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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

CASWELL FILE

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

PC 129013

JUN 30 1986

005237

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Application for Registration of Gokilaht™ (Forte; S-2703) Manufacturing Use Product; Review of Submitted Toxicology Data

EPA No. 10308-RN
Record No. 161016

Project No. 926
Tox. Chem. No. 725A

TO: George LaRocca (PM Team #15)
Registration Division (TS-767c)

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

John E. Whalan 6-9-86

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Budd 6/30/86

Sumitomo Chemical Company, Limited has applied to register a synthetic pyrethroid, Gokilaht™ [Forte; S-2703; (RS)- -cyano-3-phenoxybenzyl (1RS)-cis,trans-chrysanthemate]. Toxicology studies have been submitted in support of the registration of the technical product. The review of these reports follow. No tolerances were requested in this request.

The toxicology data base required to support the registration of Gokilaht™ is adequate. The Toxicology Branch has no objection to the registration of this product. The product label (attached) is acceptable.

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Front Panel

GOKILAHT(TM) TECHNICAL

An Insecticide for Formulating Use Only.

ACTIVE INGREDIENTS:	
(RS)-∝-cyano-3-phenoxybenzyl(1R)-cis,trans-chrysanthemate	87.4%
Other isomers	4.6%
INERT INGREDIENTS:	8.0%
	100.0%

WARNING

SEE SIDE PANEL FOR PRECAUTIONARY STATEMENTS

STATEMENT OF PRACTICAL TREATMENT

If swallowed: Call a physician or a Poison Control Center. Drink 1 or 2 glasses of water and induce vomiting by touching back of throat with finger. Do not induce vomiting or give anything by mouth to an unconscious person.

If inhaled: Remove victim to fresh air. If not breathing, give artificial respiration, preferably by mouth-to-mouth. Get medical attention.

SUMITOMO CHEMICAL COMPANY, LTD.
15, 5-chome, Kitahama
Higashi-ku
Osaka, Japan

Left Side Panel

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMAN AND DOMESTIC ANIMALS

WARNING

May be fatal if swallowed or inhaled. Wash thoroughly with soap and water after handling and before eating or smoking. Remove contaminated clothing and wash before reuse.

ENVIRONMENTAL HAZARDS

This product is toxic to fish. Do not discharge into lakes, streams, ponds, or public waters unless in accordance with an NPDES permit. For guidance, contact your Regional Office of the Environmental Protection Agency.

STORAGE AND DISPOSAL

1. STORAGE: Containers should be stored in a cool, dry, well ventilated area. Do not expose to prolonged heat. Keep container closed when not in use.

2. PROHIBITIONS: Do not contaminate water, food, or feed by storage or disposal. Open dumping is prohibited.

3. PESTICIDE DISPOSAL: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

4. CONTAINER DISPOSAL: Triple rinse (or equivalent). Then offer for recycling, reconditioning, or puncture and dispose of in a sanitary landfill, or by other procedures approved by state and local authorities.

5. GENERAL: Consult federal, state, or local disposal authorities for approved alternative procedures.

EPA Registration No. 10308-

EPA Est. No. 10308-JP-02.

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Right Side Panel

DIRECTIONS FOR USE

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

Refer to technical literature for formulation of Gokilaht™ Technical for end uses.

Formulators are responsible for providing data to support registration of their formulated products.

NOTICE -- READ CAREFULLY

CONDITIONS OF SALE:
Sumitomo (and seller) offer(s) this product for sale subject to, and buyer and all users are deemed to have accepted, the following conditions of sale and warranty which may only be varied by written agreement of a duly authorized representative of Sumitomo.

WARRANTY LIMITATION:
Sumitomo warrants that this product conforms to the chemical description in the directions for use on the label subject to the inherent risks referred to below. Sumitomo makes no other express warranties: THERE IS NO IMPLIED WARRANTY OF MERCHANTABILITY and there are no warranties which extend beyond the description on the label hereof.

INHERENT RISKS:
The directions for use of this product are believed to be reliable and should be followed carefully. However, it is impossible to eliminate all risks associated with use. Buyer assumes all risks associated with use or application of this product contrary to label instructions or resulting from extraordinary weather conditions.

LIMITATION OF LIABILITY:
In no case shall Sumitomo be liable for special, indirect or consequential damages resulting from the use or handling of this product and no claim of any kind shall be greater in amount than the purchase price of the product in respect of which such damages are claimed.

Net weight_____pounds (_____Kilos)

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ACUTE ORAL TOXICITY STUDY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd., Report No. EET-30-0011; February 8, 1983; Accession No. 259842

PROTOCOL: Randomized groups of 10 male (170-190 g) and 10 female (125-145 g) six week old Sprague Dawley rats were orally dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil at levels of 0 (vehicle control), 50, 100, 250, 320, 420, 550, 710, and 930 mg/kg. They were given single doses by gavage. Food and water were available ad libitum. They were observed daily for toxic signs for 14 days, and weighed at 0, 7, and 14 days. All rats were necropsied and examined grossly. Probit analysis was performed using the method of Litchfield and Wilcoxon.

RESULTS: All deaths occurred on the day of dosing. There were no deaths at doses less than 250 mg/kg. The calculated LD_{50} values were as follows:

Sex	LD_{50} (95% Confidence Limits)
Male	318 (219-463) mg/kg
Female	419 (281-624) mg/kg

No toxicity was observed at 0 and 50 mg/kg. Toxic signs included muscular fibrillation, tremors, hyperexcitability, lethargy, ataxia, limb paralysis, hyperpnea, dyspnea, soft feces, loss of righting reflex, salivation, and urinary incontinence. The two surviving males dosed at 550 mg/kg had mean weight loss on day 7 of 15% compared to the controls; the significance of this weight loss is uncertain given the small population size. The onset of toxicity was 2 hours after dosing; all toxic signs reversed within 2-5 days. Gross lesions found in rats which died on the day of dosing included attachment of saliva around the mouth, discoloration of the stomach mucosa, and remnant of an unspecified colored material in the stomach and small intestine.

This study is CORE GUIDELINE - Toxicity Category II. This study was well reported and received Quality Assurance Review. The dosing formulation was not specified.

ACUTE DERMAL TOXICITY STUDY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd., Report No. EET-30-0012; February 8, 1983; Accession No. 259842

PROTOCOL: Randomized groups of 10 male (160-180 g) and 10 female (140-160 g) six week old Sprague Dawley rats were dermally dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil at levels of 0 (vehicle control), 2500, and 5000 mg/kg. They were dosed with single 5 ml/kg volumes on the shaved skin of their backs. The dosing sites were occluded with surgical tape for 24 hours, then the doses were removed with diethyl ether and cotton. Food and water were available ad libitum. They were observed daily for toxic signs for 14 days, and weighed at 0, 7, and 14 days. All rats were necropsied and examined grossly.

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RESULTS: There were no deaths and no signs of toxicity at doses as high as 5000 mg/kg. There were no significant gross lesions in any group.

This study is CORE GUIDELINE - Toxicity Category III. This study was well reported and received Quality Assurance Review. The dosing formulation was not specified.

ACUTE INHALATION TOXICITY STUDY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd., Report No. EET-10-0006; December 29, 1981; Accession No. 259842

PROTOCOL: Randomized groups of 10 male (120-140 g) and 10 female (90-110 g) five week old Sprague Dawley rats were dosed acutely by inhalation with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in deodorized kerosene. The nominal concentrations were not reported. There were five dose levels, a vehicle control, and a nontreated control. All exposures were for 3 hours in a 0.64 m^3 dynamic inhalation chamber. The formulated test article aerosol was generated by a glass atomizer and introduced into the chamber atmosphere. Any large particles were captured by passing the aerosol through two bottles prior to chamber introduction. Aerodynamic particle diameter was measured with a Microscopic Sedimentation Analyzer. Analytical concentrations were measured by drawing the chamber atmosphere through a silica gel column and measuring the collected compound with a gas chromatograph. Chamber aerosol concentration and particle size were measured after 30 minutes of exposure. Food and water were available ad libitum. The rats were observed daily for toxic signs for 14 days, and weighed at 0, 3, 7, and 14 days. All rats were necropsied and examined grossly.

RESULTS: The analytical chamber concentrations and particle sizes were as follows:

Analytical Concentration		Median Particle Size
mg/l	mg/m^3	
Vehicle Control		0.80u
0.224	224	0.82u
0.499	499	0.83u
1.090	1090	1.01u
1.470	1470	1.02u
1.850	1850	1.00u

Particle size was largely in the respirable range. There were no deaths at any concentration; thus the LC_{50} was >1.850 mg/l (1850 mg/m^3). The lowest dose was not toxic. Toxic signs seen at higher doses included hyperpnea, deep respiration, salivation, urinary incontinence (males only), muscular fibrillation, and tremors. Weight gain was similar in all dosed and control groups. No gross lesions were reported.

This study is CORE MINIMUM - Toxicity Category II. The exposure duration was three hours instead of the recommended minimum of four hours. The airflow rate of 50 l/min provided less than 5 air changes/hour instead of the recommended

minimum of 10 air changes/hour. Oxygen levels were not monitored, so it was not possible to determine whether the slow airflow resulted in reduced chamber oxygen. Nominal chamber concentrations were not reported, making it impossible to assess the efficiency of the exposure system. Although no compound-related lesions were found, a presentation of gross lesions should have been included.

ACUTE SUBCUTANEOUS TOXICITY STUDY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd., Report No. EET-30-0014; February 28, 1983; Accession No. 259842

PROTOCOL: Randomized groups of 10 male (160-180 g) and 10 female (140-160 g) six week old Sprague Dawley rats were subcutaneously dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil at levels of 0 (vehicle control), 2500, and 5000 mg/kg. They were dosed with single 5 ml/kg volumes into the skin of their backs. Food and water were available ad libitum. They were observed daily for toxic signs for 14 days, and weighed at 0, 7, and 14 days. All rats were necropsied and examined grossly.

RESULTS: There were no deaths or signs of toxicity in this study. The LD_{50} was >5000 mg/kg. All of the rats had remnants of the injected materials at the dosing sites, and a few of those given the test article had ulcer-like lesions.

This study is ACCEPTABLE. This study was well reported and received Quality Assurance Review. The dosing formulation was not specified.

PRIMARY EYE AND SKIN IRRITATION STUDIES OF S-2703 FORTE IN RABBITS

Sumitomo Chemical Co., Ltd., Report No. EET-10-0001; August 3, 1981; Accession No. 259842

PROTOCOL: Nine male New Zealand White rabbits (2.18-3.00 kg), judged to be free of eye and skin lesions, were dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6).

Eye Irritation - The liquid technical article (0.1 ml) was instilled into the lower lid of one eye on each rabbit, and the eyelids were held together for one second. The opposite eyes served as controls. The treated eyes of three rabbits were flushed with lukewarm water for 1 minute following 30 seconds of exposure. The other six rabbits' eyes were not flushed. The eyes were observed and graded for irritation by the method of Draize at 1, 24, 48, 72, and 96 hours, and 1 week. Severity classification was by the technique of Kay and Calandra (1962).

Skin Irritation - Six rabbits were prepared for dosing by shaving four dosing sites on their backs and abrading the stratum corneum of two of them. They were dosed by applying 1 inch square lint patches containing 0.5 ml aliquots of the technical material to each dosing site. The patches were occluded with surgical tape. The rabbits were restrained in cages for 24 hours, after

which the patches were removed and the dosing sites wiped clean. Skin irritation was graded by the method of Draize at 24, 48, and 72 hours, and 1 week after application.

RESULTS:

Eye Irritation - One hour after instilling the doses, two rabbits with unrinsed eyes had injected conjunctival vessels and discharge with moistening of the lids and hairs just adjacent to the lids. No other irritation was seen in any other rabbits or at any other time. Toxicity Category IV.

Skin Irritation - The test article was nonirritating in all animals. Toxicity Category IV.

These studies are CORE MINIMUM. The report was unclear as to whether the same animals were used for testing both skin and eye irritation. The use of the same animals for two or more studies is generally poor laboratory procedure and will not be tolerated on future studies. This study received Quality Assurance review.

DERMAL SENSITIZATION STUDY OF S-2703 FORTE IN GUINEA PIGS

Sumitomo Chemical Co., Ltd., Report No. EET-30-0009; January 17, 1983; Accession No. 259842

PROTOCOL: Fifty male Hartley guinea pigs (220-260 g) were tested for dermal sensitization by the method of Buehler (1965). [Note - intradermal injection of test article >2% caused erythema so the topical Buehler method was selected for this study.] The backs of all guinea pigs were shaved. Thirteen guinea pigs were dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6), while another 12 served as controls. The treated animals were sensitized by attaching 1.5 inch square lint patches moistened with 0.5 ml aliquots of the test article. The patches were occluded with surgical tape for a period of 24 hours. The sensitizing doses were applied 3 times/week for a total of 10 doses. Challenge doses of 0.5 ml aliquots of S-2703 Forte were given to all sensitized and control (nonsensitized) animals. Dermal irritation was evaluated 24 and 48 hours after the challenge, and graded according to the method of Draize (1959). A positive control was similarly performed by dosing 13 guinea pigs with 0.5% DNCB (2,4-dinitro-chlorobenzene) in acetone, while another 12 guinea pigs served as negative controls.

RESULTS: There was no dermal irritation in any sensitized or control animals when challenged with S-2703 Forte. In contrast, all guinea pigs sensitized with DNCB in acetone had moderate to severe erythema and swelling with the greatest effect being seen at 24 hours. None of the DNCB controls had dermal lesions resulting from a single challenge dose. One of the guinea pigs dosed with S-2703 Forte died after receiving the third sensitizing dose. The cause of death, although not determined, was probably not compound-related.

This study is CORE MINIMUM. There was no mention of the method of dose removal. This study received Quality Assurance review.

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29-DAY SUBACUTE INHALATION TOXICITY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd.; Report No. EET-30-0020 (amended) - August 20, 1984 and EET-40-0044 (individual animal data) - January 9, 1985; Accession No. 259844

PROTOCOL: Randomized groups of 10 male (120-140 g) and 10 female (100-120 g) five week old Sprague Dawley rats were dosed by inhalation with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in deodorized kerosene. They were dosed 4 hours/day over 29 consecutive days in a 0.64 m^3 dynamic chamber. The nominal concentrations were not reported. There were three dose levels (using 1%, 3%, and 7% test article dilutions), a vehicle (kerosene) control, and a nontreated control. The formulated test article aerosol was generated by a glass atomizer and introduced into the chamber atmosphere. Large particles were captured by passing the aerosols through two bottles prior to chamber introduction. Aerodynamic particle diameters were measured with a Microscopic Sedimentation Analyzer. Analytical concentrations were measured twice weekly by drawing chamber atmospheres through silica gel columns and measuring the collected compound with a gas chromatograph. Samples were drawn thirty minutes into the exposures. The analytical samples and particle size samples were drawn at the same time. Food and water were available ad libitum, except during exposure.

The rats were observed daily for toxic signs for 29 days, and weighed twice weekly. Food and water consumption were measured on two consecutive days each week. The eyes of all rats were examined ophthalmologically on days 27 and 28. Urine samples were collected on day 28 and analyzed for the following parameters:

Urinalysis:

Occult blood	Bilirubin
Ketones	Protein
Glucose	pH

At the end of the study, the rats were fasted and anesthetized, and blood samples drawn from the abdominal aorta. The following clinical pathology parameters were measured:

Hematology:

Erythrocytes	Mean corpuscular volume
Hematocrit	Leukocytes (total and differential)
Hemoglobin	Platelets

Clinical Chemistry:

Alkaline phosphatase	Total protein	Cholinesterase
SGOT	Blood urea nitrogen	Sodium
SGPT	Albumin	Potassium
Total bilirubin	Glucose	Calcium
Total cholesterol		

Each rat was examined grossly and the following tissues were examined histopathologically (the asterisked organs were weighed):

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*Heart	*Thyroid	Pancreas
*Spleen	Thymus	Small intestine
*Liver	*Adrenals	Large intestine
*Kidneys	Eye	Salivary glands
*Testes	*Brain	Urinary bladder
Epididymides	Spinal cord	Skin
Prostate	Sciatic nerve	Tongue
Preputial glands	*Lungs	Mesenteric lymph nodes
*Ovaries	Trachea	Nasal cavity
Uterus	Esophagus	Bone marrow
*Pituitary	Stomach	*Gross lesions

RESULTS: The mean analytical concentrations and aerodynamic particle diameters were measured to be as follows:

Dilution	Analytical Concentration mg/l	Analytical Concentration mg/m^3	Aerodynamic Particle Diameter (um)
1%	0.015	15.1	N/R
3%	0.054	54.0	0.92
7%	0.152	152	0.80

Nominal concentrations were not reported. There were fluctuations in chamber concentrations. In the 1% chamber, the analysis during the fourth week was 163% of that seen in the preceding analyses. Other analyses deviated as much as 31% from the means. In addition, no analyses were performed in the second week (second analyses) for the 3% and 7% chambers, or in the fourth week (second analyses) for the 1% and 3% chambers. No explanations were offered for the missing or deviant values. Particle size in the 3% and 7% chambers was similar and fluctuated little during the study. Particle sizing was not accomplished in the 1% dose group due to the insufficient number of captured particles; apparently, no attempt was made to remedy this situation. Particle size distributions were not reported. Nevertheless, it appeared that mean particle size was in the respirable range.

No rats died during this study. Clinical observation data for the nontreated controls was missing from the report, so it was impossible to ascertain the extent of vehicle toxicity. All dosed and vehicle control rats had decreased spontaneous activity which may have been caused by the kerosene vehicle. All rats dosed with the test article had irregular respiration throughout the study. Additional toxic signs seen in the mid- and high-dose groups included nasal discharge, lacrimation, salivation, and urinary incontinence. Decreased weight gain of 10-11%, and decreased food consumption of 21% (compared to the vehicle controls) was seen in the last study week in the mid-dose males. Since this weight loss was slight and not dose-related, it was probably not significant. No corresponding effect was seen in any dosed females. Water consumption was similar in all groups.

One high-dose male rat was found during an ophthalmologic examination to have lenticular opacity in its left eye. This finding was confirmed during gross examination at the end of the study. Since no pretest examinations were conducted, the significance of this finding is doubtful. There were no significant changes in urinalysis or clinical pathology parameters. There were several anomalies in relative organ weights, but these were all due to weight

loss, and not to changes in absolute organ weights. There were no dose-related gross lesions. The presentation of histopathology data was too poor to permit evaluation. A NOEL was not defined since frank toxicity was observed at the lowest dose used.

This study is CORE SUPPLEMENTARY. Clinical observations were not reported for the nontreated controls. Particle sizing was unsuccessful for the low-dose (1%) chamber because of the small quantity of compound accumulated; more compound could have been accumulated if sampling had continued for a longer period of time, but this was not done. Analytical chamber concentration values were not reported for 17% of the sampling intervals, and there were a number of deviant values which suggest a lack of precision in dosing; these matters were not addressed in the report. The nominal chamber concentrations were not reported. Although tissues were examined histopathologically, the report was lacking a summary of the findings. All the histopathologic findings were included in 69 pages of individual findings that failed to even define the range of severities. Thus, the histopathology data were uninterpretable. The core classification of this study may be upgraded upon submission of an acceptable histopathology summary and justification for the anomalous analytical concentration values. These reports received Quality Assurance Review.

SUBACUTE INHALATION TOXICITY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd.; Report No. EET-40-0045; December 20, 1984; Accession No. 259844

[NOTE: This report is called a "Supplementary Report," but it is actually describing a repeat of the preceding study. The repeat study was performed using lower doses in order to define a NOEL.]

PROTOCOL: Randomized groups of 10 male (130-150 g) and 10 female (90-110 g) five week old Sprague Dawley rats were dosed by inhalation with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in deodorized kerosene. They were dosed 4 hours/day over 28 consecutive days in a 0.64 m^3 dynamic chamber. The nominal concentrations were not reported. There were two dose levels (using 1% and 0.5% test article dilutions), a vehicle (kerosene) control, and a nontreated control. The formulated test article aerosol was generated by a glass atomizer and introduced into the chamber atmosphere. Large particles were captured by passing the aerosols through two bottles prior to chamber introduction. Aerodynamic particle diameters were not measured due to low particle concentration. Analytical concentrations were measured twice weekly by drawing chamber atmospheres through silica gel columns and measuring the collected compound with a gas chromatograph. Samples were drawn sixty minutes into the exposures. Food and water were available ad libitum, except during exposure.

The rats were observed daily for toxic signs for 28 days, and weighed twice weekly. Food and water consumption were measured on two consecutive days each week. The eyes of all rats were examined ophthalmologically on day 28. Urine samples were collected on day 27 and analyzed for the following parameters:

Urinalysis:

Occult blood	Urobilinogen
Ketones	Protein
Glucose	pH
Bilirubin	

At the end of the study, the rats were fasted and blood samples drawn from the abdominal aorta while under anesthesia. The following clinical pathology parameters were measured:

Hematology:

Erythrocytes	Mean corpuscular volume
Hematocrit	Leukocytes (total and differential)
Hemoglobin	Platelets

Clinical Chemistry:

Alkaline phosphatase	Total cholesterol	Total protein
SGOT	Blood urea nitrogen	Sodium
SGPT	Albumin	Potassium
Total bilirubin	Glucose	Calcium

Each rat was examined grossly and the following tissues were weighed:

Heart	Testes	Adrenals
Spleen	Ovaries	Brain
Liver	Pituitary	Lungs
Kidneys	Thyroid	Gross lesions

The report states that, "Histopathological examination was not conducted in this study because no remarkable findings were observed in rats exposed to the highest level of S-2703 Forte (152 mg/m^3) in the previous study," (Report Nos. EET-30-0020 and EET-40-0044 - see the preceding review).

RESULTS: The mean analytical concentrations and aerodynamic particle diameters were measured to be as follows:

Dilution	Analytical Concentration mg/l	Analytical Concentration mg/m^3	Aerodynamic Particle Diameter (um)
0.5%	0.008	7.76	N/D
1.0%	0.020	20.2	N/D

Nominal concentrations were not reported, and aerodynamic particle diameter measurements were not done (N/D). Chamber concentration in the high-dose (1%) chamber was within 8% of the study mean. The low-dose (0.5%) chamber had greater fluctuations. In the 0.5% chamber, the second analysis during the fourth week was 156% of that seen in the preceding analyses. Other analyses deviated as much as 27% from the means. No explanations were offered for the deviant values. Because there was no attempt to measure particle size, it is impossible to determine whether the aerosols were respirable.

No rats died during this study. The nontreated controls had no toxic signs. The majority of vehicle control and low-dose rats had decreased activity which commenced on day 8 and continued throughout the study. This sign was probably caused by the kerosene vehicle. The high-dose rats had decreased activity and irregular respiration which began on day 1 and persisted throughout the study. In addition, several high-dose females had urinary incontinence on days 3-8, which readily reversed within one hour after dosing. Weight gain and food consumption was comparable in all groups. Water consumption in the high-dose females was 14-25% greater than in the vehicle controls. There were no ophthalmologic anomalies.

There were no significant changes in urinalysis or clinical pathology values. Organ weights were comparable for all dosed and control groups. There were no compound-related gross lesions.

The NOEL is defined as 0.008 mg/l (7.76 mg/m^3). The LEL is defined as 0.020 mg/l (20.2 mg/m^3) at which dose toxicity included decreased activity, irregular respiration, urinary incontinence (females only), and increased water consumption (females only).

This study is CORE SUPPLEMENTARY. Particle sizing was not attempted, based on sampling problems in the preceding study; the particles were probably respirable, however. There were a number of deviant analytical concentration values which suggests a lack of precision in dosing; this matter was not addressed in the report. The nominal chamber concentrations were not reported. Because of the negative findings in the preceding study, no tissues were preserved, and no attempt was made to examine any tissues histopathologically. Since the report of the preceding study was lacking a summary pathology table and definitions of the range of severities used in scoring lesions, it was not possible to concur with the "negative" histopathology findings. The preceding report must be changed to better present the data before the core classification of either study can be upgraded. This report received Quality Assurance Review.

SIX-MONTH SUBCHRONIC FEEDING TOXICITY STUDY OF S-2703 FORTE IN RATS

Sumitomo Chemical Co., Ltd., Report No. EET-40-0038; February 6, 1985; Accession No. 259843

PROTOCOL: Randomized five week old male (129-160 g) and female (105-133 g) CD rats were assigned to study groups of 21 rats/sex (designated the "main group") and 12 rats/sex (designated the satellite group). The main group was on study for 6 months, and the satellite group was on study for 3 months. They were dosed with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in their feed at concentrations of 0 (nontreated controls), 100, 300, and 1000 ppm. Food and water were available ad libitum. All rats were observed twice daily for clinical signs, and palpated once a week. They were weighed weekly, and on days 1, 2, and the last day of administration. Food and water consumption were measured weekly for a 3 day period for each cage (3 rats/cage). Urine samples were collected during the last study week and examined for the following parameters:

Urinalysis:

pH	Leukocytes
Occult blood	Erythrocytes
Glucose	Crystalline components
Bilirubin	Casts
Ketone bodies	Epithelial cells
Protein	Sperm
Urobilinogen	

At the end of the study, the rats were fasted and anesthetized, and blood samples drawn from the abdominal aorta. The following clinical pathology parameters were measured:

Hematology:

Erythrocytes	Mean corpuscular hemoglobin concentration
Hematocrit	Mean corpuscular volume
Hemoglobin	Leukocytes (total and differential)
Mean corpuscular hemoglobin	Platelets

Clinical Chemistry:

Blood urea nitrogen	Total protein
Creatinine	Triglyceride
Creatinine phosphokinase	Albumin/Globulin ratio
Glucose	Leucine amino peptidase
Alkaline phosphatase	Cholinesterase
SGOT	Phospholipid
SGPT	Sodium
Total bilirubin	Potassium
Total cholesterol	Calcium
Albumin	Chlorine
Lactic dehydrogenase	

The eyes of all surviving rats were examined ophthalmologically during the final study week. All rats were examined grossly. The following tissues were examined histopathologically for control and high-dose satellite rats at 3 months and all main group rats at 6 months (the asterisked organs were weighed):

Heart	*Thyroid	Pancreas
*Spleen	Thymus	Small intestine
*Liver	*Adrenals	Large intestine
*Kidneys	Eye	Salivary glands
*Testes	*Brain	Urinary bladder
Epididymides	Spinal cord	Skin
Seminal vesicles	Sciatic nerve	Tongue
Prostate	*Lungs	Mesenteric lymph nodes
Preputial glands	Trachea	Submaxillary lymph nodes
*Ovaries	Esophagus	Muscle (thigh)
Uterus	Femur	*Gross lesions
*Pituitary	Stomach	

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RESULTS: The mean oral doses during the course of the study were as follows:

Dose (ppm)	Daily Dose (mg/kg/day) Males	Daily Dose (mg/kg/day) Females	6-Month Cumulative Dose (mg/kg) Males	6-Month Cumulative Dose (mg/kg) Females
0	0	0	0	0
100	5.64	6.60	1015	1188
300	16.8	19.6	3024	3528
1000	56.4	65.2	10152	11736

Dose concentration analyses indicate that dose formulations were 88-97% of the nominal concentrations.

Food and water intake was similar among the groups for each sex. The rats received reasonably consistent doses throughout the study without any food palatability problems. Weight gain for each sex was similar in all groups. The observed clinical signs were due to normal fighting and accidents; there were no compound-related clinical signs. Two rats had eye lesions at 6 months. A high-dose male had an indistinct fundus oculi, and a low-dose female had corneal opacity and incomplete mydriasis in the right eye. The significance of these lesions was not discussed. The low incidence of ocular lesions preclude any biological signicance.

There were few clinical pathology anomalies. The high-dose males had markedly increased urinary protein at 3 months, but not at 6 months. The mid- and high-dose females had 8-10% increases in serum potassium at 3 months compared to controls, but normal levels at 6 months. Monocyte percentage was normal in the high-dose males at 3 months, but elevated 54% at 6 months compared to controls. These anomalies appeared to be isolated cases and probably were not compound-related.

There were no compound-related gross lesions, although there may have been a tendency for dosed animals to have slightly enlarged livers. There were no compound-related lesions evident in any groups. Organ weight anomalies were generally mild, often not dose-related, and unsubstantiated by other observations. The NOEL is defined as 1000 ppm (56.4 and 65.2 mg/kg/day for males and females, respectively), the highest dose tested. The LEL was not defined.

This study is CORE MINIMUM. Although a NOEL was defined, the doses selected were inadequate for establishing a LEL. The report stated that in a separate 5-week feeding study, the 1000 ppm dose caused slight tremors in all rats, presumably because the rats ate 15% more feed and therefore 15% more compound. Tremor was not observed in any rats in this study. Tremor is a tenuous clinical sign which can be caused by environmental as well as toxicologic factors. The selection of the highest dose should have been based on a sign of frank toxicity. This study received Quality Assurance Review.

TERATOLOGY STUDY OF S-2703 FORTE IN RABBITS

Bozo Research Center, Inc.; Report No. EET-41-0029; May 31, 1984; Accession No. 259845

PROTOCOL: Twenty male (unspecified weight) and 85 female (3.30-4.37 kg) 6 month old New Zealand White rabbits were used in this study. They were dosed subcutaneously with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil. The females in estrus were mated by housing each with a male. Evidence of copulation established gestation day 0. Gravid females were assigned to four groups of 15 rabbits each. They were dosed into the skin of their backs on gestation days 6-18 at doses of 0 (vehicle control), 25, 50, and 125 mg/kg/day. Food and water were available ad libitum.

The females were observed daily for clinical signs during the gestation period. They were weighed on gestation days 0, and 6 through 28. Food consumption was measured during a 24 hour period prior to gestation days 6, 10, 14, 17, 21, 24, and 28. On gestation day 28, the dams were sacrificed by air embolism, and their organs grossly examined. Their ovaries and uteri were examined for the number of corpora lutea, implantation sites, live, dead, and resorbed fetuses, and placenta weights. Organ weights were measured prior to fixation for heart, lung, liver, kidney, spleen, and ovary.

All fetuses were weighed, sexed, and examined for external and oral malformations. The thoracic and abdominal organs were examined and fixed. The hearts and kidneys were further examined by microdissection. The eviscerated fetuses were then fixed, clarified, and stained with alizarin red-S by the Method of Dawson (1926) for skeletal examination.

RESULTS: No dams died during this study. Loss of hair and/or scabbing at the dosing sites was seen between gestation days 17 and 28 in 2 to 4 dams/group in all dosed and control groups. One dam in the mid-dose group (50 mg/kg/day) aborted on gestation day 23. Body weight gain was similar for all the groups. The high-dose group weights were 4-5% lower than the controls during most of the dosing period; this slight decrease was not biologically significant. Food consumption was reduced (as much as 24%) in the mid- and high-dose groups, but this is not uncommon in pregnant rabbits. The only compound-related gross lesions were found at the injection sites and were seen mostly in the high-dose rabbits. These lesions included viscosity [sic], retention of yellowish oily liquid, and yellowish abscess, and were probably due to incomplete absorption of the doses. There were no significant differences in organ weights.

The pregnancy rate was 93% for the controls and low-dose group, 87% for the mid-dose group, and 100% for the high-dose group. Group mean litter data were as follows:

Dose mg/kg/day	No. of Litters	Implants	Live Young	Embryonic Deaths Early	Late	Total	Post-Implant Loss %
0	14	11.1	10.0	0.9	0.5	1.4	9.68
25	14	10.1	9.4	0.3	0.8	1.1	7.75
50	12	10.5	9.8	0.5	0.4	0.9	7.14
125	15	10.5	9.1	1.6	0.5	2.1	13.92

Dose mg/kg/day	No. of Litters	% Males	Mean Fetal Wt. (g) Male	Female
0	14	48	37.9	38.1
25	14	52	37.2	38.1
50	12	47	37.8	36.4
125	15	42	40.8	37.6

These data demonstrated that the high-dose dams had slightly increased incidence of early embryonic death, but the incidence was not biologically significant. In all other respects, the litters were similar for all groups. The high-dose and control groups both had incidences of serious external abnormalities, but there was no indication that any of these lesions were caused by the test article. The incidence of visceral and skeletal anomalies and variations were similar for all groups.

Teratogenic NOEL ≥125 mg/kg/day (highest dose tested)
Embryotoxic NOEL ≥125 mg/kg/day (highest dose tested)
Fetotoxic NOEL ≥125 mg/kg/day (highest dose tested)
Maternal Toxicity NOEL ≥125 mg/kg/day (highest dose tested)

This study is CORE GUIDELINE. This study received Quality Assurance Review.

TERATOLOGY STUDY OF S-2703 FORTE IN RABBITS [Repeat Study]

Bozo Research Center, Inc.; Report No. EET-41-0036; October 29, 1984; Accession No. 259845

[NOTE: The summary of the preceding study (Report No. EET-41-0029) stated that, "No abnormality supposed to be due to administration of the test compound was detected in findings of external, visceral and skeletal examinations of fetuses." The incidences of external abnormalities (facial and cranial abnormalities) were within normal limits for rabbits, and were similar for both the control and high-dose groups. A repeat study was performed to, "elucidate the relationship between the occurrence of these abnormalities and the dosage, by administering the test compound not only under the same conditions as before, but also in a higher dose and during a particular critical phase during which the [external] abnormalities have been known to occur."]

PROTOCOL: Twenty male (unspecified weight) and 85 female (2.86-3.92 kg) 6 month old New Zealand White rabbits were used in this study. They were dosed subcutaneously with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil. The females in estrus were mated by housing each with a male. Evidence of copulation established gestation day 0. Gravid females were assigned to four groups of 15 rabbits each. They were dosed into the skin of their backs at doses of 0 (vehicle control), 50, and 125 mg/kg/day on gestation days 6-18, and at 250 mg/kg/day on gestation days 6-10 (a critical phase during which excess Vitamin A or X-radiation can induce facial and cranial abnormalities in rats and mice). Food and water were available ad libitum.

The females were observed daily for clinical signs during the gestation period. They were weighed on gestation days 0, and 6 through 28. Food consumption was measured during a 24 hour period prior to gestation days 6, 10, 14, 17, 21, 24, and 28. On gestation day 28, the dams were sacrificed by air embolism, and their organs grossly examined. Their ovaries and uteri were examined for the number of corpora lutea, implantation sites, live, dead, and resorbed fetuses, and placenta weights. Organ weights were measured prior to fixation for heart, lung, liver, kidney, spleen, and ovary.

All fetuses were weighed, sexed, and examined for external and oral malformations. The thoracic and abdominal organs were examined and fixed. The heart and kidneys were further examined by microdissection. The eviscerated fetuses were then fixed, clarified, and stained with alizarin red-S by the Method of Dawson (1926) for skeletal examination.

RESULTS:

No dams died during this study. Loss of hair and/or scabbing at the dosing sites was seen between days 15 and 28 in 4 low-dose, 7 mid-dose, and 1 high-dose rabbits. Spontaneous abortions occurred in 1 low-dose rabbit (gestation day 25), and 2 mid-dose rabbits (gestation days 22 and 24). Weight gain in the high-dose group was slightly less than that seen in the controls, but the loss was not biologically significant. There were no dose-related effects in food consumption. The only compound-related gross lesions were found at the injection sites and included viscosity and retention of yellowish oily liquid. These lesions were probably due to incomplete absorption of the doses. Ovary weights were reduced 10-17% in the mid- and high-dose groups. The significance of this effect is uncertain, especially since ovary weights did not decrease in the preceding study.

The pregnancy rate was 93% for the controls and 100% for the three dosed groups. Group mean litter data were as follows:

Dose mg/kg/day	No. of Litters	Implants	Live Young	Embryonic Deaths Early	Late	Total	Post-Implant Loss %
0	14	9.4	9.3	0.1	0.1	0.2	1.52
50	14	8.6	7.9	0.6	0.6	1.2	8.33
125	13	10.0	9.4	0.2	0.6	0.8	6.15
250	15	9.2	8.7	0.7	0.2	0.9	5.80

Dose mg/kg/day	No. of Litters	% Males	Mean Fetal Wt. (g) Male	Female
0	14	42	38.7	35.7
50	14	58	41.2	40.1
125	13	45	32.5	32.0
250	15	47	38.5	39.0

These data demonstrate that there were no significant litter differences in any dosed or control group. None of the fetuses had any external or visceral malformations. There were no significant group differences in the incidence of skeletal malformations and variations.

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This study reinforced the contention that the external malformations observed in the preceding study were spontaneous malformations and not compound-related.

Teratogenic NOEL ≥250 mg/kg/day (highest dose tested)
Embryotoxic NOEL ≥250 mg/kg/day (highest dose tested)
Fetotoxic NOEL ≥250 mg/kg/day (highest dose tested)
Maternal Toxicity NOEL ≥250 mg/kg/day (highest dose tested)

This study is CORE GUIDELINE. This study received Quality Assurance Review.

REPRODUCTION AND TERATOLOGY STUDY OF S-2703 FORTE IN RATS

Bozo Research Center, Inc.; Report No. EET-41-0026; April 25, 1984; Accession No. 259845

PROTOCOL: Fifty male (unspecified weight) and 186 virgin female (216-309 g) 12 week old Crj: CD (SD) rats constituted an F_0 generation. They were dosed subcutaneously with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil. The females in estrus were mated by housing them overnight with a male. Evidence of vaginal plugs and sperm in vaginal smears established gestation day 0. Gravid females were assigned to four groups of 38 rats each. They were dosed into the skin at an unspecified site at doses of 0 (vehicle control), 50, 150, and 500 mg/kg/day on gestation days 7-17. Food and water were available ad libitum.

The F_0 females were observed daily for clinical signs beginning on day 7. They were weighed on gestation days 0, 4, and 7 through 20, and on lactation days 0, 4, 7, 11, 14, 17, and 21. Food consumption was measured during 48 hour periods prior to gestation days 4, 9, 11, 14, 17, and 20, and on lactation days 2, 4, 7, 11, 14, 17, and 21. On gestation day 20, some of the dams were sacrificed by cervical dislocation, and their organs grossly examined. Their ovaries and uteri were examined for the number of corpora lutea, implantation sites, and the number of live, dead, and resorbed fetuses. Organ weights were measured for heart, lung, liver, kidney, spleen, ovary, and placenta prior to fixation.

All F_1 fetuses collected on gestation day 20 were weighed, sexed, and examined for external and oral malformations. Half of each litter (live pups) were fixed in Bouin's solution and examined for visceral anomalies of the head, thorax, and abdomen by the Method of Wilson (1965). The remaining live fetuses were fixed, clarified, and stained with alizarin red-S by the Method of Dawson (1926) for skeletal examination.

The F_0 dams allowed to deliver were observed during delivery, and the length of their gestation periods calculated. They were observed for their rearing behavior during lactation. On lactation day 21, all surviving dams were sacrificed by cervical dislocation, and their organs grossly examined. Their uteri were examined for the number of implantation sites. Organ weights were measured for heart, lung, liver, kidney, spleen, and ovary prior to fixation.

The delivered F_1 litters were examined for live and stillborn pups, sex, weight, and external malformations. The pups were weighed twice weekly until lactation day 21, and weekly from day 21 to 70. They were observed periodically for pinna detachment, the appearance of abdominal hair, eruption of lower incisors, eyelid separation, descent of testicles, and vaginal opening. The litters were culled to 4 pups/sex on lactation day 4. The culled pups were examined for visceral abnormalities prior to being fixed. On lactation day 21, 1 pup/sex/litter was sacrificed by ether anesthesia; the viscera of these pups were examined, and organ weights were measured for brain, heart, lung, liver, kidney, adrenals, spleen, testes, and ovary prior to fixation. The remaining 3 pups/sex/litter were weaned on lactation day 21, and subsequently used for spontaneous activity studies and breeding.

One F_1 pup/sex/litter was observed on day 21 for spontaneous activity (revolving wheel method) and neuromuscular activity (inclined plane method and rotor rod method). Also, visual (pupillary reflex) and auditory (Preyer's reflex) responses were observed. Additional studies were conducted using one pup/sex/litter, including emotional studies (open field test) at 8 weeks, learning studies (water "T" maze) at 9 weeks, and conditioned avoidance response (shuttle box conditioned avoidance response) at 10 weeks.

Two F_1 pups/sex/litter (F_1 generation) which had not participated in the functional studies were selected for breeding. Upon reaching the age of 70 days, these rats were mated by housing females with males from different litters in order to assess any reproductive effects. Whenever an insufficient number of F_1 rats of a particular sex were available for mating, untreated rats (10 weeks old) were substituted (the report claimed that the data for these matings were handled separately, but in fact they were combined with the other data). The F_1 dams were measured for body weight changes on gestation days 0, 4, 7, 11, 14, 17, and 20, and on lactation days 0-4. On day 0, the litters were counted, weighed, and evaluated for live and dead F_2 pups, and pups with external malformations. On day 4, the litters were again counted and weighed. Half of each litter was fixed in Bouin's solution, while the other half were fixed in 95% alcohol; they were not further examined. The F_1 dams were sacrificed on day 4 and their uteri examined for the number of implantations.

The 2 F_1 rats/litter used in behavioral studies were sacrificed at 11-12 weeks, and the F_1 males used to assess reproductive effects were sacrificed at 12-13 weeks or 14-15 weeks; they were examined grossly, and their organs fixed and stored. The testes, epididymides, prostates, and seminal vesicles were examined histopathologically for the males which failed to copulate. The ovaries and uteri were examined histopathologically for the females which successfully mated but did not become pregnant.

RESULTS: Two high-dose F_0 dams died during this study. One died on lactation day 3, and was found to have scabbing and retention of an oily liquid at the subcutaneous injection sites, and "retention of hair-like substance in two stomachs." The other dam was sterile and died after receiving 8 doses. There were reportedly no clinical signs in the other dams (including retention of doses at the injection sites) during the gestation and lactation periods. Body weight change and food consumption were similar for all groups during the gestation and lactation periods.

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Gross findings of retained oily liquid (presumably the corn oil vehicle) at the injection sites were seen in nearly all dosed and control rats sacrificed on gestation day 20. A mid-dose dam had injection site scabbing. There were no other compound-related gross lesions or organ weight changes in these dams. Approximately one-third of the dams sacrificed on lactation day 21 had subcutaneous retention of an oily liquid. This demonstrated that the test article formulations were slowly and incompletely absorbed. One mid-dose dam had injection site scabbing and alopecia. There was a slight (15%) increase in splenic weights in the high-dose group, compared to the controls.

The pregnancy rates for the F_0 dams were 95% for the controls, 87% for the low-dose group, 97% for the mid-dose group, and 89% for the high-dose group. Group mean litter data for the dams sacrificed on gestation day 20 were as follows:

Dose mg/kg/day	No. of Litters	Implants	Live Young	Embryonic Deaths Early	Late	Total	Post-Implant Loss %
0	24	13.3	12.4	0.75	0.08	0.83	6.3
50	21	15.1	14.4	0.71	0.00	0.71	4.7
150	25	14.0	13.2	0.64	0.12	0.76	5.4
500	21	13.1	11.9	1.10	0.14	1.23	9.4

Dose mg/kg/day	No. of Litters	% Males	Mean Fetal Wt. (g) Male	Female
0	24	55	3.34	3.21
50	21	55	3.42	3.22
150	25	50	3.41	3.23
500	21	54	3.29	3.20

These data demonstrate that there were no significant F_0 litter differences between the control and dosed groups on gestation day 20. External malformations included micrognathia and club foot in a control fetus, and tail hypoplasia and cleft palate in each of two mid-dose fetuses. There were no significant group differences in the incidence of visceral and skeletal malformations and variations.

Group mean litter data for the F_0 dams sacrificed on lactation day 21 were as follows:

Dose mg/kg/day	No. of Litters	Implants	Live Young	Stillborn	% Males	Mean Birth Wt. (g) Male	Female
0	12	14.3	13.0	2.3	51	6.2	5.9
50	12	13.8	12.5	1.2	47	6.2	5.9
150	12	15.0	13.6	1.1	43	5.9	5.6
500	13	15.8	14.2	1.5	51	6.0	5.7

These data demonstrate that there were no significant F_0 litter differences between the control and dosed groups on lactation day 21. Gestation periods were similar for all groups, and all dams appeared to have normal delivery and lactating behavior. One dam in the high-dose group died on an unspecified day during the lactation period; the cause of death was not mentioned.

There were no external malformations found in any of the weaned pups. F_1 pup viability was similar for all groups during the intervals of days 0-4, 4-21, and 21-70. Weight gain in the pups was similar for all groups between days 0 and 70. Postnatal development (based on pinna detachment, the appearance of abdominal hair, eruption of lower incisors, eyelid separation, descent of testicles, and vaginal opening) was similar for all groups. No visceral malformations were found in any of the pups culled on lactation day 4. The pups culled on lactation day 21 had no dose-related gross lesions or organ weight changes.

There were no compound related effects observed during the functional tests regarding spontaneous activity, neuromuscular activity, pupillary reflex, and audition in the F_1 pup on day 21. In an open field test (emotional studies) conducted at 8 weeks, the high-dose F_1 males had higher incidences of grooming, urination, and defecation. A female in the mid-dose group developed hindlimb paralysis during the shuttle box experiment and subsequently died. In the water "T" maze test (learning studies) conducted at 9 weeks, all groups were comparable in their ability to learn the maze, and in the time they required to traverse it. In the shuttle box test (conditioned avoidance response studies) conducted at 10 weeks, the percentage of avoidance response time increased in all groups during subsequent trials, indicating the acquisition of conditioned avoidance response. When these rats were examined grossly, no compound-related lesions were found.

Upon reaching the age of 70 days, the F_1 rats were bred in order to assess reproductive performance. All groups had similar mating and fertility indices. The pregnancy rates were 78% for the controls, 75% for the low-dose group, 83% for the mid-dose group, and 83% for the high-dose group. Their weight gain during gestation and the first 4 days of lactation were comparable, as were the lengths of their gestation periods. Group mean litter data for the F_1 dams sacrificed on lactation day 4 were as follows:

Dose mg/kg/day	No. of Litters	Implants	Live Young	Stillborn	% Males	Mean Birth Wt. (g) Male	Female
0	18	15.7	13.7	0.3	51	5.9	5.6
50	18	14.8	13.1	0.4	53	6.0	5.6
150	19	14.8	13.6	0.3	51	6.1	5.6
500	20	14.2	13.1	0.2	48	6.1	5.7

These data demonstrate that there were no significant F_1 litter differences between the control and dosed groups. Gestation periods were similar for all groups, and all dams appeared to have normal deliveries. F_2 pup weight gain and survival during the first 4 days of lactation were similar for all groups. There were no external malformations found in any F_2 pups. Gross examinations of the F_1 males and females on day 4 did not show any compound-related lesions.

The testes, epididymides, prostates, and seminal vesicles of the males which failed to copulate were examined histopathologically. Prostatic lesions of mild interstitial lymphocytic infiltration and mild focal atrophy were found in each of two high-dose males (there were two sterile high-dose males). Testis and epididymis weights were similar among the sterile control and dosed males. No compound-related histopathologic lesions were found in the females which were sterile or failed to copulate. There was no definite compound-

related effect on ovary and uterus weights in these females. The small population size and lack of comparison data on sterile and noncopulating F_0 rats made evaluation dubious.

Defined doses:

Maternal Toxicity NOEL (F_0) = 150 mg/kg/day
Maternal Toxicity LEL (F_0) = 500 mg/kg/day (lethality)

Reproductive NOEL (F_0) = 500 mg/kg/day
Reproductive NOEL (F_1) - undefined due to insufficient dosing.

Embryotoxic NOEL (F_1) = 500 mg/kg/day

Fetotoxic NOEL = (F_1) = 500 mg/kg/day

Teratogenic NOEL = 500 mg/kg/day

Functional Teratogenic NOEL (F_1) = 500 mg/kg/day

Behavioral Teratology:
Emotional NOEL (F_1) = 150 mg/kg/day
Emotional LEL (F_1) = 500 mg/kg/day (increased incidences of grooming, urination, and defecation in males in an open field test)
Learning NOEL (F_1) = 500 mg/kg/day
Conditioned Avoidance NOEL (F_1) = 500 mg/kg/day

The teratology portion of this study is CORE MINIMUM. The reproduction portion is CORE SUPPLEMENTARY because the F_0 generation was only dosed during organogenesis, and the F_1 generation was not dosed at all after birth. The report was difficult to read and interpret due primarily to the quality of the translation. Two high-dose F_0 dams died, but there was no explanation of the probable cause of death. Organ weights were only presented as absolutes; relative organ weights should have been presented as well. There were no gross pathology or organ weights reported for the F_0 dams sacrificed on lactation day 21. Whenever an insufficient number of F_1 rats of a particular sex were available for mating, untreated rats (10 weeks old) were substituted (the report claimed that the data for these matings were handled separately, but in fact they were combined with the other data). This study received Quality Assurance Review.

PHARMACOLOGIC ACTIVITY OF S-2703 FORTE IN MICE, RABBITS, DOGS, AND CATS

Hiroshima University School of Medicine; April 28, 1983; Accession No. 259845.

Tomio Segawa, Yasuyuki Nomura, Hiroaki Nishio, and Yoshihiro Nakata. Pharmacological Activities of (RS)-α-Cyano-3-phenoxybenzyl (1R)-cis, trans-chrysanthemate (S-2703 forte). Pharmacometrics, Volume 26. No 4. October, 1983. [Reprint]

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PROTOCOL: This study was performed using S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6, Lot No. PK-81051). Several pharmacologic activities were evaluated:

1. Experiments on CNS Activity -

A. Groups of five male ddY mice (15-20 g) were orally dosed with 0 (vehicle control), 50, 100, and 200 mg/kg of the test article dissolved in corn oil. An ANIMEX activity meter was used for 10 minute periods every 10 minutes to measure locomotor activity.

B. The impact of the test article on sodium pentobarbital induced "sleep" was evaluated by predosing mice with 0 (vehicle control), 5, 20, and 50 mg/kg of the test article 10 minutes before dosing the mice intraperitoneally with 50 mg/kg of sodium pentobarbital. The duration of sleep (>20 seconds of a loss of the righting reflex) was measured.

C. Spontaneous EEG effects were evaluated in female rabbits (2.3-3.0 kg) which had been pretreated intravenously with 10 mg/kg of sodium pentobarbital. The rabbits were aroused, then dosed intravenously with 0 (vehicle control), 1.0, 2.0, and 5.0 mg/kg of the test article. Electrodes were placed at the motor cortex, sensory cortex, hippocampus, and midbrain reticular formation positions. Recordings were made at unspecified times.

D. Body temperature changes were monitored in female rabbits (2.1-2.5 kg) dosed subcutaneously with 500, 1000, and 2500 mg/kg of the test article in corn oil. Rectal temperatures were recorded prior to dosing, and 30, 60, 120, 180, and 240 minutes after dosing.

2. Experiments on Cardiovascular Activity -

A. Respiratory rate, blood pressure (femoral artery), and heart rate were monitored in dogs (8.5-9.0 kg) dosed intravenously with 0 (vehicle control), 0.05, 0.5, 5.0, and 10.0 mg/kg of the test article in Sorpol. The dogs were pretreated with 25 mg/kg of intravenously administered sodium pentobarbital. Further experiments were conducted to assess blood pressure effects in the presence of atropine, ACh, isoproterenol, and propranolol, but since the procedures were not described, the results will not be discussed.

B. ECG's were taken for rabbits. Since there was no further description of the procedures, the results will not be discussed.

C. Effects on the isolated atria of guinea pigs were assessed by suspending the atria in a Ringer-Locke solution bubbled with 95% O_2 and 5% CO_2.

Since there was no further description of the materials and methods used, the results will not be discussed.

3. Experiments on Autonomic Activity -

A. The nictitating membranes of cats (3.0-3.5 kg) were evaluated for the effect of the test article on the autonomic system. Cats were intraperitoneally pretreated with 25 mg/kg of sodium pentobarbital. They were dosed intravenously with 0.5 mg/kg of the test article (a dose of 1.0 mg/kg would have elicited convulsions, so it was not attempted). A Recticorder and an isotonic transducer were used to measure contractions in the nictitating membranes. The superior cervical nerves were electrically stimulated.

B. Effects on the isolated ilium of guinea pigs and rabbits were assessed by suspending the ilium in a Tyrode solution bubbled with air. Since there was no further description of the materials and methods used, the results will not be discussed.

4. Experiments on Peripheral Activity -

A. Effects on the neuromuscular junction of the isolated Wistar rat diaphragm were assessed by suspending the muscle in Krebs-Ringer solution bubbled with 95% O_2 and 5% CO_2. The muscle was electrically stimulated for 0.1 msec every 10 seconds, and the contraction was recorded. The muscle was dosed with the test article, the Sorpol vehicle, and other chemicals. Since there was no further description of the materials and methods used, the results will not be discussed.

B. The effects on the eyes of rabbits was determined by instilling 0.2 ml aliquots of 1% and 50% test article in corn oil, corn oil, and 1% cocaine into the eyes of 6 rabbits/group. There was no mention of whether the eyes were rinsed. The corneal reflex was assessed immediately after dosing. The eyes were examined after 10 and 30 minutes, and 1, 2, and 24 hours. There was no attempt to score eye lesion severity.

5. Experiments on Hematological Effects

A. The effects on rabbit blood coagulation were assessed by combining 0.1 ml of 0.5% Sorpol, and 0.1 and 0.5% test article in Sorpol with 1 ml of arterial blood. The mix was agitated every 30 seconds, and timed for the onset of coagulation.

B. The hemolytic effects on rabbit erythrocytes was assessed by drawing three blood samples from the cervical artery and preserving it with 3.8% sodium citrate. The blood was centrifuged, and the precipitate washed with saline. The blood was again centrifuged, then diluted with 9 volumes of saline. A 0.25 ml volume of the resulting suspension was mixed with an equal volume of the test article at concentrations of 0 (distilled water - positive control), 0.05, 0.1, 0.5, 1.0, and 2.0%, then incubated for 20 minutes at 37°C, and centrifuged. The supernatant was then diluted with 3.3 ml of distilled water, and the optical density (at 550 mu) measured with a spectrophotometer.

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RESULTS:

1. Experiments on Central Activity -

   A. Mice orally dosed with 0 (vehicle control), 50, 100 mg/kg of the test article had similar levels of activity as measured with an ANIMEX activity meter. The mice dosed at 200 mg/kg, however, became far more active 75 minutes after dosing and continued to be relatively active during the next 45 minutes. Clinical signs in these animals included increased exploratory behavior, grooming, and preening in 2 mice which began 50 minutes after dosing at the 100 mg/kg dose; one of these mice had sudden convulsions 4.5 hours after dosing and died. Four mice dosed at 200 mg/kg had tremors (70 minutes after dosing), and jumping and severe convulsions (90 minutes after dosing); these mice were dead 140 minutes after being dosed.

   B. Predosing mice with the test article at doses of 5, 20, and 50 mg/kg 10 minutes before dosing the mice I.P. with sodium pentobarbital had little effect on sleeping time, although there was a slight dose-related decrease in sleeping time (23% in the high-dose group).

   C. An unspecified number of anesthetized rabbits had convulsions after being dosed with 5.0 mg/kg of the test article, but there were no changes in any EEG readings. No EEG or toxic effects were reported in rabbits at the lower doses.

   D. There were no fluctuations in rectal body temperature in any rabbits dosed subcutaneously with the test article at 2500 mg/kg.

2. Experiments on Cardiovascular Activity -

   A. The respiratory depth and frequency were unaffected by the Sorpol vehicle and test article doses of 0.05 and 0.5 mg/kg. There were no data on higher doses.

   Blood pressure was unchanged by the Sorpol vehicle and the test article at the 0.05 mg/kg dose. The 0.5 mg/kg dose elicited a 2 mm Hg increase in diastolic pressure and a 12 mm Hg increase in systolic pressure 30 seconds after injection; blood pressure returned to normal after 60 seconds. There were no further evaluable data.

   B. Rabbit ECG's data cannot be presented due to deficiencies in the publication.

   C. Data on the effects on the isolated atria of guinea pigs cannot be presented due to deficiencies in the publication.

3. Experiments on Autonomic Activity -

   A. Dosing anesthetized cats with 0.5 mg/kg of the test article did not affect the tone of the nictitating membranes, nor did it alter the effect of electrically stimulating the superior cervical nerves. Further experiments to assess adrenalin modulation effects were not described, so the results will not be discussed.

B. Data on the effects on the isolated ilium of guinea pigs and rabbits cannot be presented due to deficiencies in the publication.

4. Experiments on Peripheral Activity -

A. Data on the effects of the test article on the neuromuscular junction of the isolated rat diaphragm cannot be presented due to deficiencies in the publication.

B. Hyperemia was seen in one rabbit dosed with the 50% test article solution 10 minutes after dosing. The 1% cocaine solution eliminated the corneal reflex for all rabbits. No other eye lesions were observed.

5. Experiments on Hematological Effects

A. There was a slight increase in coagulation time in blood mixed with 0.5% test article in Sorpol, compared to the vehicle (Sorpol) controls. The 0.1% test article mixture did not affect coagulation time. There were no negative controls.

B. The test article did not cause hemolysis in rabbit erythrocytes at the doses used. The positive control did cause hemolysis.

This publication is CORE SUPPLEMENTARY. It was inadequate for describing the pharmacologic effects of the test article. It was a poor translation of a poorly written report. The descriptions of the study protocols and results were seriously lacking in detail, making data interpretation difficult or impossible. Generally missing from the many studies reported in this publication was information on the test materials and formulations, and the strain, sex, age, and number of animals used. The primary vehicle used, Sorpol, was not defined. It is surprising that this study was accepted for publication in its present format. Because of the poor scientific procedure evident in this publication, none of the data should be considered reliable.

AMES ASSAY OF S-2703 FORTE

Sumitomo Chemical Co., Ltd.; Report No. EET-20-0008; October 21, 1982; Accession No. 259845

PROTOCOL: Reversion assays were performed according to the Methods of Ames et al (1975) and Yahagi et al (1975) using Salmonella typhimurium TA98, TA100, TA1535, TA1537, and TA1538 strains, and the Escherichia coli WP-2 (trp^-) uvrA strain. The test article, S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6), was dissolved in DMSO and administered to each culture at concentrations of 10 to 5000 ug/plate. A S-9 mixture was prepared from the liver of a male rat which had been activated with PCB. Positive and vehicle control groups were run for the activated and nonactivated systems. The positive control agents were as follows:

	Nonactivated Systems	Activated Systems
TA98	2-nitrofluorene (1 ug/plate)	benzo(a)pyrene (5 ug/plate)
TA100	methyl methanesulfonate (200 ug/plate)	benzo(a)pyrene (5 ug/plate)
TA1535	ethylnitronitrosoguanidine (5 ug/plate)	2-aminoanthracene (2 ug/plate)
TA1537	9-aminoacridine (80 ug/plate)	benzo(a)pyrene (5 ug/plate)
TA1538	2-nitrofluorene (2 ug/plate)	benzo(a)pyrene (5 ug/plate)
WP-2	ethylnitronitrosoguanidine (2 ug/plate)	2-aminoanthracene (80 ug/plate)

The test compounds were mixed in a test tube with 0.1 ml of indicator cell suspension, and 0.5 ml of either a Na-phosphate buffer (nonactivated systems) or S-9 mixture (activated systems) and incubated with shaking for 20 minutes at 37°C. The mixtures were then mixed with 2 ml of melted soft agar which contained minimal amounts of histidine (for the S. typhimurium strains) or tryptophan (for the E. coli strain) in order to sustain growth, and poured onto minimal agar plates. The plates were incubated for 2 days, after which the revertant colonies were counted with an automatic colony counter. Each assay was performed twice.

RESULTS: There were no indications of cytotoxicity in any of the assays. There were no increases in revertant colonies in the nonactivated or activated assays dosed with the test article. All of the positive assays had significant increases in revertant colonies (3 to 83-fold, compared to the vehicle controls), thus demonstrating the sensitivity of the systems. The test article was thus not mutagenic in either the nonactivated or activated systems up to the limit dose (5000 ug/plate).

This study is ACCEPTABLE. The mean values for each assay were reported instead of the individual assay data. This study received Quality Assurance Review.

MICRONUCLEUS TEST OF S-2703 FORTE

Sumitomo Chemical Co., Ltd.; Report No. EET-30-0021; October 11, 1983; Accession No. 259845

PROTOCOL: Seven week old male ICR mice (30-38 g) were assigned to groups of six, and dosed intraperitoneally with S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6) in corn oil at doses of 0 (vehicle control), 200, 400, and 800 mg/kg. The doses were selected based on a preliminary acute toxicity study with the highest dose (800 mg/kg) being approximately 80% of the LD_{50}. A positive control group was dosed with 2 mg/kg of Mitomycin C. Mice of all groups were sacrificed by cervical dislocation 24 hours after being dosed, and bone marrow samples were drawn from the femurs. The marrow was smeared on glass slides, allowed to sit for a day, then fixed with methanol and stained with 5% Giemsa in phosphate buffer for 30 minutes. Each slide was then evaluated microscopically for incidence of micronucleated cells per 1000 whole erythrocytes, and per 1000 polychromatic erythrocytes (PCE's). The ratio of PCE's to total erythrocytes was calculated (as a measure of cytotoxicity. The experiment was repeated to evaluate any time course effect at the highest dose (800 mg/kg). These mice were sacrificed 24, 48, and 72 hours after dosing; the vehicle controls were sacrificed 24 hours after dosing. A positive control

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was not used in the repeat study.

RESULTS: There was no test article effect on PCE ratios in either trial. None of the groups dosed with the vehicle or the test article had any significant changes in the parameters measured. Lengthening the post-dosing interval had no effect on micronucleus induction. The positive control group had a 14-fold increase in the micronucleated erythrocytes:whole erythrocytes ratio, and a 34-fold increase in the micronucleated PCE:PCE ratio, thus demonstrating the sensitivity of the system. A mouse died of unspecified causes after receiving a 400 mg/kg dose of the test article.

This study is ACCEPTABLE. The Materials and Methods failed to give the doses used, and to mention that the study was repeated using different doses and sacrifice intervals. Despite the poorly written report, it was possible to determine the study design from the data tables. There was no mention of the cause of death for the one mouse that died on study. The study received Quality Assurance Review, yet the report deficiencies were not corrected.

IN VITRO SISTER CHROMATID EXCHANGE TEST OF S-2703 FORTE IN CHO-K1 CELLS

Sumitomo Chemical Co., Ltd.; Report No. EET-30-0022; October 11, 1983; Accession No. 259845

PROTOCOL: This test was performed using Chinese hamster ovary cells (CHO-K1). The test article, S-2703 Forte (93.6% pure; cis/trans = 18.4/81.6), was dissolved in DMSO. The positive control articles, mitomycin C (nonactivated systems) and cyclophosphamide (activated systems), were dissolved in saline. Vehicle controls were dosed with DMSO. A S-9 mixture was prepared from the liver of a male rat which had been activated with PCB. CHO-K1 cells were cultured for 24 hours in plastic dishes in an atmosphere of 5% CO_2, after which the media were discarded and the cells washed with Ham's F12 medium (without serum). The cells in the nonactivated systems were then mixed with 0.05 ml of a test or control article and 5 ml of medium without serum. The cells in the activated systems were mixed with 0.05 ml of a test or control article, 4.5 ml of medium without serum, and 0.5 ml of S-9 mixture. Each test was performed twice. The concentrations used for dosing in the two separate experiments were as follows:

	Dose (Moles)			
	First Experiment		Second Experiment	
	Nonactivated	Activated	Nonactivated	Activated
S-2703 Forte	10^{-6}	10^{-6}	10^{-5}	10^{-5}
	10^{-5}	10^{-5}	$3x10^{-5}$	$3x10^{-5}$
	10^{-4}	10^{-4}	10^{-4}	10^{-4}
	10^{-3}	10^{-3}	$3x10^{-4}$	$3x10^{-4}$
Positive controls:				
mitomycin C	10^{-7}	-	10^{-7}	-
cyclophosphamide	-	10^{-6}	-	10^{-6}
Vehicle controls	-	-	-	-

These mixtures were then poured into dishes, incubated for 2 hours, then washed and cultured for 40 hours in complete medium supplemented with 10 uM of 5-bromodeoxyuridine. Colcemid (0.1 ug/ml) was added for the final two hours of culturing. The cells were harvested by trypsinization, centrifuged, treated with KCL, fixed with methanol/acetic acid, and spread onto glass slides. The cells were differentially stained by the FPG method (Perry and Wolff, 1974). The slides were then prepared for microscopic examination. Fifty metaphases per slide were examined for SCE (25/slide for positive controls). Cytotoxicity was assessed at the same time by culturing treated cells for 48 hours, harvesting by trypsination, staining with Crystal Violet solution, and microscopically counting cells.

RESULTS: In the first experiment, cytotoxicity following treatment with the test article was unaffected at doses of 10^{-6} to 10^{-4} M, but reportedly increased sharply at 10^{-3} M (no cytotoxicity data were reported); no SCE evaluation was attempted at this dose. The cytotoxicity profiles were similar for cells which were nonactivated and activated. The incidence of SCE in the cells treated with the test article was similar to the vehicle controls in both experiments up to concentrations of $3x10^{-4}$ M (the highest dost tested in experiment 2). The sensitivity of the system was demonstrated by the 3 to 5-fold increase in SCE incidence in the positive controls compared to the vehicle controls. Thus, the test article did not induce SCE in this study.

This study is ACCEPTABLE. Some attempt should have been made to sample SCE in the non-lethal range ($>3x10^{-4}$ to $<10^{-3}$ M). This study received Quality Assurance Review.

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Chemical:	**Cyclopropanecarboxylic acid, 2,2-dimethy**
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