2-11-77

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 001968

SUBJECT: Registration #9687-RR DATE: 2/11/77

PRODUCT: B-47 Powdered Bacteriostat Concentrate

FROM:

OTHER NAMES: IRGASAN DP 300, Triclosan, 5-chloro-2-(2,4-Dichlorophenoxy) phenol

TO:

ACTION: Registration of product for use on diaper.

Registrant: Re-oda Chem. Engineering

FROM: Toxicology Branch (Registration Division)
C. Frick

TO: Mr. Elmer Geathers, PM #32

Recommendation: Toxicology Branch objects to the registration of this product for use on baby diapers.

Comments: For further consideration the following data will be required.

1. Acute Rat (oral) LD_{50} (Technical & Formulation)
2. Acute Dermal LD_{50} (Technical & Formulation)
3. Eye Irritation (Tech. & Formulation)
4. Neurotoxicity (Technical) using Hen as test animal
5. Lifetime Feeding Study (Technical)
6. Teratology (Technical)
7. Eighteen Month Skin Painting in Mice using 0, X, 10X, where X is calculated level of exposure
8. Phototoxicity (Tech. & Formulation)
9. Acute Primary Dermal Irritation (Tech. & Formulation)
10. Reproduction Study (Technical)

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EPA Form 1320-6 (Rev. 6-72)

Registration: 9687-RR

Product: Irgasan DP-300

Subject: 90 day subacute oral toxicity study with Irgasan DP-300 in Beagle Dogs.

Conclusion: On the basis of this study NEL = 12.5 mg/kg

Test Organization:

Group	No. of Animals Male	Female	Dose Level (mg/kg/day)
Control	4	4	None
T-I	4	4	12.5
T-II	4	4	25
T-III	4	4	50
T-IV	4	4	100
T-V*	2	2	50

*Allowed a four week recovery period (off test) after termination of the 90-day investigation.

Dosage administered orally via gelatin capsule seven days per week.

Parameters: The following determinations were conducted upon each dog from the untreated control group and five test groups just prior to the inception of the study and after 42 and 85 days of testing.

Hematologic Studies

total leukocyte count
erythrocyte count
hemoglobin
hematocrit
differential leukocyte count

Blood Chemistry Studies

blood urea nitrogen
serum alkaline phosphatase
SGOT
serum glucose
serum bilirubin
SGPT

Urine Analyses

albumin
pH
erythrocytes
glucose
specific gravity
crystals
bilirubin
leukocytes

At the conclusion of 90 days of testing, the dogs from the untreated control group and Test Groups I, II, III and IV were sacrificed by electric shock. All major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland, pituitary gland.

The following tissues and organs excised from these animals were examined histologically.

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lungs
Lymph Nodes (cervical, mesenteric)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (Submaxillary)
Small Intestin
Spinal Cord
Spleen
Stomach
Trachea
Thyroid Gland
Uterus
Urinary Bladder
Muscle (skeletal)

Animals in Test Group V were taken off test after 90 days and allowed a four-week recovery period, after which time they were sacrificed and examined according to procedures described above.

Results: The mean overall body weight gain for females receiving 12.5 mg/kg Irgasan DP-300 was significantly lower than that for untreated control females. However, mean gains seen at higher dose levels showed no significant deviation from untreated controls.

One male receiving 100 mg/kg died after 23 days on test; another 100 mg/kg male was sacrificed in extremis after 26 days. One female receiving 50 mg/kg was sacrificed in extremis after 57 days - Each of the three animals that died or was sacrificed during the study displayed weight loss, anorexia, lethargy and symptoms of jaundice (a distinct yellow cast to ocular and oral mucous membranes) three to five days prior to death. No abnormal reactions were noted among any of the other test animals during the study.

Increases in serum alkaline phosphatase activity were noted among animals receiving 100 mg/kg Irgasan DP-300 similar elevations were noted in the two groups (T-III, T-V) dosed with 50 mg/kg. Serum alkaline phosphatase activities among dogs at lower levels (25 or 12.5 mg/kg) were comparable to the untreated control. Animals in test group V (50 mg/kg) were allowed a four-week withdrawal period (off test) after which time formerly elevated serum alkaline phosphatase activities returned to normal.

With respect to the other blood chemistry studies, data revealed no significant abnormalities at any of the levels tested.

Histopathologic examination of tissues from the three animals that died or were sacrificed in extremis during the study, revealed that death was attributed to hepatotoxicity which resulted in abstructive jaundice. No table lesions in other major organs (kidneys, spleen) were also seen and are considered to be related to the systemic effects of the hepatotoxicity and inanition.

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Histopathologic examination of tissues derived from animals surviving the 90-day test period revealed treatment related morphologic changes in the livers of most animals at the 25, 50, 100 mg/kg dose levels. Changes consisted of focal acidophilic to granular degeneration of the cytoplasm of a few individual hepatocytes or small numbers of adjacent hepatocytes. Histopathologic examination of tissues from the animals dosed with 12.5 mg/kg and the four 50 mg/kg dogs allowed a four-week recovery period (off test) revealed no abnormalities attributable to the ingestion of the test material.

Blood Levels: 200 mg/kg Dog - single dose oral
Blood Drawn 0, 1, 2, 4, 8, 24, 48, 72 hours
Blood Level Max. 0.322 ppm (free) at 4 hours - 72 hours .023 ppm

100 mg/kg - Single dose (oral) Max. conc. 50 ppm total
0.50 ppm Free - Reached 7 hours

Compound forms B-glucuronide or sulfate fairly rapidly.

Excretion complete in approx. 5 days.

Excretion major route feces to a lesser extent urine. Smaller animals relative more excretion takes place in the urine.

Tissue distribution after 50 mg/day for 6 days

Brain = 0.17 ppm
Liver = 12.6 ppm
Kidneys = 6.37 ppm
Muscle = 0.80 ppm
Fat = 2.06 ppm

90 day Beagle study
Blood Levels after 90 days:

Dose Levels		Free	Total
	12.5 mg/kg/day	.111 ppm	36.1 ppm
	25.0 mg/kg/day	.218 ppm	67.4 ppm
	50.0 mg/kg/day	.350 ppm	87.7 ppm
	100.0 mg/kg/day	.489 ppm	107.4 ppm

Registration: 9687-RR

Product: Irgasan DP-300

Subject: Oral Administration of Irgasan DP-300 to Albino Rats (3 months)

Conclusion: On the basis of this study NEL = 50 mg/kg

Test: Irgasan DP-300 was administered orally by stomach tube to three groups of 35 male and 35 female Charles River CD albino rats - each in single daily doses of 50, 125 and 315 mg/kg. The compound was administered as a suspension in 1% gum tragacanth and a control group of 25 rats of each sex received daily doses of gum tragacanth vehicle alone. All doses were adjusted to a volume of 10 ml/kg. The rats were dosed daily and received a maximum of 92 doses in 94 days.

Rats were weighed daily during the first week and weekly thereafter. They were observed for any changes in appearances or behavior, and food consumption was determined for 10 males and 10 females in each group throughout the study.

Parameters: Observations and tests for effects included; body weights, food consumption, behavior, systolic blood pressure on day 89, and the following hematologic determinations on peripheral blood on day 28 from five rats of each sex of the 0, 125 and 315 mg/kg groups:

- Number blood cells (white & red)
- Hemoglobin concentration
- Hematocrit
- Differential

Terminal studies included the following determinations from arterial blood from 0, 125 and 315 mg/kg levels:

Prothrombin Time	Glucose
SGOT	Urea Nitrogen
SGPT	Total Protein
Serum Alk. Phos.	Albumin
Na	Urinalysis
K	
cl	

Other determinations studies included absolute and body to organ ratios of the following organs:

Liver	Pituitary	
Kidneys	Thymus	
Adrenals	Spleen	
Heart	Sex Organs	Also included in this study (organ wt.) five males & five female rats from the 50 mg/kg group.

At the end of the study all rats not already sacrificed for other studies were autopsied. The brain, eyes, bone marrow, mammary tissue and thoracic and abdominal viscera of all rats in the study were examined macroscopically. Tissues from five of each sex from the control and 315 mg/kg groups were sectioned for microscopic examination. The brain, spinal cord, optic and sciatic nerves from the remaining rats used for organ weight data (5 of each sex from control, 50, 125 mg/kg groups) were similarly prepared for microscopic study.

At one month and at three months blood was drawn from the abdominal aorta of five male and five female rats from each group, 6 and 12 hours of dosage for the determination of plasma level of Irgasan.

Blood Levels (Highest)*

Dose 315 mg/kg (one month) =	♀ 6 hr.	= 1.21 ppm
	♀ 12 hr.	= .202 ppm
	♀ 6 hr.	= .331 ppm
	♀ 12 hr.	= .117 ppm
Dose 315 mg/kg (3 months) =		
	♀ 6 hr.	= .208 ppm
	♀ 12 hr.	= .110 ppm
	♀ 6 hr.	= .326 ppm
	♀ 12 hr.	= .098 ppm

*Free or conguated form not indicated.

Comments: No dose dependent or dose related effects were noted in any of the studies with the exception of organ weights. In both sexes the 315 mg/kg group and in males in the 125 mg/kg group, the liver was heavier than controls ($P = 0.05$). The changes were probably compound related stress as no histopathologic alterations were noted.

Registration: 9687-RR

Product: CH3565 (Irgasan DP-300)

Data Source: Geigy - Dept. of Toxicology - Notebook P-3c, pages 56-60, 4/30/68

Subject: Acute Intravenous LD-50 in male rats

Conclusion: Acute Intravenous LD-50, in male rats = 29.9 mg/kg

Protocol: No. of animals: 30 male albino rats of the Sprague-Dawley Strain

Wt. of animals: 120 to 160 gm

Route and Site: The compound was administered in a single intra-venous dose into the tail vein

Rate: 0.4 to 0.8 ml. per 100 gm. of body weight

Concentration: 0.5% aqueous solution

Observations: The animals were observed for 14 days for mortality, body wt. changes, and toxic effects. The LD-50 was calculated on day 14. Necropsy examinations were performed on the animals which succumbed and on day 14 when the surviving animals were killed with carbon monoxide.

Results:

Values below represent the number of animals dead/number of animals used:

Dosage Level mg./kg. b.w.	Time of Death Days 1 - 14
0 (Control)	0/5
20	0/5
25	0/5
30	3/5
35	4/5
40	5/5

LD_{50} - 29.9 mg./kg. b.w.

95% Confidence Limits - 26.0 - 34.4 mg./kg.

No. Affected / No. Used or Surviving	Effect	Time After Administration
5/5	Exhibited no effects	
5/5	Extension of hind limbs, opisthotonic convulsions	1 minute
5/5	Pupils very pale, lethargic	30 to 45 minutes
5/5	Bloody urine	
1/5	Large tail sore	4 to 7 days
5/5	Kyphosis followed by tonic convulsions, pupils white	
	Hind limb muscular weakness, followed by recovery	15 to 30 minutes
	Bloody urine	1 to 3 hours
1/5	Large portion of tail necrotic	5 to 8 days
5/5	As at 25 mg./kg.	
3/5	Dead	1 to 3 minutes
1/2	Large (long) tail sore	5 to 8 days
	As at 25 mg./kg.	
4/5	Dead	1 to 3 minutes
1/1	Large portion of tail necrotic	5 to 8 days
5/5	Convulsive deaths	1 to 3 minutes

Registration: 9687-RR

Product: CH 3565 (Batch No. 9) Geigy

Data Source: Industrial Bio-Test - IBT No. J4773, Dec. 15, 1966

Subject: Acute Oral Toxicity - Dogs

Conclusion: Study not acceptable.
See comment.

Procedure:

To test groups, each consisting of two male and two female dogs and corresponding to dose levels of 2500 and 5000 mg/kg respectively were employed in the study. Healthy adult mongrel dogs were used. On the morning of the first test day, after a 16-hour fast (water permitted) all dogs received their respective doses of the undiluted test material, the dogs were observed for the succeeding 14 days.

Results: Emesis occurred among all animals at both dosage levels within eleven hours after dosage and all animals appeared normal for the remainder of the study.

Comments: As these animals were fasted, except for water and test compound - The emesis seen for eleven hours must have consisted of water and the test compound. There is no way to accertain how much of the test compound remained in the dogs stomach for obsorption.

Registration #9687-RR

Product: Irgasan DP-300

Data Source: Industrial Bio-Test IBT No. A1993, Sept. 29, 1972

Subject: Acute Oral Toxicity Study in New Born Albino Rats

Conclusion: Review by L. Chitlik - Elaboration on some specific difficulties appear necessary in this case, the new born rat (acute oral study) - It would seem to satisfy the requirement for an acute oral study. It does not, because the compound was administered as a 1% w/v suspension and the resulting LD_{50} value is very biased. The study also concluded that the LD_{50} was 580.0 mg/kg, when only 2 of the animals were dead 600 mg/kg. This procedure in LD_{50} determination is difficult to understand. This reviewer concurs.

Procedure: The test material (Batch 351) was administered as a 1.0% w/v suspension in corn oil at dose levels of 256.7, 400.0, 600.0, 750.0 and 900 mg/kg. Ten new born albino rats were tested at each level. The acute oral median lethal dose (LD_{50}), expressed in terms of Irgasan DP-300, was calculated to be 580 mg/kg. General signs of intoxication exhibited by the rats following dosing included: hypoactivity, ruffed fur, labored breathing, and muscular weakness. Necropsy of the animals that died revealed inflammed gastrointestinal tracts. No gross pathologic alterations were noted among the animals sacrificed at the end of the 14-day observation period.

Dose mg/kg	Number dead/number tested
266.7	1/10
400.0	2/10
600.0	2/10
750.0	8/10
900.0	10/10

Registration: 9687-RR

Product: [CH 3565] Irgasan, -300 [5-chloro-2-(2,4-dichlorophenoxy) phenol]

Petitioner: Re-nda Chem. Engineering Co. Data Ref. Ciba-Geigy

Subject: 14 day Subacute Dermal Toxicity Study on CH 3565 in Albino Rabbits.

Test:

Group	Number of Animals M	F	Material Tested	Conc. % w/v	Dose ml	Appl
Untreated Control	5	5	-	-	-	
Treated Control I	5	5	corn oil	-	10	
Treated Control II	5	5	corn oil	-	10	
Test I	5	5	CH 3565	3%	10	

Results: Hypoactivity, hyperirritability, muscular weakness, emaciation, cyanosis, loss of righting reflex were noted among animals exposed to corn oil alone. The skin at the site of contact was moderately irritated by corn oil and slightly irritated by CH 3565.

Comments:

Sixty percent of both control groups (corn oil only) died - while no test animals died.

Only one dose level used in the experiment.

No animals with abraded dermis were used.

Moderate to severe weight loss in both the controls (corn oil) and treated animals.

Conclusion:

T/B questions the value of this study and will not accept a dermal NEL of 3% in corn oil for CH 3565.

Registration: 9687-RR

Product: IRGASAN DP-300

Subject: 90-Day Toxicity Study with IRGASAN DP-300 in Newborn Rhesus Monkeys

Outline of Experiment

Group	Test Material	Dose Levels (ml/day)	(mg/day)	Number of Animals
Vehicle Control	None*	15	-	5
Positive Control	Hexachlorophene (3% formulation)	5	150	7
T-Ia	IRGASAN DP 300 (1% formulation)	5	50	5 (1)**
T-IIa	IRGASAN DP 300 (3% formulation)	5	150	7
T-I	IRGASAN DP 300 (1% formulation)	15	150	5
T-II	IRGASAN DP 300 (3% formulation)	10	300	7

* The vehicle control group animals received daily applications of a formulation (surgical scrub soap solution) containing no IRGASAN DP 300 or hexachlorophene.

** An extra animal (T-Ia female) which had been treated in the same manner as the other monkeys was included to provide frozen tissues for CIBA-GEIGY when tissues from another T-I animal became available.

Conclusion: On the basis of this study, T/B can make no recommendations regarding the Toxicity of IRGASAN DP 300.

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DOSING PROCEDURE: The formulation with or without the respective test material (IRGASAN DP 300) was applied to the unshaven trunk of the monkey. The formulation was worked into the fur and skin for a period of 5 minutes. This was followed by a thorough warm water rinse. Each animal was then thoroughly dried in a warm chamber before being returned to its mother.

D. Body Weights

The body weight of each animal was recorded at birth and at weekly intervals for 13 weeks. Weekly body weights were also recorded during a 4-week recovery period for 2 positive control, 2 T-IIa, and 2 T-II monkeys. Total weight gains were computed at the conclusion of the investigation.

E. Mortality and Reactions

Checks for mortality and abnormal behaviorial reactions were recorded daily.

F. Hematologic, Clinical Blood Chemistry Studies, and Urinalysis

1. Group A Animals (Test Groups T-I and T-II)

Blood and urine samples were collected individually from each animal after 30, 60, 84, and 90 days of treatment. Blood and urine samples were also collected individually from the 4 animals (2 each) of the positive control group and the T-II group at weekly intervals during a 4-week recovery period (following the 90-day treatment period.) The blood and urine samples collected after 30, 60, and 90 days of treatment and during the recovery period were shipped to CIBA-GEIGY Corporation for analysis.

2. Group B Animals (Test Groups T-Ia and T-IIa)

Blood, urine, and fecal samples were collected individually from each animal after 30, 60, and 90 days of treatment. Blood, urine, and fecal samples were collected weekly from the 2 T-IIa animals during a 4-week recovery period. Also, blood, urine, and fecal samples

were collected from extra animals treated at the high dose level (T-IIa). These samples were collected from 1 animal after 3, 7, 14, 21, and 28 days and 2 animals at 14, 21, and 28 days of treatment. All of these samples were shipped to CIBA-GEIGY Corporation.

The following hematologic, clinical blood chemistry studies and urinalyses were conducted on the blood and urine samples collected from animals in both Groups A and B after 84 days of treatment:

1. Hematologic Studies

   a. Hematocrit Value
   b. Erythrocyte Count
   c. Hemoglobin Concentration
   d. Total Leukocyte Count
   e. Differential Leukocyte Count
   f. Mean Corpuscular Volume (MCV)
   g. Mean Corpuscular Hemoglobin (MCH)
   h. Mean Corpuscular Hemoglobin Concentration (MCHC

2. Clinical Blood Chemistry Studies

   a. Blood Urea Nitrogen Concentration
   b. Blood Glucose Concentration
   c. Serum Alkaline Phosphatase Activity
   d. Serum Glutamic-Pyruvic Transaminase Activity
   e. Serum Glutamic-Oxalacetic Transaminase Activity
   f. Total Bilirubin

3. Urine Studies

   a. pH
   b. Specific Gravity
   c. Glucose
   d. Occult Blood
   e. Ketones
   f. Albumin
   g. Microscopic Elements - Leukocytes
      Erythrocytes
      Crystals
      Casts (Group A only)

G. Pathologic Studies

Following 90 days of treatment, 5 animals from each group were sacrificed and autopsied. The remaining animals (2 each) from the positive control group, the T-IIa group, and the T-II group were sacrificed at the end of a 4-week recovery period following the 90-day treatment period. At the time of gross examination, a complete set of organs and other tissues were recomved from each animal and preserved in a 10 percent buffered formalin solution. The weights of the liver, kidneys, spleen, gonads, heart, brain, adrenals, thyroid and pituitary were also determined and recorded.

Microscopic examination was conducted on tissues taken from all animals in each group. These tissues, stained with Hematoxylin-Eosin, were:

Adrenals	Mesenteric Node
Aorta	Muscle
Bladder	Optic Nerve
Brain (cerebrum, cerebellum, and pons)	Pancreas
Caecum	Parathyroids
Colon	Peripheral Nerve
Duodenum	Pituitary
Esophagus	Prostate
Eye	Salivary Gland
Gall Bladder	Seminal Vesicle
Gonads	Skin
Heart	Spinal Cord
Ileum	Spleen
Jejunum	Sternum
Kidneys	Stomach
Liver	Thymus
Lungs	Thyroid
Lymph Node	Tongue
	Trachea

Blood Levels: Blood levels of IRGASAN DP 300 were determined after 30, 60 and 90 days for 24 newborn rhesus monkeys. A short-term study was carried out on one group of 3 monkeys, with sampling after 3, 7, 14, 21, 28 days of daily scrubbing. Withdrawal determination was carried out on three

groups (each consisting of 2 monkeys chosen randomly) for 4 additional weeks after application had ceased. Doses were 50, 150, 300 mg. At the 300 mg dose use a blood level as high as 35 ppm was seen after 23 days from treatment cession blood levels as high as 1.12 ppm were noted. The correlation between blood concentration and dose levels were erratic.

Comments:

a) The effects or no effects observed in this study cannot be correlated with a known dose level, as by the method of application used in this experiment, much of the applied compound would be found on the hair of the animal and we are unable to acertain how much of the compound actually came into contact with the skin.

b) The area of skin exposed to treatment is not known.

c) It is not clear to T/B as to what type toxicological data was to be generated by a dermal exposure of 5 minutes/day.

1963

Registration: 9687-RR

Product: CH 3565 (Irgasan DP-300)

Subject: Subacute Dermal Toxicity Study of CH 3565 in New Born Beagle Pups

Conclusion:

This is not an acceptable subacute dermal toxicity study for the use pattern of this compound in this registration request.

Protocol:

The CH 3565 was made into a 0.1% sol. with Ivory soap (4.9%) and distilled water (95.0%). The test group pups were immersed in this solution for 30 seconds. Treated control group pups were immersed in a 5.% Ivory soap solution with distilled H^{20} for 30 seconds. Pups were immersed up to their necks; the test of treated control solution covered the entire body of the pups with the exception of their heads. Following the immersion the pups were thoroughly dried with clean towels under an air dryer and returned to their respective mothers. The above schedule was followed five days per week for three weeks for a total of 15 applications. The pups were weighed twice weekly and length measurements recorded weekly during the investigational period. They were under observation and examined daily for clinical signs indicative of toxic effects. The following determinations were conducted upon each pup from the treated control and test group prior to the inception of the test, two days after the immersion period (Day 21) and again 12 days after the immersion period (Day 32): hematocrit, hemoglobin, crythrocyte count, total and differential leukocyte counts, methemoglobin and platelet counts. Two days after the immersion period (Day 21), three pups from each group were sacrificed and 17 days following the immersion period (Day 36) the remaining three pups in each group were sacrificed. At both autopsies all major tissues and organs were grossly and microscopically examined.

The treated control pups were initiated on test 10 days after the test pups.

Results:

Elevate and erratic methemoglobin levels seen in both test and control pups.

Gross and histopathologic findings included: hyperemia, edema, vacuolar changes, fatty degeneration and other pathology - These conditions were present in both test and control groups - Not enough animals were used in this study to give these findings any statistical meaning.

In the other parameters studied i.e., body weights
food consumption
body length
survival
behavioral reactions
skin reactions - macroscopic
hematology
No significant adverse effects were noted.

Comments:

The experiment was set up using a 30 second dip on 15 occasions, a total of 7.5 minutes. The actual concentration of CH 3565 that these animals came in contact with is by the nature of the experiment, not known - such factors as how much of the compound was wiped off, in the hair or licked off, is not addressed.

Registration: 9687-RR

Product: CH 3565 (Irgasan DP-300)

Subject: Repeated Insult Patch Test

Conclusion: This study, as submitted, is not acceptable. See Comments½ at end of review.

Protocol: Test items

1. Wool containing 7,200 ppm, CH 3565
2. Wool control
3. Nylon containing 18,900 ppm, CH 3565
4. Nylon control

Patch occluded-1 inch sq.

This study was set up to demonstrate whether the test material was a 1. primary irritant, 2. fatiguing agent, 3. sensitizer on the basis of the visible clinical responses.

No. of subjects: 56 Human Volunteers (21 Males & 35 Females - ranging in age from 1.5 to 35 years.

The test was performed on and applied to a predesignated site on the upper arm of each subject and covered with lintine(?) gauze. The lintine, in turn, was covered with polypropylene, the edges of which were then affixed to the skin with tape to seal the contents completely. At the end of 24 hours, the patches were removed. The contact sites were examined and the observations were assigned scores according to a criteria of "grading".

Following removal, the contact sites were left untreated and uncovered and allowed to rest. At the end of the rest period, they were re-examined to ascertain whether there were any changes. The procedure was adapted to a weekly routine consisting of:

1. Application of patches on Mondays, Wednesdays and Fridays.
2. Removal of patches after 24-hour contact periods on Tuesdays, Thursdays and Saturdays respectively.

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3. Rest periods of 24-hours following the Tuesday and Thursday removals, and 48-hours following the Saturday removals.

After removal of the last application preceding the challenge, the sites were examined immediately and once daily for at least two days.

The challenge was applied to the original contact site after 14-days of no contact with the test material. The challenge application was terminated after 24-hours. The sites were examined for immediate reactions which, if present, were graded and recorded. These sites were re-examined 24- and 48-hours later for delayed reactions. Grading of Responses 0 to 4+ was used.

#1 = No visible irritation, or no difference from surrounding untreated skin.

#2+ = Erythema confined to the contact site and barely exceeding that of untreated skin.

#3+ = Erythema confined to contact site, and definitely exceeding that of untreated skin.

#4+ = Erythema and edema with one or more complications such as: extension beyond margins of contact area, vesiculation and ulceration.

Results:

For all categories, primary irritant, fatiguing agent, sensitization, all test subjects had a scoring of 0 (no reaction).

Comments:

As to the amount of compound the skin of the test subject were exposed to, the following information is given: "wool containing 7,200 ppm CH 3565 and Nylon containing 18,900 ppm CH 3565 and Patch occluded - 1 square inch". From the above information this reviewer is unable to accertain the quantity of CH 3565 applied to the test subjects skin.

After each study the statement - ..."since 50 subjects were used, we may predict with 95% certainty that at least 92.89% of a general population will not be sensitized by this material" - As it is clearly seen that in all three studies 56 subjects are used, TB request an

explanation of these conflicting statements. Reference study Reg. # 100-491, A # 051150, Report #4 Title: Repeated Insult Patch Test on Nylon and Wool Impregnated with CH 3565, by Industrial Biology Laboratories, Inc., March 29, 1968.

The rest periods between compound application is not compatible to an experiment designed to show the fatigue phenomenon.

Registration: 9687-RR

Product: CH 3565

Data Source: Ciba-Geigy Corp., Ardsley, New York. Vol. X, Sept. 7, 1970

Subject: 90-Days-Dermal-Toxicity of CH 3565 in New Zealand White Rabbits.

Conclusion: No objections.

Protocol:

The local and systemic tolerance of a 3% CH 3565 sol. in propylene-glycol in comparison with propylene glycol alone was investigated in 24 New Zealand White rabbits. The study involved 90 days of treatment (local application on the intact back skin) and 14 days of observation.

The doses amounted to 0.1, 0.5 and 1.0 ml of 3% CH 3565 sol./kg bw. per day local. Control animals were treated with the solvent (1.0 ml propylene glycol/kg bw. per day.

Behavior, outward appearance, food consumption, development of body weight, hematological and clinical-chemical investigations, composition of urine, ophthalmic, hearing, and macroscopic inspection and comparison of the wt. of internal organs at the autopsy after 90 days of treatment was carried out.

Group	CH 3565-doses: 3% solution in propylene glykol/kg b.w./day local	Number of animals	Mean initial weight in kg X)
(I)			
Male rabbits	0, 1 ml	3	2, 3 ± 0, 1
Female rabbits		3	
(II)			
Male rabbits	0, 5 ml	3	2, 3 ± 0, 2
Female rabbits		3	
(III)			
Male rabbits	1, 0 ml	3	2, 4 ± 0, 1
Female rabbits		3	
(IV)			
Male rabbits	1,0 ml propylene glykol/kg	3	2, 4 ± 0, 1
Female rabbits		3	

The application area was depilated by shearing without injury 3 days before beginning and then twice every week during experiments. It was situated between the fore and hind extremities on the back of the animals and had a size of 20 x 20 cm.

Within this area, the solutions were applied onto 15 x 16 cm (10% of the body surface) of the intact skin with a brush. They were held in contact with the skin by means of a four-fold layer of cotton gauze and a rubber sleeve according to DRAIZE, which was imposed very loose. The daily time of exposure amounted to 8 hours.

heart	thymus	parotis	rib junction
vessels (aorta)	lymph nodes	adrenal	skeletal muscle
lungs	esophagus	gonads	brain
trachea	stomach	pancreas	
liver	small & large intestine	bladder	
gall bladder	pituitary	tongue	
kidney	thyroid	eye	
spleen	prostutal uterus	spinal cord	

Of all animals paraffin sections of the preceeding organs were prepared and given histological examinations.

The following investigations were performed:

Hemoglobin	SGPT
RBC	SGOT
WBC	Glucose
Differential	Na, k, Total Protein
Hematocrit	Bun
Reticulocytes	Urine Studies
Thrombocytes	
Prethrombin-Time	
Heinz bodies	

Results:

Skin reactions

0.1 ml of 3% sol./kg/bw/day = slight erythema
0.5 ml of 3% sol./kg/bw/day = slight erythema and edema (low grade symptoms) reaction increased until 2nd-6th test week - reaching macimum grade 0.6 (Draize) - diminished slowly, till it had completely disappeared from 7th-9th week on - No Eschar or rhagades observed.

1.0 ml 3% sol./kg/bw/day = growing erythema and edema showed from the 1st test day on, in single values surpassing grade 2 according to DRAIZE. From the 1st to the 8th test week, it was accompanied by eschar formation and rhagades. From the 3rd-6th week on, erythema and edema faded till at the end of application the mean value was grade 0.7 DRAIZE.

Behavior and external changes

No alterations in behavior or external changes noted.

Food consumption

No significant difference between test and control groups.

Blood and Urine studies

Not extraordinary.

Histology

Not extraordinary.

Comments:

This study did demonstrate local dermal reactions at dose levels used.

Registration: 9687-RR

Product: GP 41-353-(triclosan) (Irgasan DP-300)

Subject: 14 Day Neurotoxicity study - Oral Administration to Albino Rats

Conclusion:

Cannot accept this study as fulfilling the neurotoxicity requirement this specie is not particularly sensitive to neurotoxic effects. Studie should be performed on hens.

Procedure:

Test compound GP 41-353 was administered orally to albino rats (SI/50) in 2% carboxymethylcellulose once a day, seven days a week, for two weeks. The doses of 0 (controls), 100, 300, 1000 and 2000 mg/kg/day were given. Each dose group except the top dose consisted of five male and five female rats. In the top dose group (2000 mg/kg/day there were ten male and ten female rats. At the beginning of the experiment the rats weighed 151 to 158 gm (mean 154 gm). All animals were kept in cages in groups of five rats per cage and given food and water ad libitum. Body weights, clinical symptoms and mortality were observed every day. The rats reaching a morfbund state were sacrificed. The spontaneously dead rats were autopsied. On day 15 (ca. 24 hours following the last treatment) the remaining animals were sacrificed and exsanguinated under light ether norcosis. The brains were dissected and their fresh weights measured. The left nervus ischiadicus was stretched on wooden plates and fixed together with the brain in 4% neutral formaldehyde. For embedding in paraffin the brains were sliced in the following transversal levels:

A) - in the level of the commissura anterior and the chiasma opticum

B) - in the level of the commissura posterior and of the nucleus intorpeduncularis

C) - in the level of the cerebellar nuclei and of the oliva inferior.

Paraffin sections were cut at a thickness of 5-7 um and stained with luxol fast blue (myelin stain and cresyl violet (NNissl stain). The nervus ischiadicus was examined on longitudinal paraffin sections stained with luxol fast blue and Bodian's stain (impregnation of axons). The nervus ischiadicus of the dose groups 1000 and 2000 mg/kg/day was also cut into 20-11 thick frozen sections that were stained according to Benda-Spielmeyer Method (visualisation of myelin sheaths). All sections were examined in the light microscope.

Results:

- The average body weight of rats administered 2000 mg/kg/day decreased during the first days of the experiment and remained distinctly lower than controls at the end of the experiment.

- Some inhibition of movement, decreased muscular tone, polydipsia and polyuria were observed. These symptoms appeared to a slight extent in the group administered 300 mg/kg/day. They were more advanced in the groups treated with 1000 mg/kg/day and with 2000 mg/kg/day.

- The rats administered 2000 mg/kg/day revealed a mortality rate of 17/20. Five of these 17 rats were sacrificed in a moribund state and 12 died spontaneously.

- The average brain weights of the treated rats did not differ from the controls.

- There were no pathological changes in the brain of the treated rats.

- Peripheral nerve (sciatic) of the treated rats did not reveal any pathological changes.

Microscopical Findings in the Brain

The brain of the rat No. 4 (0 1000 mg/kg/day) revealed a marked dilatation of both lateral ventricles with atrophic changes of the surrounding ependyma, the white and the grey matter. This finding was considered to be an incidental spontaneous change, unrelated to the effect of the test compound. There were no pathological changes in the other examined brains. All the areas that were examined showed myelinated nerve fibres arranged according to known anatomical structures and revealing an even staining pattern. The nerve cells showed no change in the structure and distribution of Nissl substance. There were no pathological changes in the nuclei of nerve cells or glia cells nor changes of the structure of blood vessels or the tissue in the vicinity of the membrana gliae perivascularis. Some of the sections revealed preparation artifacts and autolytical changes.

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Microscopical Findings in the Peripheral Nerve

The proximal (femoral) part of nervus ischiadicus was examined. The myelin sheaths showed an entirely normal structure as revealed by specific staining. The appearance of the axons showed no deviation from the normal pattern. No changes were seen in the structure and localisation of the Schwann cells.

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Registration: 9687-RR

Product: CH3565-Irgason DP-300

Subject: Three-phase guinea pig photosensitization tests

Conclusion: TB cannot accept these studies
see comments

Procedure:

Phase #1
A test group and a positive control group consisting of six guinea pigs each were employed. The dorsal area of each animal was shaven. Exactly 0.05 ml of a 2.0% (w/v) absolute alcohol solution of CH3565 of TCSA (3,3,4',5-tetrachlorosalicylanilide)(positive control) was applied daily to the prepared sites of each animal for five consecutive days. After each application the animals were irradiated with UV (2900-3200A°) for 15 minutes via sun lamp from a distance of 18 inches. Erythematous reactions in the skin were recorded 24 hours after each application according to the following scoring criteria:

Description	Grade
Barely perceptible (area not defined)	1
Pale red (pink) in color and area definable	2
Definite red in color and area well defined	3
Beet or crimson red in color and/or injury in depth (necrosis, escharosis)	4

After a period of ten days, the animals in the test and positive control groups received three challenge applications of 0.05 ml of a 0.1% w/v sol. of CH 3565 or TCSA in olive oil. Irradiation for 15 minutes followed each challenge application and readings for erythema were made the next day. After the skins of the guinea pigs appeared normal, they were shaven on the flanks and treated with a 0.2% w/v solution of CH3565 in 8.0% w/v solution of Ivory soap for a total of three applications. An 8.0% w/v solution of Ivory soap without the test material was applied on the opposite flank, UV irradiation for 15 minutes followed each application and skin readings were made 24 hrs. after each treatment.

CH 3565

Results Animal Number	Initial application in the Form of 2.0% Abs ETOH					Challenges in 0.1% olive 8.1			Rechallenge in 2.0% sol. in 8. % soap		
	Days					Days			Days		
	1	2	3	4	5	1	2	3	1	2	3
1	0	0	0	0	1	1	1	2	0	0	0
2	0	1	1	1	1	1	1	2	0	0	0
3	0	1	1	1	1	1	2	2	0	0	0
4	0	1	1	1	1	1	2	2	0	0	0
5	0	1	1	1	1	1	1	2	0	0	0
6	0	0	0	0	0	1	1	2	0	0	0
Mean	0.0	0.7	0.7	0.7	0.8	1.0	1.3	2.0	0.0	0.0	0.0

TCSA

	Initial Application in a 2.0% in absol. ETOH					Challenges in a 0.1 % olive oil			Rechallenge in the Form of a 0.2% sol. in in 8.0% soap			Challenge with CH3565 in 0.1% in olive oil	
	Days					Days			Days			Days	
	1	2	3	4	5	1	2	3	1	2	3	1	2
1	0	1	1	1	1	1	2	2	0	1	1	2	2
2	0	1	1	1	1	1	2	2	0	0	1	2	2
3	0	1	1	1	1	1	2	2	0	0	0	2	2
4	0	1	1	2	2	1	2	2	0	1	1	2	2
5	0	0	0	1	1	1	2	2	0	1	1	2	2
6	0	1	1	1	1	1	2	2	0	1	1	2	2
Mean	0.0	0.8	0.8	1.2	1.2	2.0	2.0	0.0	0.0	0.7	0.8	2.0	2.0

Comments on Phase I:

Initial Application of test compound was 1.0 mg per day, for 5 days, for a total of 5 mg. This is a very small amount of compound for total dose in this study. However, if this exposure level is comparable or higher to the levels found in actual use situations it will be acceptable. At this point the acutual use and exposure levels are not known to the reviewer and judgement on this matter must be reserved.

After the challenge doses of the 0.05 ml of the 0.1 % solution of CH3565 and TCSA in olive oil the skin of the animals were allowed to heal back to normal. TB would like to know how long this healing process took.

In the result charts the data "challenge with product R in the form of a 0.1% olive oil solution" for two days of exposure. This reviewer cannot find in the submitted experimental procedure, when or from where this data is generated.

In this scale of grading the interpretation of readings between 0-1 are most subjective. The difference between the test compound and known sensitizer was seen in the soap solution applications in te magnitude of the 0 to 1 scale and to such small extent that the effects seem between the two compounds are not significantly different.

In the olive oil treatment both the test compound and the positive control,- erythema was noted.

Three-phase guinea pig photosensitization test.

Phase II Procedure:

One test group consisting of six guinea pigs was employed. Exactly 0.05 ml of absolute alcohol was applied to the prepared site of each animal for five consecutive days after a rest period of ten days, each animal received one application of 0.05 ml of olive oil and three applications of 0.05 ml of a 0.1% w/v solution of product $ (CH3565) in olive oil. UV irradiation for 15 minutes followed each challenge application and readings for erythema were made on the next day.

Results:

After the challenge application of olive oil, there was erythema (Grade 1 in all animals). After the challenge applications of product $ (CH3565) in olive oil, there was the same amount of erythema (Grade 1 in all animals at all three days).

Comments:

The animals in this study were sensitized with aboolute alcohol not CH 3565 - this study may demonstrate the irritating quality of the olive oil used in this test but provides no additional information on the photosensitization properties of the test compound CH3565.

Subject: Three-Phase guinea pig photosensitization test

Phase III Procedure:

Test group consisting of six guinea pigs were employed for CH3565. Exactly 0.05 ml of a 2.0% solution of CH3565 in absolute alcohol was applied daily to the prepared sites of the animals. The animals were ultraviolet-irradiated after each application in exactly the same manner as in Phases I and II except only a GE sun lamp was utilized. Skin readings for erythema were made after each application. After a rest period of ten days the animals insulted with CH3565 received three challenge applications of 0.05 ml of a 0.1% w/v solution of CH 3565 in olive oil.

In addition to this test group, two treated control groups consisting of six guinea pigs each were employed. Exactly 0.5 ml of absolute alcohol or Virgin Press olive oil respectively was applied daily to the prepared sites of animals in each group for five consecutive days. After each application the animals were UV irradiated in exactly the same manner as the animals in the test group and the skin sites read for erythema. After a rest period of ten days, both the animals insulted with absolute alcohol and olive oil received three challenge applications of 0.05 ml of virgin press olive oil plus UV-irradiation after a second rest period of three days the animals insulted with absolute alcohol received three challenge applications of 0.05 ml of a 0.1% solution of CH 3565 in olive oil on the original site. At the same time a challenge application of 0.05 ml of 0.2% CH 3565 in a solution of 8.0% Ivory soap was made on one flank and a challenge application of 0.05 ml of Ivory soap on the other flank. The animals originally insulted with olive oil received three challenge applications of 0.05 ml of a 0.1 % sol. of CH3565 in olive oil on the original site. At the same time a challenge application of 0.05 ml of 0.2 % CH3565 in a sol. of 8.0% Ivory soap was made on one flank and a challenge application of 0.05 ml of Ivory soap on the other flank.

Results:

These data show that CH3565 did not produce photosensitization when challenges are applied as 0.2% sol. in a sol. of 8.0% Ivory soap in water.

Challenges of CH3565 in olive oil produced erythema in skin which had been insulted with CH3565 in alcohol, with absolute alcohol only or with olive oil only. Olive oil, itself, as a challenge produced the same degree of erythema in skin which had been insulted with absolute alcohol only or with olive oil only.

Comments:

These data show since olive oil produced erytherma on olive oil insulted skin, and on alcohol insulted skin, it appears that it (9live oil) may

be a weak sensitizer, cross-sensitizer or photosensitizer-this reviewer must question these results.

In all three phases of this experiment the insult exposure was only five day - this exposure should be maintained for at least ten days.

The positive control TCSA did not work well in this experiment.

This reviewer does not understand why a vehicle such as absolute alcohol was used in this thpe of experiment.

In an experiment of this type the test compound vehicle cannot be an irritant, or photo toxic or a photosensitizer as the olive oil seem to be in this particular study.

The UV range of radiation from the GE sunlamp used in phases II and III is not given.

Conclusion: This three phase study is unacceptable.

Registration: 9687-RR

Product: GS14703=CH3565 (Irgesan DP 300)

Date Source: Industrial Bio-Test Laboratories, Inc. 8/24/68
IBI #J4915

Subject: 18 Month Study of the Carcinogenic Potential of GS14703

Conclusion: This study is not acceptable.
see comments:

Procedure:

The sutdy was conducted on a sample of GS14703, employing Charles River CD-1 Swiss white mice. The study utilized an Untreated Control Group, Treated Control Group (acetone), Postive Control Group (9,10-dimethyl-1,2-benzanthracene, 0.01% in acetone) and Test Groups I and II (0.5 and 1.0% GS14703 in acetone resepctively) applications of 0.1 ml of the respective solutions were applied to the interscapular region three times weekly. Test groups of 50 mice each (25 male & 25 female) were used. Control groups were 100 animal 1 0 : 1 0. The animals were clipped once a week on the day prior to administration of the test material.

The solutions were applied three tiems weekly for the duration of the experiment.

Animals were weighed weekly and observed for detection of toxic effect and/or tumors.

Food consumption was measured.

At the end of the experiment, all surviving animals were sacrificed and subjected to complete gross examination, plus microscopic tissue studies as dictated by gross findings. In addition, the skin from all positive control group animals, all other animals showing gross abnormalities was examined histologically.

Group	Number of Animals Tested Males	Females	Test Material*	Concentration Tested (per cent)	Volume Solution (ml)
UC (Untreated Control)	50	50	None	-	Volume of solution applied in ml -
TC (Treated Control)	50	50	Acetone	Undiluted	0.1

Group	Number of Animals Tested Males	Females	Test Material*	Concentration Tested (per cent)	Volume Solution (ml)
PC (Positive Control)	50	50	9, 10 Dimethyl 1, 2 Benzanthracene	0.01	0.1
Test I	25	25	GS 14703 (GP 41353 P.1)	0.5	0.1
Test II	25	25	GS 14703 (GP 41353 P.1)	1.0	0.1

* Positive control and test materials administered as acetone solutions.

Results:

Body weight:
Nothing extraordinary noted

Food Consumption
Nothing extraordinary noted

Mortality
Positive control had an accelerated mortality rate.
No drug related (CH3565) effects were noted.

Behavioral Reactions
No abnormal behavioral reactions were noted among animals in any group during the experiment.

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TEST MATERIAL: GS 14703 (GP 41353 P.1)

Carcinogenic Study - Swiss White Mice

Summary of Tumor Incidence - Males

Type of Tumor	Class	Number of Tumors per Group: UC	TC	PC	T-I	T-II
Squamous cell carcinoma of the skin, lung or liver	I	-	-	14	-	-
	II	-	-	19	-	-
	III	-	-	15	-	-
	IV	-	-	2	-	-
Fibrosarcoma	-	-	-	1	-	1
Neurofibrosarcoma	-	-	-	1	-	-
Lymphoma	-	3	4	2	-	4
Adenoma	-	1	-	-	1	1
Bronchiolar carcinoma	II	4	3	-	-	-
Hepatoma	-	-	-	-	2	-

TEST MATERIAL: GS 14703 (GP 41353 P.1)

Carcinogenic Study - Swiss White Mice

Summary of Tumor Incidence - Females

Type of Tumor	Class	Number of Tumors per Group: US	TC	PC	T-I	T-II
Squamous cell carcinoma of the skin, lung, spleen or lymph nodes	I	-	-	10	-	-
	II	-	-	26	-	-
	III	-	-	16	-	-
	IV	-	-	[illegible]	1	-
Lymphoma	-	8	8	-	-	-
Bronchiclar carcinoma	III	1	2	-	-	-

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Type of Tumor	Class	Number of Tumors per Group: UC	TC	PC	T-1	T-11
Arterine carcinoma	III	-	-	2	-	-
Adenocarcinoma	II	1	-	-	-	2
Fibrosarcoma	I	-	-	-	-	1

Skin Reactions

Local skin reaction were noted among all animals of all groups-these were limited to slight transient erythema and slight drying of the skin at the dosing site.

Gross Pathology

Organs examined:

Liver	Spleen
Lungs	GI Tract
Pleura	Salivary Glands
Kidney	Epididymis
Thymus	Mesentary
Ovaries	Uterine Horns

Skin

Histopathology

All organs or tissues exhibiting macroscopic lesions were examined histologically.

Comments:

This reviewer would like to know why the highest dose used in this study was only 1 mg.

The use exposure of this product will be seven days a week - the exposure in this study was only three applications per week.

In this study all organs or tissues exhibiting macroscopic lesions were examined histologically - TB will require complete histology, not just those tissue showing gross pathology.

(c) 1968

In the treated control group and the untreated control group, the gross pathology changes notes the ovaries ie. cysts 15/50 and the Epididymis ie. Hypertrophy 23/50. In the Positive Control Group and Test Group #1 and Test Group #2 no mention is made of pathology observed in those areas. No explanation is given as to whether all were normal (which is questionable) or whether they were examined at all.

In the treated control group and the untreated con ol group, the gross pathology changes notes the ovaries ie. cysts 15/5[illegible] and the Epididymis ie. Hypertrophy 23/50. In the Positive Control Group a d Test Group #1 and Test Group #2 no mention is made of pathology obse ed in those areas. No explanation is given as to whether all were nor l (which is questionable) or whether they were examined at all.

Registration: 9687-RR

Product: Irgasan DP-300

Data Source: Bio-Test Labs Inc. IBT Nos. P7113 and J7112

Subject: Teratogenicity and Reproduction Study Using Albino Rats and New Zealand Rabbits

Conclusion: Studies, as submitted, not acceptable. See comments.

1. Experimental Animals

The animals employed were young Charles River albino rats, received at 33 days of age and acclimatized to these laboratory conditions for one week. At that time, a total of 90 rats (30 male and 60 female animals) were divided into three groups, two test and one control.

2. Organization of Groups

A structural arrangement of this phase of the experiment is shown below in Table I.

TABLE I

TEST MATERIAL: CH3565

Outline of Experiment

Albino Rats

Group	Dose Level of Drug (mg/kg of body weight/day)	Number of Animals Male	Female
C	None	10	20
T-I	50	10	20
T-II	100	10	20

Note: Test material was administered as a 10 percent (w/v) solution in corn oil.

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