DATA EVALUATION RECORD

STUDY 7

CHEM 069001 Pyrethrins I §165-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 43302301
Schocken, M.J. 1994. Bioconcentration study with [^{14}C]pyrethrin 1 in bluegill sunfish. Laboratory Study No.: 11572.0993.6125.140. Report No.: 94-5-5258. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA, and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC.

Ancillary Data
Dimenna, G.P. and G.P. Schoenig. 1993. Summary of work performed to determine appropriate storage and handling procedures for [^{14}C]pyrethrin 1. Unpublished study performed by Toxicology/Regulatory Services, Charlottesville, VA and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC.

DIRECT REVIEW TIME = 16

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CONCLUSIONS:

Laboratory Accumulation - Fish

1. This study can be used towards the fulfillment of data requirements.

2. [^{14}C]Pyrethrins 1 [Pyrethrin 1; 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid 2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester] residues accumulated in bluegill sunfish continuously exposed to cyclopropane-labeled [^{14}C]pyrethrin 1, at a mean concentration of 74.2 ppt, for 28 days under flow-through aquarium conditions. Maximum bioconcentration factors were 127x for the edible tissues, 873x for the nonedible tissues, and 471x for whole fish. Maximum mean concentrations of total [^{14}C]residues were 9.43 ppb for edible tissues, 64.8 ppb for nonedible tissues, and 34.9 ppb for whole fish. The metabolite identified in the nonedible tissues was chrysanthemic acid. Depuration was rapid; by day 10, the accumulated [^{14}C]residues were

eliminated to below the level of detection in from the edible tissues, and 97.7% from the nonedible tissues.

3. This study is acceptable and contributes towards the fulfillment of EPA Data Requirements for Registering Pesticides by providing information on the accumulation of cyclopropane-labeled [^{14}C]pyrethrins 1 in laboratory fish.

4. No additional information on the accumulation of cyclopropane-labeled [^{14}C]pyrethrins 1 in laboratory fish is required at this time. If there are concerns about toxicological or ecological effects, information may be needed on the fish accumulation of residues originating from the cyclopentene portion of the molecule.

METHODOLOGY:

Bluegill sunfish (Lepomis macrochirus; mean length and wet weight, 53 mm and 1.8 g, respectively) were held in culture tanks on a 16-hour photoperiod for 14 days prior to the initiation of the study. Flow-through aquatic exposure systems were prepared using three 75 x 39 x 30 cm aquaria maintained at a 25 cm (73-L) exposure volume. Aerated well water (17 ± 1 C; Table V) was provided to each aquarium at a rate of 8.3 turnovers per day. The flow-through systems were allowed to equilibrate for 15 days prior to the start of the study.

Bluegill sunfish (200) were transferred into each aquarium. One aquarium was continuously treated with cyclopropane-labeled [^{14}C]Pyrethrin 1 [2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid 2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester]; (radiochemical purity 98.8%, specific activity 74286 dpm/µg, Pyrethrin Joint Venture], dissolved in acetone and reagent grade water at 9.51 µg/ml. The remaining aquarium was treated with an equal volume of acetone:reagent grade water (50:50, v:v) to serve as a control. The toxicant delivery system was calibrated to deliver a nominal concentration of 156 ng/L in order to achieve a target concentration of 90 ng/L. The difference between target and nominal concentrations is believed to be due to adsorption of pyrethrin 1 to the glass surfaces of the aquarium. During the exposure period, single 250-mL water samples were collected and five fish were sampled from both exposure and solvent control tanks at 0, 1, 3, 7, 10, 14, 21, and 28 days of exposure. In addition, 2-L water samples were collected on days 14 and 21 for metabolite identification. The 14-day samples were abandoned due to HPLC column contamination. Following the 28-day exposure period, the fish remaining in the exposure aquarium were transferred into an identical aquarium with flowing pesticide-free water. During the depuration period, 250-mL water samples and five fish from the depuration and control tanks were removed at 1, 3, 7, 10, and 14 days. Only solvent control fish removed on day 28 of exposure and day 14 of depuration were analyzed.

Although the study was meant to evaluate bioconcentration factors and identify metabolites at the same time, difficulty in maintaining water concentration of pyrethrins led to the termination of the initial bioconcentration experiment, which was then used as a metabolite identification experiment only. In the chemical stock for this experiment, radiochemical purity was 96.4%, specific activity 47528 dpm/μg dissolved in acetone and reagent grade water at 19.0 μg/ml. The toxicant delivery system was calibrated to deliver a nominal concentration of 113 ng/L in order to achieve a target concentration of 90 ng/L. At day 21, the nominal concentration was boosted to 226 ng/L in order to reach the target concentration of 90 ng/L. Solvent control was maintained as before. 165 bluegill sunfish (mean length and wet weight, 54 mm and 2.1 g, respectively) were placed in the metabolite tank. Sampling for water was the same as in the BCF experiment, but fish were only sampled on days 16, 22, 24, 27 and 28. Five fish were collected on day 16, and three fish on the other sampling days.

The 250-ml water samples were extracted once with 15 ml of hexane, the hexane layer added to a scintillation vial and concentrated under nitrogen to < 1 ml. Method recoveries ranged from 83.4 to 122.6%, and the method detection limit was 3.12 ng/L (ppt). Aliquots of the water samples were analyzed for total [^{14}C]residues using LSC. The 2-L water samples were divided into 1-L portions and extracted twice with 250 ml of hexane. Each set of two extracts was combined and concentrated until only water remained. The water was then extracted twice with 5 ml of hexane, combined with 2 ml saturated salt solution and centrifuged. Each hexane layer was transferred to a glass tube, evaporated to dryness, reconstituted with 1 ml of methanol, then vortexed and sonicated. Aliquots of the methanol fraction were analyzed by HPLC-UV and HPLC-RAM. The normal phase HPLC was performed on a silica column (250 mm x 4.6 mm I.D.). The mobile phase was 96% hexane, 4% dioxane. The reverse phase HPLC was performed on a Beckman Ultrasphere ODS column (250 mm x 4.6 mm I.D.). The mobile phase was 85% methanol, 15% reagent grade water. To quantify the concentration of ^{14}C residues in fish tissue, the sample fish were dissected into edible and non-edible tissue, air dried overnight, combusted and analyzed by LSC.

In the metabolite identification study, the fish from each sampling interval were dissected into edible and nonedible tissues. The fish tissues were analyzed for total radioactivity by LSC following combustion. The counting efficiencies were not reported. Based on Table III, the method detection limits appear to be approximately 1 ppb for edible tissues, nonedible tissues, and whole fish samples.

Two replicate samples (approximately 20 g each) of the day 28 edible tissue was extracted two times with 80 ml hexane:acetone (1:1) using a biohomogenizer. The homogenate was centrifuged, then combined in a separatory funnel. The hexane layer was concentrated to a small volume (not specified). This sample was analyzed by both normal phase and reverse phase HPLC. The reverse phase for identification of

pyrethrin 1 and chrysanthemic acid was a Beckman Ultrasphere ODS column (250 mm x 4.6 mm I.D.) with a mobile phase of 85% methanol, 15% reagent grade water. The mobile phases for isolation of chrysanthemic acid were phosphoric acid, 0.05% for 20 minutes followed by methanol:water:phosphoric acid, 90:10:0.1. The normal phase confirmatory HPLC for pyrethrin 1 was a silica column (250 mm x 4.6 mm I.D.)with a mobile phase of 96% hexane, 4% dioxane. To confirm chrysanthemic acid, the silica column was used with a mobile phase of 100% acetonitrile. The tissue was further extracted with 60 ml of methanol. The methanol extract was combined with the acetone layer of the first extraction. The combined extract was concentrated to small volume and analyzed by both normal phase and reverse phase HPLC. Aliquots of each fraction were also radioassayed by LSC. The remaining tissue was allowed to dry overnight, then suspended in 50 ml of TRIS-HCl buffer (pH 7.6) containing 0.3 g protease enzyme. The tissue suspension was shaken overnight at 37 °C. The sample was centrifuged and radioassayed by LSC. The remaining tissue was extracted with 50 ml of methanol:water (1:1) and the extract quantified by LSC. The remaining tissue was combusted to quantify bound residues. (See Figure 4 for scheme)

Two replicate samples (approximately 5 g each) of the day 28 viscera tissue was extracted two times with 20 ml hexane:acteone (1:1) using a biohomogenizer. The homogenate was centrifuged, then combined in a separatory funnel. The hexane layer was concentrated to a small volume (not specified). This sample was analyzed by both normal phase and reverse phase HPLC (as detailed above for edible tissue extracts). The tissue was further extracted with 60 ml of methanol. The methanol extract was combined with the acetone layer of the first extraction. The combined extract was concentrated to small volume and analyzed by both normal phase and reverse phase HPLC. Aliquots of each fraction were also radioassayed by LSC. The remaining tissue was allowed to dry overnight, then suspended in 20 ml of TRIS-HCl buffer (pH 7.6) containing 0.1 g protease enzyme. The tissue suspension was shaken overnight at 37 °C. The sample was centrifuged and radioassayed by LSC. The remaining tissue was extracted with 20 ml of methanol:water (1:1) and the extract quantified by LSC. The remaining tissue was combusted to quantify bound residues. (See Figure 5 for scheme)

Ancillary data

A study was done to evaluate the stability of [^{14}C]pyrethrin 1 in various solvents under ambient laboratory conditions.

100-ml aliquots of [^{14}C]pyrethrin 1 were dissolved in hexane:ethyl acetate (19:1, v:v) and exposed to yellow or white laboratory lighting or maintained in the dark as control. Samples were taken from the dark controls and the vial exposed to yellow light at 0, 0.5, 1, 2, and 4 hours of exposure; samples were taken from the vial exposed to white light at 0, 0.5, 1, 2, 4 and 24 hours and 4, 7, 14, and 21 days of exposure.

Aliquots of radiolabeled and non-labeled pyrethrin 1 in hexane: ethyl acetate solution were added to a 3-ml conical vial and the solvent evaporated under N_2. 1-2 ml of corn oil was then added, and the mixture stirred until dissolution. Eight vials were then stored in the dark, in yellow light, or in white light. Analysis of the vial contents was performed in either yellow or white light. Samples were taken at 0, 0.5, 1, 2, 4 and 24 hours for all vials; additional samples were taken at 2, 4, 7, and 17 days of exposure for a duplicate vial that had been stored and analyzed under white light.

Aliquots of radiolabeled pyrethrin 1 in hexane: ethyl acetate solution were added to a 5-ml conical vial and the solvent evaporated under N_2. 3-4 ml of acetone or DMF was then added, and the mixture stirred until dissolution. Eight vials were then stored in the dark, in yellow light, or in white light. Analysis of the vial contents was performed in either yellow or white light. Samples were taken at 0, 0.5, 1, 2, 4 and 24 hours and 3, 7, 14, 21, 28 and 35 days.

All samples were analyzed by reverse phase HPLC. The analysis was performed on a Beckman Ultrasphere ODS column (250 mm x 4.6 mm I.D.) with a mobile phase of 85% methanol, 15% reagent grade water. Quantification was by radiodetection and UV at 245 nm.

DATA SUMMARY:

[^{14}C]pyrethrin 1 residues accumulated in bluegill sunfish continuously exposed to cyclopropane-labeled [^{14}C]pyrethrin 1 [2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid 2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester, radiochemical purity 98.8%] at a mean concentration of 74.2 $\pm$ 14.5 ppt, for 28 days under flow-through aquarium conditions. Total [^{14}C]residues were highest in the nonedible tissues. Mean bioconcentration factors were 127x for the edible tissue, 873x for the nonedible tissue, and 471x for the whole fish (original LSC analysis; Table I). Maximum mean concentrations of total [^{14}C]residues were 11.8 ppb for edible tissues (day 10), 93.6 ppb for nonedible tissues (day 3), and 48.8 ppb for whole fish (day 3). The only metabolite in the fish tissues or the aquarium water was

chrysanthemic acid

In the edible fish tissues, total radioactivity residue at 28 days posttreatment was 7.2 ppb; reported reverse phase HPLC recovery totalled 109.1% of this quantity. Of the extracted radioactivity, pyrethrin 1 was 56.4%, chrysanthemic acid was 29.5%, and three Unknowns totalled 23.2%. (Table X)

In the nonedible fish tissues, total radioactivity at 28 days posttreatment was 196 ppb; reported reverse phase HPLC recovery totalled 74.3% of this quantity. Of the extracted radioactivity,

pyrethrin 1 was 19.6%, chrysanthemic acid was 32.9%, and five Unknowns totalled 21.8%. (Table X)

Depuration was rapid; by day 1, 77% of the accumulated [^{14}C]residues were eliminated from the edible tissues, 66% from the nonedible tissues, and 68% from the whole fish. By day 14, residues in edible tissues were below the detection limit (apparently 1 ppb) and were close to the detection limit (average = 1.29 ppb) in viscera (Tables I and III).

[^{14}C]Residues in the water were 49.5-107.0 ppt during the study period (Table I). Based on HPLC analyses of extracts from water sampled on day 21 of the exposure period, pyrethrins 1 was present at 54% of the total radioactivity (Figure 6). The major metabolite, chrysanmtheimc acid, represented 44% of the total radioactivity at day 21. Unextracted [^{14}C]residues were $\leq$ 5% in edible tissue and $\leq$ 0.8% in viscera.

Throughout the study, the temperature of the treated water was 17 C, the pH ranged from 6.9 to 8.1, and the dissolved oxygen content average from 7.7 $\pm$ 0.7 mg/L; values were identical for the control water (Table V). No abnormal behavior was observed in the test fish and only one of the original 400 fish died during the study.

Ancillary Data

At the termination of the exposure period, [^{14}]C pyrethrin 1 was >97% pure by radiodetection and >98% pure by UV detection when exposed to white light at ambient temperatures for 21 days in hexane:ethyl acetate (19:1, v:v), 17 days in corn oil, 35 days in acetone and 35 days in DMF. (Table 2)

COMMENTS:

1. Pyrethrin 1 is highly insoluble in water; with a log K_{ow} of 5.9, this compound would be expected to accumulate in the fatty tissues of fish. However, the results of this study support the study author's conclusion that fish do not accumulate sub-lethal concentrations of pyrethrins because the fish are capable of catalyzing the hydrolysis of pyrethrin 1 to chrysanthemic acid. Several factors point to the metabolic production of chrysanthemic acid from pyrethrin 1 by fish, and its subsequent excretion: 1) the recovery of large amounts of chrysanthemic acid from the day 21 water sample, although fresh pyrethrin was constantly flowing into the aquarium; 2) the relatively high concentration of chrysanthemic acid in fish viscera and low concentration in muscle tissue, suggesting breakdown of pyrethrin 1, formation of a hydrophilic product, and excretion; 3) the rapid depuration of pyrethrin 1 from fish tissue, with depuration occurring most rapidly from the muscle tissue and a residue remaining in the viscera; and 4) the establishment of a steady state concentration of [^{14}C] residues in fish tissue by day 3, suggesting that the fish

adapted to the exposure after a brief accumulation period and were then able to metabolize and excrete radiolabeled material at a rate equal to the rate of exposure.

2. The study author stated that, "The minimum detectable [^{14}C]residue concentration was dependent on the counting efficiency, sample size (milliliters or grams) and the acceptable minimum net cpm [57 cpm for water and 78 cpm for oxidized samples]." The counting efficiency and sample size for each sample were not reported. However, based on Table III (the only table where results below the detection limit were reported), it appears that the detection limit was approximately 1 ppb in fish tissue.

3. Because pyrethrin 1 was shown to degrade in pH 7 buffer solution exposed to natural sunlight (MRID 43096601, reviewed in this package) there was concern that ambient laboratory light might also promote pyrethrin 1 degradation, reducing fish exposure to the test substance. The ancillary data provided by the registrant demonstrates that pyrethrin 1 is stable under ambient laboratory conditions, although the case of pyrethrin 1 exposed to ambient light in pH 7 aqueous solution is not covered. However, as pyrethrin 1 was stable to light exposure in acetone, which is a photosensitizer, it appears reasonable to believe that little if any pyrethrin 1 degradation was promoted by ambient light during the brief residence time of the applied chemical in the aquarium.

4. The study author reported that the target concentration of 90 ng/L is approximately 1/100 of the pyrethrin I LC50 for bluegill sunfish.

5. No mention is made of cold storage of any of the samples, so storage stability data is not required.

6. Although metabolites other than chrysanthemic were found during the HPLC analysis of tissue extracts, the study author stated that "these minor metabolites could not be identified due to their low concentrations and the presence of co-extractives from fish tissue." Even though this argument is not especially convincing for Unknowns 2 and 4 (recovered at 20.3 and 20.2 ppb, respectively), the overall low bioconcentration of pyrethrin residues in fish renders further degradate identification unnecessary.

7. The reported bioconcentration factors (127x for the edible tissue, 873x for the nonedible tissue, and 471x for the whole fish) were determined by dividing the mean measured equilibrium (steady state) ^{14}C tissue concentration by the mean measured exposure water concentration calculated over the entire exposure period. Reviewer calculated maximum mean BCF's determined by dividing the mean tissue concentration by the mean exposure water value for each sampling day were: edible tissue, 193x (day 7), non-edible tissue, 1467x (day 7), and whole body, 774x (day 7). Contributing to the high BCF's recorded on day 7 was the fact that recorded water concentrations on that day had dropped to only 50.9 ng/L, while the fish tissue concentrations

may have been accumulated during a time when ambient concentrations were higher. The fact that the highest concentrations occurred on day 7, rather than at the end of the study, is another indication that metabolism of pyrethrin in fish tissue prevents the compound from accumulating to excessive levels.

8. The test fish were fed a standard commercial fish food daily in an amount equivalent to approximately 2% of their body weight during the acclimation and test periods except during each 24 hours prior to sampling.

9. The data from the analyses of untreated water and fish were not provided for each sampling interval. However, the study author stated that, "The concentrations of [^{14}C] residues in exposure water and fish tissues (edible and nonedible) from the solvent control aquarium were found to be below the minimum detection limit at each sampling interval." EFGWB generally requires evidence (chromatograms, LSC counts, etc.) that control has been achieved. However, the overall study control appears to be acceptable based upon the consistency of all reviewed results.

Pyrethrins review

Page ____ is not included in this copy.

Pages __9__ through __60__ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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_____ Claimed Confidential by submitter upon submission to the Agency.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

REFERENCES

The following studies were reviewed:

Reynolds, J.L., and R.A. Robinson. 1994. XBL Study No. 93064; XBL Report No. RTP00156. Unpublished study performed by XenoBiotic Laboratories, Inc., Plainsboro, NJ; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC. (43096603)

Selim, S. 1994a. Aqueous photolysis of pyrethrin 1. BTC Study No. P1192006. Unpublished study performed by Biological Test Center, Irvine, CA; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC. (43096601)

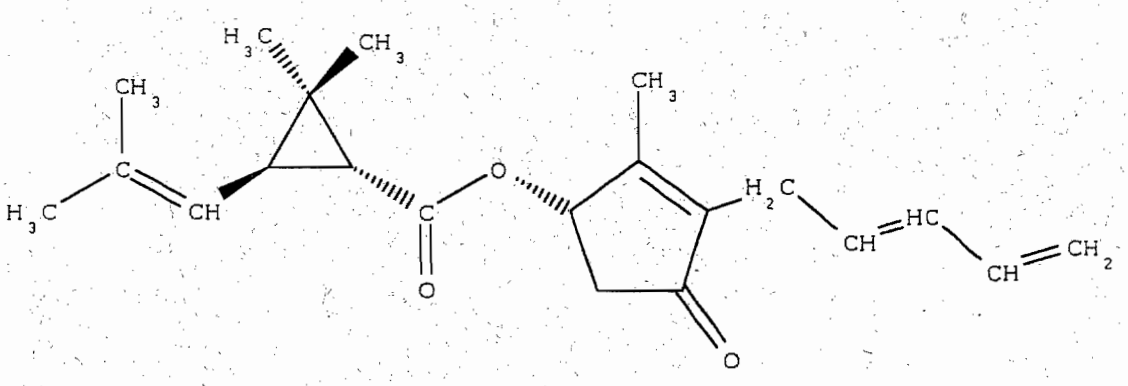
Selim, S. 1994b. Hydrolysis of pyrethrin 1 as a function of pH at 25°C. BTC Study No. P1092011. Unpublished study performed by Biological Test Center, Irvine, CA; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC. (43188201)

Selim, S. 1994c. Laboratory volatility of pyrethrin 1 from soil. BTC Study No. P0693011. Unpublished study performed by Biological Test Center, Irvine, CA; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC. (43096604)

Testman, R. 1994. Soil surface photolysis of pyrethrin 1. BTC Study No. P1192007. Unpublished study performed by Biological Test Center, Irvine, CA; and submitted by Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC. (43096602)

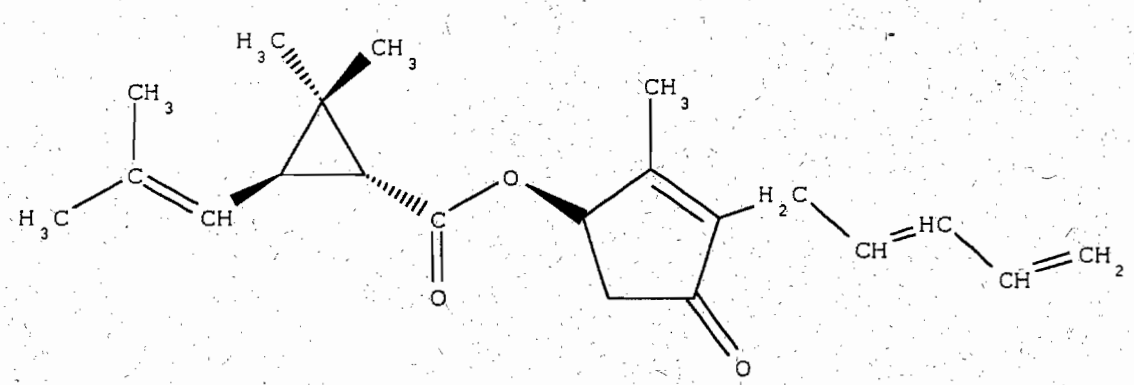
APPENDIX

PYRETHRIN 1 AND ITS DEGRADATES



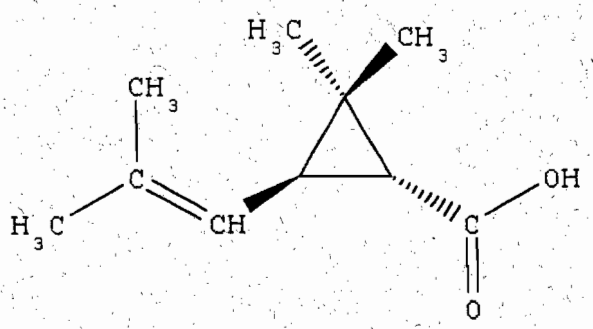
2,2-Dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid
2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester

(Pyrethrin 1)



(E)-isomer of Pyrethrin 1

("formed by the cis to trans isomerization at the 2-position of the 2-pentadienyl side chain of the alcohol moiety")



Chrysanthemic acid