10	1	0
Positive Control (DMBA)	12	51	69
C-9211M			
0.12 nl/ml	9	4	0
0.075 nl/ml	5	1	0
0.050 nl/ml	2	14	0
0.010 nl/ml	7	2	0

*Type I foci stained more densely than the sorrounding monolayer and composed of tightly packed cells.

**Type II foci show extensive piling up of cells, and cells more densely stained than type I, no crisscrossing or swirling is observed at the edge of the focus.

***Type III foci densely piled up of cells withd a stelate morphology and marked crisscrossing and swirling of the cells at the edge of the focus. Cells are highly polar (elongated).

Conclusion:

No significant increase in the number of type II and type III foci were observed in the treated C3H 10T 1/2 mouse cell system when compared to the negative control. The total number of foci "14" observed in the 0.050 nl/ml treated group appears to be an individual incidence and is not a dose related response. The positive control (DMBA) gave the expected response. Therefore, the test compound Marine Antifoulant C-9211M did not induce any significant level of transforming activity in the C3H 10T 1/2 mouse cell system at the dose levels tested.

Classification: Acceptable

10. The submitted Protocol, No. 822-233 (Exhibit C7), to determine the teratogenic potential of C-9211M when administered orally to rats during organogenesis is acceptable. A second teratology in another species is also required.

Carlos A. Rodriguez CAR 9/8/83.
Review Section #1
Toxicology Branch/HED (TS-769)

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Classification: Core-Minimum

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Irrigation of eyes 20-30 seconds after dosing reduced both the intensity and duration of the ocular effects.

Classification: Core-Minimum
(Although only 4 rabbits were used, sufficient information is obtained to determine the toxicity via eye contact).

Tox. Category: I

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Necropsy Observations

Red-mottled lungs and livers, foamy fluid in the trachea and lungs, gas filled gastrointestinal tract, dilation of the kidney medulla and corneal opacities.

Classification: Core-Minimum Study

Tox. Category: II