Original

PETITION DATA EVALUATION REPORT

Prepared by

Sciences International, Inc.

(b) (4)

Prepared for:

U.S. FOOD AND DRUG ADMINISTRATION
Office of Premarket Approval

August 1998

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I. EXECUTIVE SUMMARY

This petition data evaluation report (PDER) summarizes Sciences' review of the chemistry and toxicology portions of food additive petition (FAP) 8B4574. (b) (4) is seeking to amend the food additive regulation at 21 CFR §178.2010, "Antioxidants and/or stabilizers for polymers," to include bis(2,2,6,6-tetramethyl-4-piperidinyl) sebacate, marketed as Tinuvin 770, as a thermal/light stabilizer in adhesives and pressure-sensitive adhesives complying with 21 CFR §177.105 and 21 CFR §177.125. The maximum use level proposed for Tinuvin 770 in these applications is 0.1 % by weight with no other restrictions.

GENERAL

The test material used in the toxicology studies is designated as (b) (4), Tinuvin 770, (b) (4) (b) (4) There is no discussion of the designations (b) (4) or (b) (4) in the petition. These are most likely different forms of Tinuvin 770. Also, the pharmacology study "Effects of Tinuvin 770 on ^{3}H-desmethoxyverapamil binding to guinea-pig heart membranes" (page 000791) included a Tinuvin 770 fragment as a test agent which was designated as (b) (4). There is no CAS Registry Number, molecular formula, or structure given in the petition for this fragment. Sciences believes the omission of data on the Tinuvin 770 fragment is without toxicological significance.

CHEMISTRY

Conclusions

The petition adequately describes the identity and manufacture of the additive.

Deficiency

The petitioner should be notified that adhesives cleared under 21 CFR §175.105 are to be used at room temperature or below.

TOXICOLOGY

Conclusions

1. The acute oral LD_{50} for Tinuvin 770 in rats is 3.7 g/kg BW (page 20 of the PDER).

2. The acute dermal LD_{50} for Tinuvin 770 in rats is >3.17 g/kg BW (page 25 of the PDER).

3. The acute LC_{50} for Tinuvin 770 dust in rats is >960 mg/m^3 (page 32 of the PDER).

4. Tinuvin 770 causes moderate irritation when applied to rabbit eye mucosa (page 77 of the PDER).

5. Tinuvin 770 did not cause dermal sensitization in guinea pigs (page 82 of the PDER).

6. Tinuvin 770 did not cause dermal sensitization in human subjects (page 88 of the PDER).

7. Tinuvin 770 was nonmutagenic when tested in *Salmonella* strains TA98, TA 100, TA1535, or TA1537 (page 103 of the petition).

8. The NOEL for inhalation of Tinuvin 770 dust in rats is 4.1 mg/m^3 (page 116 of the PDER).

9. The NOAEL for administration of Tinuvin 770 to rats by gavage over a 4 week period is 50 mg/kg/day (page 126 of the PDER).

10. The LOEL for administration of Tinuvin 770 in feed to rats is 29 mg/kg/day (page 136 of the PDER).

11. The results of three pharmacological studies indicate has the high affinity binding of Tinuvin 770 to L-type calcium channels of isolated heart membranes is of minor biological significance in the intact heart.

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II. INTRODUCTION

This petition data evaluation report (PDER) summarizes Sciences' review of the chemistry and toxicology portions of food additive petition (FAP) 8B4574. (b) (4) [redacted] is seeking to amend the food additive regulation at 21 CFR §178.2010, "Antioxidants and/or stabilizers for polymers," to include bis(2,2,6,6-tetramethyl-4-piperidinyl) sebacate, marketed as Tinuvin 770, as a thermal/light stabilizer in adhesives and pressure-sensitive adhesives complying with 21 CFR §177.105 and 21 CFR §177.125. The maximum use level proposed for Tinuvin 770 in these applications is 0.1 % by weight with no other restrictions.

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III. CHEMISTRY

Identity

The chemical name, CAS Registry Number, molecular formula, and structural formula are given in Section A, page 000002 of the petition.

Chemical Name: Bis(2,2,6,6-tetramethyl-4-piperdinyl) sebacate

CAS Registry Number: 52829-07-9

Trade Name: Tinuvin 770 (b) (4)

Molecular Formula: $C_{28}H_{52}N_2O_4$

Structural Formula:

(b) (4)

There are no infrared, NMR, or other spectra of the additive included in the petition to further identify the additive. The test material used in the toxicology studies is designated as (b) (4) Tinuvin 770, (b) (4), or (b) (4). There is no discussion of the designations (b) (4) or (b) (4) in the petition. These are most likely different forms of Tinuvin 770. Also, the pharmacology study "Effects of Tinuvin 770 on ^{3}H-desmethoxyverapamil binding to guinea-pig heart membranes" (page 000791) included a Tinuvin 770 fragment as a test agent which was designated as (b) (4). There is no CAS Registry Number, molecular formula, or structure given in the petition for this fragment. Sciences believes the omission of data on the Tinuvin 770 fragment is without toxicological significance.

Manufacture

(b) (4)

(b) (4)

(b) (4)

Description of Process

(b) (4)

(b) (4)

Additive Composition and Impurities

The additive has the following typical composition (page 00005).

Tinuvin 770 (C_{10} diester)	98.5% min
Monoester (C_{10})	1.3%
Homologs (C_{11}, C_{12} diester)	0.6%
Volatiles (ISOPAR G, water)	0.2%

Physical Properties of the Additive

The physical properties of the additive are described on page 000006 of the petition as follows.

Appearance	White to off-white granules
Odor	No discernible odor
Purity	98.5% minimum
Melting range	81–85°C
Vapor pressure	1.3×10^{-8} mmhg at 20°C
Octanol/water partition coefficient	Log $P_{o/w}$: 0.35
Solubility in water	<1 ppm at 20°C
Specific gravity	1.0–1.1

Proposed Use

The petitioner is proposing that 21 CFR §178.2010 be amended to permit use of the Tinuvin 770 as a stabilizer in adhesives complying with 21 CFR §175.105 and in pressure-sensitive adhesives complying with 21 CFR §175.125 (Section B, page 000008 of the petition). The requested clearance includes a maximum use level of 0.1% by weight for pressure sensitive adhesives complying with 21 CFR §175.125, but no such restriction for adhesives complying with 21 CFR §175.105. In further describing the intended use of the additive, the petitioner states that "...finished adhesives under §175.105 could be used with all food types at all temperatures - generally dry foods at less than 150°F. Pressure-sensitive adhesives are only used with raw or processed fruits and vegetables, poultry and dry foods at room temperature or below."

The above description of the intended use of the additive in pressure-sensitive adhesives is consistent with 21 CFR §175.125. However, the statement that the additive is to be used as a component in finished adhesives at all temperatures is not consistent with 21 CFR §175.105. Adhesives cleared under 21 CFR §175.105 are to be used at room temperature or below. Adhesives used in laminate constructions intended for use above room temperature are regulated under 21 CFR §177.1390 and 21 CFR §177.1395.

The petitioner should be notified that adhesives cleared under §175.105 are to be used at room temperature or below. The description of the proposed use provided on page 000008 of the petition is otherwise consistent with the proposed amendment to 21 CFR §178.2010 shown in Section G.1, page 000799.

Intended Technical Effect

A graph is shown in Section C (page 000011) of the petition indicating that for a polyolefin-based hot melt adhesive, 0.1 wt % Tinuvin 770 reduces the Gardner Color Index to half that of an adhesive with no stabilizer. The test was conducted on an adhesive of 1 inch thickness at 170°C for 24 hours. Results for other concentrations of Tinuvin 770 are not shown.

Dietary Exposure

The petitioner makes the following statement regarding dietary exposure to the additive (Section D.1, page 000013), "The FDA has concluded in previous petitions, e.g. FAP 9B4144, that [the] additive in this adhesive use contributes negligibly to the estimated daily intake."

Tinuvin 770 will be used as a stabilizer in adhesives complying with 21 CFR §175.105. The adhesive systems under 21 CFR §175.105 containing Tinuvin 770 will be used with all food types at room temperature or below. For use of their stabilizer in adhesives (at room temperature or below), extraction studies are not required since (as stated in 21 CFR §175.105) Sciences assumes that the adhesive either will be separated from the food by a functional barrier or will contact the food only in trace amounts at the seams and at the edges between packaging laminates. Therefore, the migration of the additive into food at room temperature or below would be considered virtually nil (<50 ppb).

Additionally, Tinuvin 770 will be used as a stabilizer in pressure-sensitive adhesives complying with 21 CFR §175.125 at a maximum use level of 0.1 % by weight. The pressure-sensitive adhesives under 21 CFR §175.125 containing Tinuvin 770 will be used with raw or processed fruits, vegetables, poultry, and dry foods at room temperature or below. Under refrigerated or frozen storage conditions (for poultry products), migration rates for Tinuvin 770 would be quite low. For refrigerated storage of poultry where storage times are less than a week, the amount of migration is expected to be trivial. Many fruits and vegetables bear pressure-sensitive labels. Of those fruits and vegetables that do, some bear them on a part of the food that is not eaten (i.e., bananas and grapefruit) and some bear them on a peel or skin that *may* be eaten (i.e., apples,

pears, and tomatoes). Typically, labeled fruits and vegetables with edible peels or skins are washed or peeled prior to eating. Consequently, it is expected that migration of Tinuvin 770 from pressure-sensitive adhesives would be virtually nil (<50 ppb).

Exposure to Tinuvin 770 from its use in all adhesives may be estimated by multiplying the concentration in food (<50 ppb) by the consumption factor (CF) for all adhesives of 0.14. The maximum dietary concentration of Tinuvin 770 from its use in all adhesives is 7×10^9 g/g, or 7 ppb. This corresponds to an estimated maximum daily intake (EDI) of 21 μg/p/d (7×10^9 g/g x 3000 g/p/d).

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

David G. Gray, Ph.D.	Primary Reviewer	8/20/98 Date
Elizabeth L. Anderson, Ph.D.	Independent Reviewer	8/20/98 Date

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IV. TOXICOLOGY

ACUTE ORAL TOXICITY TO RATS OF (b) (4)

A. TITLE: Acute Oral Toxicity to Rats of (b) (4)
Dated August 15, 1972

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP Statement was provided.

2. Quality Assurance Statement: A statement attesting to the correct recording of results and adherence to study protocol was signed , but not dated, by the authors and is found on page 000019.

3. Study Objective: The study was conducted to assess the acute oral toxicity of (b) (4) in the rat.

4. Test Article: (b) (4) The chemical name was provided on page 000020 of the study report. The lot number was not reported. No description was provided of either the physical appearance of the test substance or the storage conditions.

a. Purity: The purity of the test substance was not stated. The study authors did not state the composition of the test article.

b. Stability: The stability was not reported.

5. Protocol

a. Animal Diet

Type: The type of diet was not reported.

Source: The source of the animal diet was not reported.

Frequency of Administration: The frequency with which the animals were fed was not reported, however, animals were fasted overnight prior to dosing.

b. Test Animals

Species and Strain: Five male and five female rats of the CFY strain were used.

Source: The source of the rats was not reported.

Age at Initiation of the Study: The age of the rats was not reported.

Weight: The body weights of the rats on the day before dosing were 93-127 g.

Quarantine: The acclimatization period was not described.

Animal Maintenance: Housing of animals was not described. Temperature, relative humidity, and photoperiod of the room in which the animals were housed was not stated.

c. Experimental design

Dose Levels: In the preliminary test, animals were initially dosed with 5 g of the test article/kg body weight. Because this dose exceeded the median lethal dose, a range-finding test was conducted using doses of 0, 1.25, or 2.5 g/kg. It was determined that the median lethal dose was in the range of 2.5 to 5.0 mg/kg. For the main study, doses of 0, 2.5, 3.2, 4.0, and 5.0 g/kg were used.

Number of Animals Sex per Group: A group of 5 male and 5 female rats were used for the preliminary test. Groups of 2 males and 2 females were used for the range-finding test and groups of 5 male and 5 female rats were used for the main study.

Preparation of Test Article and Mode of Administration: The test material was administered orally as a single dose, presumably by gavage, although this was not stated. The test substance was prepared as a 30% suspension in 0.5% carboxy methyl cellulose and administered at a maximum dosage volume of 16.7 ml/kg bodyweight. Control animals were dose with the vehicle alone (16.7 ml/kg). It was not stated when the dose formulations were prepared.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed for death and clinical signs of toxicity throughout the observation period of 14 days. It was not stated how frequently the animals were observed.

Body Weight: Body weights were recorded at least at dosing and at the end of each of the 2 weeks of observation, as these data were shown. However, it was not stated whether body weights were recorded more frequently within these intervals.

e. Gross Pathology

Necropsy: The study author reported that the animals that died were examined macroscopically to attempt to identify target organs. The extent of the examination was not described. Surviving animals were subjected to a gross necropsy at an unspecified day; the euthanasia method was not described.

Histopathology Examination: Not performed.

f. Statistical Evaluation

From the mortality data for the main study, the LD_{50} was calculated using the method of Litchfield and Wilcox (1949). No other statistical analyses were performed.

g. Critical Appraisal of the Protocol

This study was designed as an acute oral toxicity study; very few details on this study were provided. It appears that the conduct of the study was appropriate to determine the LD_{50}, although important data are missing regarding the purity, stability, and storage of the test material. Information on the source and housing of the animals was not provided. Housing conditions were not described. Although it was appears that the animals were treated via gavage, this was not stated. Specific information of the conduct of the necropsy was also lacking. The dosing volume was 16.7 ml/kg rather than the FDA recommended 10 ml/kg. The frequency of observation for clinical signs was not stated. Clinical signs that were reported were not attributed to specific doses or experiments.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** It appears that the clinical signs that were noted in the report were observed in the main study. These were observed shortly after treatment and included salivation, diarrhea, and diuresis. The dosages at which these signs were observed was not reported. It was stated, however, that survivors recovered within 2 days of dosing, judging by their external appearance and behavior.

2. **Mortality:** In the preliminary study, 9 of 10 rats dosed with 5 g/kg died. In the range-finding study, 0/4 rats (both sexes combined) dosed with 1.25 g/kg died and 0/2 female rats dosed with 2.5 g/kg died, but ½ male rats dosed with 2.5 g/kg died 4 days after dosing. In the main study, males again appeared to be more sensitive than females, as shown in Table 1 below.

Table 1. Mortality of Rats Dosed with (b) (4) in the Main Study

Sex	Dosage (g/kg)	Mortality	Time of death after dosing (hours)
male	0	0/5	---
male	2.5	2/5	<96
male	3.2	2/5	<42
male	4.0	3/5	<42
male	5.0	5/5	<43
female	0	0/5	---
female	2.5	0/5	---
female	3.2	0/5	---
female	4.0	2/5	<42
female	5.0	4/5	<67

3. **Body Weight Changes:** In the main study, body weights were depressed (9, 14, and 23% at 2.5, 3.2, and 4.0 g/kg , respectively, in males; 8, 10, 4, and 12% at 2.5, 3.2, 4.0, and 5.0 g/kg, respectively, in females) during the first week of the observation period compared to controls, but were normal during the second week. Data on the other studies were not reported.

4. **Gross Pathology**: Pale patches were found on the lobes of the liver of all animals that died. There were no findings in animals that survived to terminal sacrifice.

E. ASSESSMENT

Overall, the design and conduct of the study were appropriate for an acute oral toxicity study in the rat. There were several deficiencies in the reporting of the study, however. Important data are missing regarding the purity, stability, and storage of the test material. Although it appears that the animals were treated via gavage, this was not explicitly stated. Information on the source and housing of the animals was not provided and specific information of the conduct of the necropsy was also lacking. The dosing volume was 16.7 ml/kg rather than the FDA recommended 10 ml/kg. The frequency of observation for clinical signs was not stated and the incidence of clinical signs per sex

and per dose group was not reported. Therefore it could not be determined whether the clinical signs were dose related. The reviewer concurs with the study author's conclusion that the LD_{50} and its 95% confidence limits in this study are 3.7 (3.1-4.4) g/kg BW.

F. CONCLUSION

The acute oral LD_{50} in male and female rats is 3.7 (3.1-4.4) g/kg BW.

G. EXECUTIVE SUMMARY

The acute toxicity of (b) (4) was evaluated in an acute oral study in rats. In the main study, (b) (4) was administered as a single dose of 2.5-5.0 g/kg BW to fasted rats. The acute oral LD_{50} in male and female rats is 3.7 (3.1-4.4) g/kg BW.

H. DATA VALIDATION CHECK

All summary data were inspected.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

8/21/98
Date

Patte Bittner, M.S. Primary Reviewer

8/21/98
Date

John Liccione, Ph.D. Secondary Reviewer

ACUTE DERMAL LD_{50} IN THE RAT OF (b) (4)

A. TITLE: Acute Dermal LD_{50} in the Rat of (b) (4)
Dated September 8, 1975
Project Number: (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP Statement was provided.

2. Quality Assurance Statement: No Quality Assurance Statement was provided. The "Results" section of the report is signed by K. Sachsse, DVM and R. Bathe, DVM (p. 000025).

3. Study Objective: The study was conducted to assess the acute dermal toxicity of (b) (4) in the rat.

4. Test Article: (b) (4) The chemical name was provided on page 000024 of the study report. The batch number was (b) (4) No description of either the physical appearance of the test substance or the storage conditions was provided.

a. Purity: The purity of the test substance was not stated. The study authors did not state the identity, stability, or composition of the test article.

b. Stability: The stability was not reported.

5. Protocol

a. Animal Diet

Type: The animals received NAFAG rat food *ad libitum* and water.

Source: The source of the animal diet was NAFAG, Gossau SG.

b. Test Animals

Species and Strain: Tif:RAIf (SPF) rats were used in this study. Three rats of each sex were used at each dose level.

Source: The rats were raised at the testing facility.

Age at Initiation of the Study: The age of the rats was not reported.

Weight: The initial body weights of the rats were 180-200 g.

Quarantine: The animals were acclimated for at least 4 days prior to the start of the experiment.

Animal Maintenance: Animals were housed individually in Macrolon cages (type 2). The rats were housed at a room temperature of 22±1 °C, a relative humidity of 55 ± 5%, and a 14 hour light cycle day.

c. Experimental design

Dose Levels: The rats were treated with 2150 or 3170 mg/kg of the test substance. The report stated that higher doses were not possible. There were no control animals used.

Number of Animals Sex per Group: Three animals/sex/dose were used.

Preparation of Test Article and Mode of Administration: The test material was administered dermally. The test substance was prepared as a suspension in 2% carboxymethyl-cellulose. Before treatment, the suspension was homogeneously dispersed with an Ultra-Turrax. It was not stated when the dose formulations were prepared. An electric clipper was used to shave an area of about 60 cm^2 on each animal's back about 24 hours prior to treatment. The test substance was evenly applied on the skin of the animal with a syringe and the area was covered with an

occlusive dressing fastened with an adhesive elastic bandage. The dressing was removed after 24 hours and the skin was cleaned with lukewarm water before evaluating it for a reaction. Animals were observed for up to 14 days.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed for clinical signs of toxicity throughout the observation period of 14 days. It was not stated how frequently the animals were observed.

Body Weight: No body weight data were reported. It was not stated whether body weights were determined during the observation period.

e. Gross Pathology

Necropsy: The study author reported that the animals were submitted to necropsy on day 14 after treatment. The extent of the examination was not described. The euthanasia method was not described.

Histopathology Examination: Not performed.

f. Statistical Evaluation

No statistical analyses were performed.

g. Critical Appraisal of the Protocol

This study was designed as an acute dermal toxicity study ("limit test"). However, important data were missing. No information was provided regarding the purity, stability, and storage of the test material. Specific information on the conduct of the necropsy was also lacking. The frequency of observation for clinical signs was not stated. Body weights, which may have been a useful indicator of toxicity, were not reported. Only 3 animals/sex/dose were used.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Rats in both dosage groups were reported to exhibit dyspnea, exophthalmus, curved position, and ruffled fur within 24 hours after treatment. It was stated, however, that the animals recovered within 13 days of treatment. No local skin irritation was observed.

2. **Mortality:** No animals died at either treatment level.

3. **Body Weight Changes:** No data were reported and it does not appear that body

weights were recorded.

4. **Gross Pathology**: No substance-related gross organ changes were observed.

E. ASSESSMENT

The design of this study was appropriate for an acute dermal toxicity study in the rat. There were several deficiencies in the reporting of the study, however. The reviewer concurs with the study author's conclusion that the LD_{50} in rats of both sexes (observed over a period of 14 days) is greater than 3170 mg/kg (slight acute toxicity). Important data are missing regarding the purity, stability, and storage of the test material. Specific information on the conduct of the necropsy was also lacking. The frequency of observation for clinical signs was not stated. Body weights, which may have been a useful indicator of toxicity, were not reported. Only 3 animal/sex were used at each of only 2 dose levels instead of 5 animals/sex/dose as recommended by FDA guidelines. No animals died at either dose level. Clinical signs (i.e., dyspnea, exophthalmus, curved position and ruffled fur) were reported in all animals within 24 hours after treatment. Animals recovered from the toxicity within 13 days. There was no irritation or necropsy findings.

F. CONCLUSION

The dermal LD_{50} in male and female rats observed over a period of 14 days is greater than 3170 mg/kg BW (slight acute toxicity). in either sex at doses up to 3170 mg/kg BW.

G. EXECUTIVE SUMMARY

The acute toxicity of (b) (4) was evaluated in an acute dermal study in rats. (b) (4) was administered as a single dose of 2150 or 3170 mg/kg BW. The acute dermal LD_{50} in rats of both sexes (observed over a period of 14 days) is greater than 3170 mg/kg..

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patte Bittner, M.S. Primary Reviewer — Date 8/21/98

John Liccione, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE DUST INHALATION TOXICITY STUDY WITH TINUVIN 770 (b) (4) (b) (4) IN ALBINO RATS

A. TITLE

Acute Dust Inhalation Toxicity Study with Tinuvin 770 (b) (4) (b) (4) in Albino Rats; Report No: IT (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® in the rat.

4. **Test Article:** Tinuvin®770 (b) (4) The reviewer notes that the designation (b) (4) was not discussed in the chemistry section of the petition. The test material was characterized as a white dust. The batch number was reported as (b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stock cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: 960 mg/m ^{3} (analytical concentration). There were no controls.

Number of Animals per Sex per Group: 5

Test Material Dust Generation and Analysis:

The report stated that dust was suspended with a specially designed dust feeder. The test material powder was then passed through a high-velocity stream of clean, dry air. The air-jet velocity was adjusted to obtain the desired concentration of suspended dust. The test atmosphere was then introduced into the exposure chamber, dispersed by a baffle plate and exhausted a t the bottom of the chamber.

The report stated that the concentration of test material dust present in the exposure chamber was determined by sampling the test atmosphere in the breathing zone of the animals being exposed. The total weight of dust collected on a glass fiber filter was divided by the total volume of air drawn

through the filter during the sampling period. Air flow rate for sampling was regulated by a calibrated limiting orifice. The average analytical concentration of airborne dust was obtained by repeated air sampling. The analytical concentration was reported to be 960 mg/m^3.

Particle size distributions were performed. Particles were counted (using a light-field technique) with respect to 4 size ranges, viz., 5 microns or smaller, 6 to 10 microns, 11 to 25 microns and larger than 25 microns. Details on the light-field technique were not provided, however, the author stated that the smallest particle size that could be detected with this technique is 1 micron. The results of particle size distribution revealed that 87% of the total particles were > 25% micron size, and 13% between 11-25 micron size. There were no particles less than 11 microns.

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after the desired dust concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 25 °C, air flow at 50 l/min. and atmospheric pressure of 30.20 inches Hg.

Length of Exposure: 4 hours. The observation period was 14 days.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs. The author did not indicate how often animals were observed for signs during the 14-day observation period.

Body Weight: The author stated that body weights were determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. The data were recorded in terms of body weight gains.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. There were no GLP compliance or Quality Assurance statements.

c. A rationale for dose selection was not provided. However, the concentration examined in the study was high (i.e., 960 mg/m^3). However, the reviewer notes that in a similar experiment an acute LC_{50} > 780 mg/m^3 Tinuvin 770 was determined.

d. The age of the animals was not specified.

e. The incidence of clinical changes by sex was not provided. There were no individual animal data.

f. There were no control animals.

g. Body weight data were not provided.

h. Details on the method used for particle size determinations were not provided.

In addition, there were no particles in the respirable range (< 10 microns). The reviewer notes that in a separate subacute inhalation study on Tinuvin 770 (entitled Subchronic Dust Inhalation Toxicity Study with TINUVIN®770 (b) (4)) in Albino Rats; Report No: IT (b) (4) [redacted] the test material dust was generated and ground to respirable size (90% < 10 microns) by using blenders and settling cylinders).

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Lacrimation and salivation were observed in nearly all the test animals during the exposure. The author tabulated the number of animals affected (summarized in Table 1 below) but did not identify the sex of the animals affected. There were no individual animal data in the study. The duration of the symptoms lasted between 205 and 230 minutes.

Table 1. Clinical signs in rats exposed to Tinuvin 770[a].

Clinical Sign	Number of Animals Affected	Time of Onset after Start of Exposure (min)	Duration (min)
Lacrimation	1	10	230
Salivation	1	10	230
Lacrimation	9	35	205
Salivation	9	35	205

2. **Mortality Rate:** No deaths were observed.

3. **Body Weight Changes:** Body weight data were not provided in the study. The author stated that the average 2-week body weight gains (86 g for males; 42 g for females) were within the normal limits.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The author stated there were no gross pathologic alterations.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

Based on the results, the acute LC_{50} is > 960 mg/m^3. However, there were no particles in the respirable range (< 10 microns).

F. CONCLUSION

Rats exposed by inhalation to 960 mg/m^3 Tinuvin® 770 for 4 hours showed clinical signs of lacrimation and salivation. There were no deaths. The acute LC_{50} is > 960 mg/m^3.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to 960 mg/m^3 Tinuvin® 770 for 4 hours. Animals were observed for a period of 14 days. Inhalation exposures did not result in any deaths. The acute LC_{50} is > 960 mg/m^3. Clinical signs during exposure included lacrimation and salivation. The average 2-week body weight gains (86 g for males; 42 g for females) were reported to be within the normal limits.

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE VAPOR INHALATION TOXICITY STUDY WITH TINUVIN 770 (b) (4) (b) (4) IN ALBINO RATS

A. TITLE

Acute Vapor Inhalation Toxicity Study with Tinuvin 770 (b) (4)). (b) (4) in Albino Rats; Report No: IT (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® vapor in the rat.

4. **Test Article:** Tinuvin®770 (b) (4) The reviewer notes that the designation (b) (4) was not discussed in the chemistry section of the petition. The batch number was reported as (b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

   Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stainless steel cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: The author reported the concentration as "312, 320 mg/m ^{3} (nominal concentration)". The reported concentration level is unclear and possibly represents two separate concentrations or a range (e.g., 369 to 440 mg/m ^{3}); however, the study contains no specific statement to this effect or does not provide any reason why the concentration would be represented as two concentrations or a range of values. There were no controls.

Number of Animals per Sex per Group: 5

Test Material Vapor Generation and Analysis:

The test material was administered as a vapor. The vapor was generated by passing a stream of clean, dry air over the undiluted test material. The test material was heated to 300 °C. The air-vapor mixture was introduced into the exposure chamber. The vapor generator was of all-glass construction. The average nominal vapor concentration was calculated by dividing the generator weight loss by the total volume of air used during the test.

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after 99 % of the maximum concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 27 °C, air flow at 4.33 l/min. and atmospheric pressure of 30.20 inches Hg.

Length of Exposure: 30 minutes.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs.

Body Weight: The study protocol stated that body weights will be determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. However, since all rats died during the exposure period, body weights following exposure were not recorded. Body weight data for the animals prior to inhalation exposure were not provided in the study.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. The exposure concentration was not clearly identified and might represent two values or a range of values.

c. There were no GLP compliance or Quality Assurance statements.

d. A rationale for dose selection was not provided. It cannot be assessed if the concentration is a "limit" concentration. However, the concentration used in the study was high.

e. The age of the animals was not specified.

f. There were no individual animal data.

g. There were no control animals.

h. Initial body weights were not reported.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Clinical signs including ptosis, enophthalmus, ruffed fur, hypoactivity, and dyspnea were observed in all the test animals during the exposure. The time of onset of these signs after the initiation of exposure was 5 minutes. The duration of the symptoms was 25 minutes.

2. **Mortality Rate:** All rats died within 30 minutes into the exposure.

3. **Body Weight Changes:** Not recorded.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The author stated there were no gross pathologic alterations.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

F. CONCLUSION

Rats exposed by inhalation to Tinuvin® 770 for 30 minutes showed clinical signs of ptosis, enophthalmus, ruffed fur, hypoactivity, and dyspnea. All rats died within 30 minutes of the exposure. The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to an undetermined high level of Tinuvin® 770 for 30 minutes. Inhalation exposures resulted in the deaths of all rats. Clinical signs during exposure included ptosis, enophthalmus, ruffed fur, hypoactivity, and dyspnea.

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE DUST INHALATION TOXICITY STUDY WITH TINUVIN 770 (b) (4) (b) (4) IN ALBINO RATS

A. TITLE

Acute Dust Inhalation Toxicity Study with Tinuvin 770 (b) (4) (b) (4) in Albino Rats; Report No: IT (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® in the rat.

4. **Test Article:** Tinuvin®770 (b) (4)). The reviewer notes that the designation (b) (4) was not discussed in the chemistry section of the petition. The batch number was reported as (b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stock cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: 780 mg/m ^{3} (analytical concentration). There were no controls.

Number of Animals per Sex per Group: 5

Test Material Dust Generation and Analysis:

The report stated that dust was suspended with a specially designed dust feeder. The test material powder was then passed through a high-velocity stream of clean, dry air. The air-jet velocity was adjusted to obtain the desired concentration of suspended dust. The test atmosphere was then introduced into the exposure chamber, dispersed by a baffle plate and exhausted a t the bottom of the chamber.

The report stated that the concentration of test material dust present in the exposure chamber was determined by sampling the test atmosphere in the breathing zone of the animals being exposed. The total weight of dust collected on a glass fiber filter was divided by the total volume of air drawn

through the filter during the sampling period. Air flow rate for sampling was regulated by a calibrated limiting orifice. The average analytical concentration of airborne dust was obtained by repeated air sampling. The analytical concentration was reported to be 780 mg/m^3.

Particle size distributions were performed. Particles were counted (using a light-field technique) with respect to 4 size ranges, viz., 5 microns or smaller, 6 to 10 microns, 11 to 25 microns and larger than 25 microns. Details on the light-field technique were not provided, however, the author stated that the smallest particle size that could be detected with this technique is 1 micron. The results of particle size distribution analyses are summarized below in Table 1.

Table 1. Particle Size Distribution Data.

Particle Size Range (Microns)	Percent of Total Counted
1-5	<1
6-10	6
11-25	29
>25	65

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after the desired dust concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 25 °C, air flow at 45 l/min. and atmospheric pressure of 30.17 inches Hg.

Length of Exposure: 4 hours. The observation period was 14 days.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs. The author did not indicate how often animals were observed for signs during the 14-day observation period.

Body Weight: The author stated that body weights were determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. The data were recorded in terms of body weight gains.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. There were no GLP compliance or Quality Assurance statements.

c. A rationale for dose selection was not provided.

d. The age of the animals was not specified.

e. The incidence of clinical changes by sex was not provided. There were no individual animal data.

f. There were no control animals.

g. Details on the method used for particle size determinations were not provided.

In addition, there was only a small amount (<7%) of the particles were in the respirable range (< 10 microns). The reviewer notes that in a separate subacute inhalation study on Tinuvin 770 (entitled Subchronic Dust Inhalation Toxicity Study with TINUVIN®770 (b) (4) in Albino Rats; Report No: IT (b) (4) the test material dust was generated and ground to respirable size (90% < 10 microns) by using blenders and settling cylinders).

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Salivation, hyperactivity, ruffed fur, lacrimation and hypoactivity were observed in all the test animals during the exposure. The author tabulated the number of animals affected (summarized in Table 2 below) but did not identify the sex of the animals affected. There were no individual animal data in the study.

Table 2. Clinical signs in rats exposed to Tinuvin 770[a].

Clinical Sign	Number of Animals Affected	Time of Onset after Start of Exposure (min)	Duration (min)
Salivation	10	10	230
Hyperactivity	10	10	60
Ruffed fur	10	10	230
Lacrimation	1	15	225
Lacrimation	9	25	215
Hypoactivity	10	70	170

2. **Mortality Rate:** No deaths were observed.

3. **Body Weight Changes:** Body weight data were not provided in the study. The author stated that the average 2-week body weight gains (80 g for males; 34 g for females) were within the normal limits.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The author stated there were no gross pathologic alterations.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

Based on the results, the acute LC_{50} is > 780 mg/m^3. However, there was only a small amount (< 7%) of the particles that was in the respirable range (< 10 microns).

F. CONCLUSION

Rats exposed by inhalation to 780 mg/m^3 Tinuvin® 770 for 4 hours showed clinical signs of salivation, hyperactivity, ruffed fur, lacrimation and hypoactivity . There were no deaths. The acute LC_{50} is > 780 mg/m^3.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to 780 mg/m^3 Tinuvin® 770 for 4 hours. Animals were observed for a period of 14 days. Inhalation exposures did not result in any deaths. The acute LC_{50} is > 780 mg/m^3. Clinical signs during exposure included salivation, hyperactivity, ruffed fur, lacrimation and hypoactivity . The average 2-week body weight gains were reported to be within the normal limits.

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE VAPOR INHALATION TOXICITY STUDY WITH TINUVIN 770 ([b) (4)] [b) (4) IN ALBINO RATS

A. TITLE

Acute Vapor Inhalation Toxicity Study with Tinuvin 770 ([b) (4)], [b) (4) in Albino Rats; Report No: IT [b) (4)

B. STUDY CITATION

[b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® vapor in the rat.

4. **Test Article:** Tinuvin®770 [b) (4)). The reviewer notes that the designation [b) (4) was not discussed in the chemistry section of the petition. The batch number was reported as [b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

   Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stainless steel cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: The author reported the concentration as "192, 130 mg/m ^{3} (nominal concentration)". The reported concentration level is unclear and possibly represents two separate concentrations or a range (e.g., 130 to 192 mg/m ^{3}); however, the study contains no specific statement to this effect or does not provide any reason why the concentration would be represented as two concentrations or a range of values. There were no controls.

Number of Animals per Sex per Group: 5

Test Material Vapor Generation and Analysis:

The test material was administered as a vapor. The vapor was generated by passing a stream of clean, dry air over the undiluted test material. The test material was heated to 300 °C. The air-vapor mixture was introduced into the exposure chamber. The vapor generator was of all-glass construction. The average nominal vapor concentration was calculated by dividing the generator weight loss by the total volume of air used during the test.

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after 99 % of the maximum concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 28 °C, air flow at 4.45 l/min. and atmospheric pressure of 30.20 inches Hg.

Length of Exposure: 30 minutes.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs.

Body Weight: The study protocol stated that body weights will be determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. However, since all rats died during the exposure period, body weights following exposure were not recorded. Body weight data for the animals prior to inhalation exposure were not provided in the study.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. The exposure concentration was not clearly identified and might represent two values or a range of values.

c. There were no GLP compliance or Quality Assurance statements.

d. A rationale for dose selection was not provided. It cannot be assessed if the concentration is a "limit" concentration. However, the concentration used in the study was high.

e. The age of the animals was not specified.

f. There were no individual animal data.

g. There were no control animals.

h. Initial body weights were not reported.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Clinical signs including ptosis, enophthalmus, ruffed fur, lacrimation, salivation, hypoactivity, and dyspnea were observed in all the test animals during the exposure. The time of onset of these signs after the initiation of exposure was between 2 to 10 minutes. The duration of the symptoms was between 20 to 28 minutes.

2. **Mortality Rate:** All rats died within 30 minutes into the exposure.

3. **Body Weight Changes:** Not recorded.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The study pathologist stated that at necropsy, the lungs of the rats that died during the study failed to collapse when the thorax was opened, and the urinary bladders were distended with urine, without any evidence of obstruction to the urethra.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

F. CONCLUSION

Rats exposed by inhalation to Tinuvin® 770 for 30 minutes showed clinical signs of ptosis, enophthalmus, ruffed fur, lacrimation, salivation hypoactivity, and dyspnea . All rats died within 30 minutes of the exposure. The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to an undetermined high level of Tinuvin® 770 for 30 minutes. Inhalation exposures resulted in the deaths of all rats. Clinical signs during exposure included ptosis, enophthalmus, ruffed fur, lacrimation, salivation , hypoactivity, and dyspnea .

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE DUST INHALATION TOXICITY STUDY WITH TINUVIN 770 ((b) (4) (b) (4) IN ALBINO RATS

A. TITLE

Acute Dust Inhalation Toxicity Study with TINUVIN 770 ((b) (4) (b) (4) in Albino Rats; Report No: IT (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® in the rat.

4. **Test Article:** Tinuvin®770 (b) (4) . The reviewer notes that the designation (b) (4) was not discussed in the chemistry section of the petition. The batch number was reported as (b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

   Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stock cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: 780 mg/m ^{3} (analytical concentration). There were no controls.

Number of Animals per Sex per Group: 5

Test Material Dust Generation and Analysis:

The report stated that dust was suspended with a specially designed dust feeder. The test material powder was then passed through a high-velocity stream of clean, dry air. The air-jet velocity was adjusted to obtain the desired concentration of suspended dust. The test atmosphere was then introduced into the exposure chamber, dispersed by a baffle plate and exhausted a t the bottom of the chamber.

The report stated that the concentration of test material dust present in the exposure chamber was determined by sampling the test atmosphere in the breathing zone of the animals being exposed. The total weight of dust collected on a glass fiber filter was divided by the total volume of air drawn through the filter during the sampling period. Air flow rate for sampling was regulated by a calibrated limiting orifice. The average analytical

concentration of airborne dust was obtained by repeated air sampling. The analytical concentration was reported to be 780 mg/m^3.

Particle size distributions were performed. Particles were counted (using a light-field technique) with respect to 4 size ranges, viz., 5 microns or smaller, 6 to 10 microns, 11 to 25 microns and larger than 25 microns. Details on the light-field technique were not provided, however, the author stated that the smallest particle size that could be detected with this technique is 1 micron. The results of particle size distribution analyses are summarized below in Table 1.

Table 1. Particle Size Distribution Data.

Particle Size Range (Microns)	Percent of Total Counted
1-5	0
6-10	3
11-25	11
>25	86

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after the desired dust concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 25 °C, air flow at 55 l/min. and atmospheric pressure of 30.18 inches Hg.

Length of Exposure: 4 hours. The observation period was 14 days.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs. The author did not indicate how often animals were observed for signs during the 14-day observation period.

Body Weight: The author stated that body weights were determined for

each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. The data were recorded in terms of body weight gains.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. There were no GLP compliance or Quality Assurance statements.

c. A rationale for dose selection was not provided.

d. The age of the animals was not specified.

e. The incidence of clinical changes by sex was not provided. There were no individual animal data.

f. There were no control animals.

In addition, there was only a small amount (3 %) of the particles were in the respirable range (< 10 microns). The reviewer notes that in a separate subacute inhalation study on Tinuvin 770 (entitled Subchronic Dust Inhalation Toxicity Study with TINUVIN®770 (b) (4)) in Albino Rats; Report No: IT (b) (4) the test material dust was generated and ground to respirable size (90% < 10 microns) by using blenders and settling cylinders).

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Hyperactivity and salivation were observed in nearly all the test animals during the exposure. The author tabulated the number of animals affected (summarized in Table 2 below) but did not identify the sex of the animals affected. There were no individual animal data in the study.

Table 2. Clinical signs in rats exposed to Tinuvin 770[a].

Clinical Sign	Number of Animals Affected	Time of Onset after Start of Exposure (min)	Duration (min)
Hyperactivity	10	15	225
Salivation	1	15	225
Salivation	9	30	210

2. **Mortality Rate:** No deaths were observed.

3. **Body Weight Changes:** Body weight data were not provided in the study. The author stated that the average 2-week body weight gains (88 g for males; 34 g for females) were within the normal limits.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The author stated there were no gross pathologic alterations.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

Based on the results, the acute LC_{50} is > 780 mg/m^3. However, there was only a small amount (3 %) of the particles that was in the respirable range (< 10 microns).

F. CONCLUSION

Rats exposed by inhalation to 780 mg/m^3 Tinuvin® 770 for 4 hours showed clinical signs of salivation and hyperactivity. There were no deaths. The acute LC_{50} is > 780 mg/m^3.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to 780 mg/m^3 Tinuvin® 770 for 4 hours. Animals were observed for a period of 14 days. Inhalation exposures did not result in any deaths. The acute LC_{50} is > 780 mg/m^3. Clinical signs during exposure included salivation and hyperactivity . The average 2-week body weight gains were reported to be within the normal limits.

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE VAPOR INHALATION TOXICITY STUDY WITH TINUVIN 770 (b) (4) (b) (4) IN ALBINO RATS

A. TITLE

Acute Vapor Inhalation Toxicity Study with TINUVIN 770 (b) (4) (b) (4) in Albino Rats; Report No: IT (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential acute inhalation toxicity of Tinuvin® vapor in the rat.

4. **Test Article:** Tinuvin®770 (b) (4). The reviewer notes that the designation (b) (4) was not discussed in the chemistry section of the petition. The batch number was reported as (b) (4) No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

   Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (Charles River)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported. The report stated that the animals were "young adult."

Weight at Day 1 of the Study: Not reported.

Quarantine: at least 5 days

Animal Maintenance: The report stated that all animals were housed individually in stainless steel cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: The author reported the concentration as "192, 130 mg/m ^{3} (nominal concentration)". The reported concentration level is unclear and possibly represents two separate concentrations or a range (e.g., 130 to 192 mg/m ^{3}); however, the study contains no specific statement to this effect or does not provide any reason why the concentration would be represented as two concentrations or a range of values. 369,440 mg/m ^{3} (nominal concentration). There were no controls.

Number of Animals per Sex per Group: 5

Test Material Vapor Generation and Analysis:

The test material was administered as a vapor. The vapor was generated by passing a stream of clean, dry air over the undiluted test material. The test material was heated to 300 °C. The air-vapor mixture was introduced into the exposure chamber. The vapor generator was of all-glass construction. The average nominal vapor concentration was calculated by dividing the generator weight loss by the total volume of air used during the test. The nominal concentration was reported as 369,440 mg/m^3.

Exposure Chambers:

The author stated that animals were exposed in a specially designed exposure chamber. The chamber was said to be designed so that the animals could be introduced into the test atmosphere after 99 % of the maximum concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. The chamber size was 80 liters. Chamber conditions included temperature of 28 °C, air flow at 4.56 l/min. and atmospheric pressure of 30.18 inches Hg.

Length of Exposure: 30 minutes.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed during the exposure period for mortality and clinical signs.

Body Weight: The study protocol stated that body weights will be determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. However, since all rats died during the exposure period, body weights following exposure were not recorded. Body weight data for the animals prior to inhalation exposure were not provided in the study.

Food Consumption: Not recorded.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: Not performed.

Blood Chemistry: Not performed.

Urinalysis: Not performed.

f. Gross Pathology

Necropsy: Necropsy examinations were performed on all animals at the end of the study. No details were provided.

Organ Weights: Not recorded.

Histopathology Examination: Not recorded.

h. Statistical Evaluation

LC_{50} was calculated by the method of Litchfield and Wilcoxon.

j. Critical Appraisal of the Protocol

There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. The exposure concentration was not clearly identified and might represent two values or a range of values.

c. There were no GLP compliance or Quality Assurance statements.

d. A rationale for dose selection was not provided. It cannot be assessed if the concentration is a "limit" concentration. However, the concentration used in the study was high.

e. The age of the animals was not specified.

f. There were no individual animal data.

g. There were no control animals.

h. Initial body weights were not reported.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Clinical signs including ptosis, enophthalmus, ruffed fur, lacrimation, hypoactivity, and dyspnea were observed in all the test animals during the exposure. The time of onset of these signs after the initiation of exposure was between 2 to 5 minutes. The duration of the symptoms was between 25 to 28 minutes.

2. **Mortality Rate:** All rats died within 30 minutes into the exposure.

3. **Body Weight Changes:** Not recorded.

5. **Food Consumption:** Not recorded.

6. **Clinical Laboratory Tests:** Not recorded.

7. **Organ Weights:** Not recorded.

8. **Gross Pathology:** The study author stated that there were no gross pathologic alterations.

9. **Histopathology**: Not performed.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol). These deficiencies and limitations limit the confidence in the study.

The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

F. CONCLUSION

Rats exposed by inhalation to 369,440 Tinuvin® 770 for 30 minutes showed clinical signs of ptosis, enophthalmus, ruffed fur, lacrimation, hypoactivity, and dyspnea . All rats died within 30 minutes of the exposure. The concentration of Tinuvin 770 in the exposure chamber was not clear and an acute LC_{50} cannot be identified.

G. EXECUTIVE SUMMARY

Rats (5 male and 5 female) were exposed by inhalation to an undetermined high level of Tinuvin® 770 for 30 minutes. Inhalation exposures resulted in the deaths of all rats. Clinical signs during exposure included ptosis, enophthalmus, ruffed fur, lacrimation, hypoactivity, and dyspnea .

H. DATA VALIDATION CHECK

All summary data were inspected. There were no individual animal data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

ACUTE SKIN IRRITATION STUDY

A. TITLE: Acute Skin Irritation Study in the Rabbit

Report No. (b) (4)

Dated: August 25, 1982

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP statement was provided.

2. Quality Assurance Statement: No quality assurance statement was provided. There is a signed statement that "the report presents the results of the laboratory investigation as compiled by the undersigned" (page 000062).

3. Study Objective: To determine the acute skin irritating potency of (b) (4) in the rabbit.

4. Test Article: The test substance was (b) (4), a stable solid. The batch numbers were (b) (4) The test article was received on July 30, 1982 and the study was begun on August 9, 1982. The content of the active ingredient was listed as "not determined".

a. Purity: The purity of the compound was not stated.

b. Stability: The compound was listed as stable.

5. Protocol

a. Animal Diet

Type: NAFAG No. 814 Tox rabbit food and water were provided *ad libitum*.

Source: The source of the diet was NAFAG AG, Gossau SG (Switzerland).

b. Test Animals

Species and Strain: New Zealand White rabbits

Source: Kleintierfarm Madoerin AG, CH-4414 Fuellinsdorf

Age at Initiation of the Study: The age of the animals at the initiation of the study was not stated.

Weight at Initiation of the Study: The rabbits weighed 2-3 kg at study initiation.

Quarantine: A quarantine period was not described.

Animal Maintenance: Animals were housed individually in metal cages. The temperature of the animal room was 22± 3 °C and the relative humidity was 55± 15%. There were 12 hours of light/day and approximately 15 air changes/hour.

c. Experimental Design

Dose Levels: Although the test substance was listed as a solid, the treatment was applied as a liquid, but the amount of solid used and the identification of the vehicle (and the volume used to moisten the solid) were not specified. Neither positive nor vehicle control animals were used.

Number of Animals/Sex/Group: 3 male and 3 female rabbits

Preparation of Test Article and Mode of Administration: Although the test substance was listed as a solid, the treatment was applied as a liquid, but the concentration was not specified. The entire back and flank of the rabbit was shaved with an electric clipper before treatment. Immediately before treatment, the shaved skin on one side was "scarified" slightly using a "Schroepfschnaepper". Gauze pads (2.5 cm^2) were soaked with 0.5 ml of the test material and applied to the abraded and nonabraded skin. The patches were covered with an impermeable material and fastened to the body with adhesive tape. Dressings were removed after 24 hours and the skin was evaluated immediately and during a subsequent observation period of 7 days.

d. Clinical Observations

Dermal Irritation: Skin was evaluated 24 hours after application and during a subsequent observation period of 7 days.

Gross Signs of Toxicity: There was no description of other observations (clinical signs, etc.) provided.

Body Weights: Body weights were not measured.

e. Pathology

Necropsy: The report does not indicate that necropsies were performed.

Histopathological Examination: Histopathological examinations were not performed.

f. Statistical Evaluation

The report does not indicate that statistical evaluations were performed.

g. Critical Evaluation of the Protocol

No controls were used in the study. There was no description of the concentration of the test article solution or the vehicle that was used for its preparation. Only 1 concentration was tested and no controls were used. There were only 3 animals/sex used. GLP and quality assurance statements were lacking.

D. IN-DEPTH EXAMINATION OF RESULTS

1. Clinical Observations: The mean scores read after 24 and 72 hours for erythema and edema (intact and abraded skin) were summed and divided by 4, as shown in Table 1 below, to give the Primary Irritation Index. The Primary Irritation Index was 0.55, indicating minimal irritation. The maximum reaction score was obtained at 24 hours with 6/6 animals showing some reaction. Evaluation of the skin at 72 hours found only 1/6 animals with irritation. No necrosis, scab formation, or extended erythema were found.

Table 1. Mean Reaction Scores for Intact and Abraded Skin 24 and 72 Hours after Application of (b) (4)

Time after Exposure (hrs)	Erythema-Intact	Erythema-Abraded	Edema-Intact	Erythema-Abraded
24	1	0.5	0.3	0.2
72	0.2	0	0	0
Total	1.2	0.5	0.3	0.2

2. Mortality: No deaths occurred in the study.

3. Body Weight Changes: Body weights were not measured.

4. Pathology: Necropsies and histopathological examinations were not performed.

E. ASSESSMENT

There were several reporting deficiencies of this dermal irritation study. These included a lack of GLP and Quality Assurance Statements. The actual amount of test material used was not specified. In addition, the vehicle was not identified, and the volume of the vehicle used to moisten the solid was not specified. No information was provided on the purity of the test material. Also, the study did not include positive and negative controls.

Based on the results, the test material caused a minimal irritation when applied to intact and abraded rabbit skin. It is not clear why the author did not include the results of the 48-hour evaluation (page 000069) in the calculation of the primary irritation index. The deficiencies and limitations of the study limit the confidence in the study.

F. CONCLUSIONS

The study author concluded that (b) (4) causes minimal skin irritation to rabbit skin.

However, there was no specification of the concentration of the test article solution or the vehicle that was used for its preparation, and no positive and negative controls were used. Also, there was no Quality Assurance and GLP Statements and no information on the purity of the test material. The reviewer believes that those deficiencies and limitations limit the confidence in the study.

G. DATA VALIDATION CHECK

Data on individual animals were presented and validated.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patte Bittner, M.S. Primary Reviewer — Date: 8/21/98

John Liccione, Ph.D. Secondary Reviewer — Date: 8/21/98

ACUTE EYE IRRITATION STUDY

A. TITLE: Acute Eye Irritation Study in the Rabbit

Report No. (b) (4)

Dated: August 10, 1982

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP statement was provided.

2. Quality Assurance Statement: No standard quality assurance statement was provided. However, there is a signed statement that "the report presents the results of the laboratory investigation as compiled by the undersigned" (page 000073).

3. Study Objective: The objective of the study was to determine the acute eye irritating potency of (b) (4) in the rabbit.

4. Test Article: The test substance was (b) (4), a stable solid. The batch number was (b) (4) The test article was received on June 1, 1982 and the study was begun on July 12, 1982. The content of the active ingredient was listed as "not determined".

a. Purity: The purity of the compound was not stated.

b. Stability: The compound was listed as stable.

5. Protocol

a. Animal Diet

Type: NAFAG No. 814 Tox rabbit food and water were provided *ad libitum*.

Source: The source of the diet was NAFAG AG, Gossau SG (Switzerland).

b. Test Animals

Species and Strain: New Zealand White rabbits. The report stated that animals were checked for normal ophthalmic conditions.

Source: Kleintierfarm Madoerin AG, CH-4414 Fuellinsdorf

Age at Initiation of the Study: The age of the animals at the initiation of the study was not stated.

Weight at Initiation of the Study: The rabbits weighed 2-3 kg at study initiation.

Quarantine: The study noted that there was a 4-day adaptation period, but no further details were described.

Animal Maintenance: Animals were housed individually in metal cages. The animal room was air conditioned with temperatures of 22± 3 °C and relative humidity of 55± 15%. There were 12 hours of light/day and there were approximately 15 air changes/hour.

c. Experimental Design

Dose Levels: The test material in an amount of 0.1 ml was used in the study.

Number of Animals/Sex/Group: 3 male and 3 female rabbits

Preparation of Test Article and Mode of Administration: The test material 0.1 ml in an amount of 0.1 ml was inserted into the conjunctival sac of the left eye and the lids were gently closed for 15 seconds. The right eye served as the untreated control. In 3/6 rabbits, the treated eye was flushed with 10 ml of sterile physiological saline about 30 seconds after the treatment.

d. Clinical Observations

Ocular Irritation: Eye irritation was assessed with a slit lamp at 24, 48, 72, and 4 and 7 days after treatment.

Gross Signs of Toxicity: There was no description of other observations (clinical signs, etc.).

Body Weights: Body weights were not measured.

e. Pathology

Necropsy: The report does not indicate that necropsies were performed.

Histopathological Examination: Histopathological examinations were not performed.

f. Statistical Evaluation

The report does not indicate that statistical evaluations were performed. Overall, the conduct of the study was acceptable for a primary eye irritation study. However, there was no information on the purity of the test material, and there were no GLP or Quality Assurance Statements.

g. Critical Appraisal of the Protocol

There was no description of the concentration of the test article solution or the vehicle that was used for its preparation. Only 1 concentration was used. There were only 3 animals/sex used. GLP and quality assurance statements were lacking.

D. IN-DEPTH EXAMINATION OF RESULTS

1. **Clinical Observations:** The mean reaction scores for cornea, iris, and conjunctiva observed after 1, 2, 3, 4, and 7 days were summed and the sum divided by 5 to give the Primary Eye Irritation Index. The Primary Eye Irritation Index in rinsed eyes was 17.4, indicating slight irritation; the Primary Eye Irritation Index in unrinsed eyes was 41.5, indicating moderate irritation. The cornea, iris, and conjunctiva were all involved. Individual animal data were provided.

2. **Mortality:** No deaths occurred in the study.

3. **Body Weight Changes:** Body weights were not measured.

4. **Pathology:** Necropsies and histopathological examinations were not performed.

E. ASSESSMENT

Table 2 of the study (p. 000081) has incorrectly reported the total score for rinsed eyes. The sum of the iris (13.2), cornea (41.7), and conjunctiva (23.3) scores is listed as 87.2, but is actually 78.2. The Primary Irritation Index, based on this number, is listed as 17.4 and should be 15.6. These errors translated into a less serious reaction (but still classifiable as slight irritation) and do not affect the assessment of the study results. Overall, the conduct of the study was reasonable for a primary eye irritation study. This study does have

reporting deficiencies including no GLP or quality assessment statements and no information on purity.

F. CONCLUSIONS

(b) (4) causes moderate irritation when applied to rabbit eye mucosa.

G. DATA VALIDATION CHECK

Data on individual animals were presented and validated.

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Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patte Bittner, M.S. Primary Reviewer — Date 8/21/98

John Liccione, Ph.D. Secondary Reviewer — Date 8/21/98

SKIN SENSITIZATION STUDY

A. TITLE: Report on Skin Sensitization (contact allergenic) effect in guinea pigs (b) (4) Maximization Test.

Study Test No: (b) (4)

Dated: January 12, 1984

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: A GLP Statement, signed by Th. Maurer and dated January 12, 1984, was provided.

2. Quality Assurance Statement: A quality assurance page listing the dates of the quality assurance inspections, was signed and dated January 9, 1984.

3. Study Objective: To determine the sensitizing potential of (b) (4) in the guinea pig.

4. Test Article: The test substance was (b) (4), a stable white powder. The batch number was (b) (4) The test article was received on July 18, 1983 and the study was begun on November 2, 1983.

a. Purity: The purity of the compound was 99.7%.

b. Stability: The compound was listed as stable.

5. Protocol

a. Animal Diet

Type: NAFAG No. 846 standard guinea pig food pellets and water were

provided *ad libitum*. The diet was supplemented with fresh carrots.

Source: The source of the diet was NAFAG AG, Gossau SG (Switzerland).

b. Test Animals

Species and Strain: Pirbright White strain (Tif:DHP) guinea pigs (male and female)

Source: The animals were bred on the premises of the test facility.

Age at Initiation of the Study: The animals were about 10 weeks old at the initiation of the study.

Weight at Initiation of the Study: The guinea pigs weighed between 332 and 478 g at study initiation.

Quarantine: Animals were acclimated for 18 days before study initiation.

Animal Maintenance: Animals were housed individually in Macrolon cages (type 3). The temperature of the animal room was 21± 2°C and the relative humidity was 50± 10%. There were 14 hours of light/day. The sensitivity of the strain is controlled every 6 months with p-phenylenediamine.

c. Experimental Design

Dose Levels: 1% (for intradermal application)
10% (for epidermal application--induction)
1% (for epidermal application--challenge)

Number of Animals/Sex/Group: 10 male and 10 female guinea pigs

Preparation of Test Article and Sensitization Procedure: For induction via intradermal injection, two intradermal injections (0.1 ml per injection) were made into the neck of the guinea pigs with a mixture of adjuvant and saline, with 1% test compound in sesame oil and with 1% test compound in adjuvant saline mixture. One week later, animals were induced via epidermal application by applying the test material (10%, about 4 g per patch) mixed in vaseline to a filter paper patch and applying it to the neck of the animals. This occlusive administration lasted 48 hours.

Two weeks following the epidermal induction application, the animals were challenged by applying to the flank 1% test material in vaseline and in the vehicle alone (24 hours occlusive application). After removing the dressing 24

hours later, the challenge reactions were graded according to the Draize scoring scale. Fur was removed chemically from the application sites 3 hours before examination using the commercial product Veet® for 5 minutes. The sites were again scored 48 hours after removal of the dressings. The control animals were treated with adjuvant and the vehicle during the induction period. During the challenge period, the controls were treated with the vehicle as well as with the test compound in order to control the maximal subirritant concentration of the test compound in adjuvant treated animals.

d. Clinical Observations

Dermal Sensitization: Guinea pig skin was evaluated for erythema and edema at 24 hours and 48 hours after the challenge, according to the methods of Magnusson and Kligman (1969, 1980).

Gross Signs of Toxicity: There was no description of other observations (clinical signs, etc.) provided.

Body Weights: Body weights were measured at the beginning and at the end of the test period.

e. Pathology

Necropsy: The report does not indicate that necropsies were performed.

Histopathological Examination: Histopathological examinations were not performed.

f. Statistical Evaluation

The report does not indicate that statistical evaluations were performed.

g. Critical Evaluation of the Protocol

The conduct of this dermal sensitization study was acceptable. However, the author did not state whether the hair of the animals was clipped from the neck region during the induction stage. Adequate numbers of animals were used and an adequate description of procedures was provided. But, the author did not provide a rationale for the selection of the dose level used for the induction and challenge. It is not certain if the dose level was based on the results of a pretest.

D. IN-DEPTH EXAMINATION OF RESULTS

1. Clinical Observations: At both 24 and 48 hours after the epicutaneous occlusive (48 hours) administration, none of the animals showed any reaction. Similarly, no animals showed a dermal reaction (erythema or edema) 24 or 48 hours after removal of the dressings in the challenge.

2. Mortality: No deaths occurred in the study.

3. Body Weight Changes: Body weights were recorded at the beginning and end of the study. Although statistical analyses were not performed on the data, it appears that animals in the control and test groups gained weight in a similar manner.

4. Pathology: Necropsies and histopathological examinations were not performed.

E. ASSESSMENT

The study author concluded that (b) (4) at a concentration of 1% in Vaseline, did not induce edema or erythema reactions after dermal challenge and can be classified, therefore, in the lowest sensitization class according to Magnusson and Kligman (1969, 1980). No reactions were observed in either control or test animals.

F. CONCLUSIONS

The test material, (b) (4) (1% in Vaseline) did not induce erythema or edema in guinea pigs in an epidermal challenge after intradermal and epidermal induction. Based on these data, (b) (4) did not cause skin sensitization in guinea pigs at this concentration.

G. DATA VALIDATION CHECK

Data on individual animals were presented and validated.

H. REFERENCES

Magnusson B., Kligman J. 1969. Invest. Dermatol. 52: 268-276.

Magnusson B. 1980. Identification of contact sensitizers by animal assay. Cont. Dermatitis 6: 46-50.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patte Bittner, M.S. Primary Reviewer — Date 8/21/98

John Liccione, Ph.D. Secondary Reviewer — Date 8/21/98

HUMAN REPEATED INSULT PATCH TEST WITH FIVE EXPERIMENTAL SAMPLES

TITLE: Human Repeated Insult Patch Test with Five Experimental Samples; Report No. IT (b) (4)

AUTHORS/LABORATORY: The report was prepared by Pamela M. Wenzel. The clinical investigator was Samuel solomon, M.D. (Consultant Dermatologist). Pages 000097 to 000120 of the petition (b) (4) There was no formal Quality Assurance or GLP compliance statement.

OBJECTIVE: The study was conducted to assess the potential dermal irritative and sensitizing effects of the test material.

TEST MATERIAL: Five experimental samples of the test material, described below, were studied.

Polypropylene (1% Tinuvin 770)
Polypropylene (0% Tinuvin 770)
Fabric (b) (4) (Polypropylene knitted fiber treated with 0.25% Tinuvin (b) (4) and 0.1% IRGANOX)
Fabric (b) (4) (Polypropylene knitted fiber treated with 0.50% Tinuvin (b) (4) and 0.1% IRGANOX)
Tinuvin 770 (b) (4)

The first four experimental samples listed above were used as received. Tinuvin 770 was evaluated as a 0.5% dilution (w/v) in corn oil.

The purity of the test materials was not specified.

PROCEDURES

PATCH TEST METHOD

A human repeated insult patch test utilizing 3 panels of 50 human subjects each was conducted with the 5 experimental samples. The characteristics of the test population were described in Tables 1, II, and III of the study (pages 000103-000105). The test population included males and females (age range: 18-66 years). The test population consisted of one hundred forty-nine Caucasians and one black.

A series of nine induction patches were placed on each of the subjects. The author did not specify the site of the body to which the patches were placed. The application schedule

was designed so that a patch of each test material was applied on Monday, Wednesday and Thursday and allowed to contact the skin for 24 hours, after which time it was removed and the skin site graded for irritation (see irritation score described in Table 1 below). Thursday's patch was placed immediately after removal and grading of Wednesday's application. Following the placement of the ninth induction patch , a nonpatching period of 12 days was allowed before the challenge patch was applied. For this 24-hour patch, a new skin site was used (adjacent to the induction site). This patch was observed 24, 48 and 72 hours following application.

Dermal applications of each test material were made using Readi-Band clear plastic patches (1-1/2" x 1-1/2" square) with nonwoven Webril centers. Test materials Polypropylene (1% Tinuvin 770), Polypropylene (0% Tinuvin 770), Fabric(b) (4) and Fabric(b) (4) were cut into ½" x ½" squares and placed on a patch premoistened with tap water just prior to application. Tinuvin 770 was patched at a concentration of 0.55 IN CORN OIL. Approximately 0.05 ml of the test material was placed on the Webril pad just prior to application.

SCORING CRITERIA

The scoring criteria for skin irritation reactions is summarized in Table 1 below.

Table 1. Assessment of Reactions.

Description	Grading
Erythema and Eschar Formation	
No reaction	0
Very slight erythema (barely perceptible)	1
Mild, well-define erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation	4
Total possible erythema score	4
Edema Formation	
No reaction	0
Very slight edema (barely perceptible)	1
Slight edema (edge of area well-defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (area raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4

RESULTS

The results of the human repeated insult patch test were summarized in Tables IX through XII of the study (pages 000109 to 000120). In addition, a table depicting the number of reactors, the number of reactions/number of patches applied, and the number of reactions with scores of 0 through 8 was provided on page 000100 of the study. The author concluded that the results showed that test materials Polypropylene (1% Tinuvin 770), Polypropylene (0% Tinuvin 770), Fabric (b) (4) and Tinuvin 770 were extremely weak primary skin irritants under the test conditions, whereas Fabric (b) (4) was not irritating to the skin. The reviewer concurs. The author also concluded that there was no evidence of skin sensitization noted following the challenge application of all of the materials tested. The reviewer concurs.

ASSESSMENT

This conduct of the human patch test was adequate. The protocol was described in sufficient detail. However, the report did not identify the site on the body to which the material was applied. Also, the purity of the test materials was not specified. In addition, there were no positive controls. A sufficient number of human subjects were examined. The characteristics of the test population were described. Nearly all the subjects (with the exception of one individual who was black) were Caucasian; thus, the study results are limited in predicting the dermal irritative and sensitizing reactivity of Tinuvin 770 in non-Caucasian populations.

The observation that Polypropylene (0% Tinuvin 770) elicited an extremely weak dermal irritative effect indicates that Polypropylene itself may be a very weak dermal irritant; however, the reaction was observed in only one individual (subject no. 33). Fabric (b) (4) (Polypropylene knitted fiber treated with 0.25% Tinuvin (b) (4) and 0.1% IRGANOX) also elicited an extremely weak dermal irritative response in only one volunteer (subject no. 16).

CONCLUSIONS

Under the experimental conditions of the human patch test, none of the test materials resulted in dermal sensitization in any of the human subjects to any of the materials tested. Polypropylene (1% Tinuvin 770), Polypropylene (0% Tinuvin 770), Fabric (b) (4) (Polypropylene knitted fiber treated with 0.25% Tinuvin (b) (4) and 0.1% IRGANOX), and Tinuvin 770 (0.5% in corn oil) can be considered extremely weak primary skin irritants. Fabric (b) (4) (Polypropylene knitted fiber treated with 0.50% Tinuvin (b) (4)) and 0.1% IRGANOX)showed no irritating reactions in any of the subjects.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

SKIN PHOTOALLERGENICITY STUDY

A. TITLE: Skin Photoallergenicity Study in the Guinea Pig of (b) (4)

Study Test No: (b) (4)

Dated: June 13, 1980

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP Statement was provided.

2. Quality Assurance Statement: No Quality Assurance Statement was provided.

3. Study Objective: To determine the photoallergenic potential of (b) (4) in the guinea pig.

4. Test Article: The test substance was (b) (4) a white powder. The identification number was EN EP 102. The test article was received on February 21, 1980 and the study was begun on April 14, 1980.

a. Purity: The purity of the compound was not provided.

b. Stability: The stability of the compound was not provided.

5. Protocol

a. Animal Diet

Type: NAFAG No. 830 standard guinea pig food pellets and water were provided *ad libitum*. The diet was supplemented with fresh carrots.

Source: The source of the diet was NAFAG, Gossau SG.

b. Test Animals

Species and Strain: Pirbright white strain (albino) guinea pigs were used.

Source: The animals were bred on the premises of the test facility.

Age at Initiation of the Study: The animals were about 10 weeks old at the initiation of the study.

Weight at Initiation of the Study: The guinea pigs weighed between 280 and 380 g at study initiation.

Quarantine: Animals were acclimated for 7 days before study initiation.

Animal Maintenance: Animals were housed individually in Macrolon cages (type 3). The temperature of the animal room was 20± 1 °C and the relative humidity was 50± 5%. There were 14 hours of light/day.

c. Experimental Design

Dose Levels: The guinea pigs were treated 4 times/week for 3 weeks with 0.1% solution of (b) (4) in 80% DAE433 (0.1 ml per application). DAE433 was the vehicle and consists of 40% dimethylacetamide, 30% acetone, and 30% ethanol. Four adjuvant injections (0.1 ml suspension of the adjuvant complete Freund and physiological saline/injection) were made to the 4 corners of the application site as indicated below. In the two challenges, animals were treated for 3 days with the same solution that they received during induction. Negative control animals received vehicle alone.

Number of Animals/Sex/Group: 10 male and 10 female guinea pigs

Preparation of Test Article and Mode of Administration: Four days before the initiation of induction, the neck of each animal was shaven and chemically depilated with Veet ®. The remainder of the induction period, animals were shaved only (1/20 mm). During the induction phase, animals were treated 4 times/week for 3 weeks by applying a 0.1% solution of (b) (4) in 80% DAE433 on a 2 x 2 cm area of the shaved neck (0.1 ml was applied to this area). The area was left open.

One hour after application of the test material, the animals were immobilized and irradiated. A light-proof bandage was applied to the area

around the skin to be irradiated to protect it. During the first induction week, animals were irradiated for 10 minutes with UV-A or visible light and during the second and third induction weeks, for the same duration, but using UV-A, UV-B, or visible light. The light was generated using a special irradiation chamber with a water cooled 6000 W Xenon burner (Osram), with a distance of 80 cm between the burner and the animal. The maximal irradiation intensity used was:

UV-A (320-400 nm)	4.55 mW/cm^2
UV-B (280-320 nm)	785 $\mu W/cm^2$
visible (400-700 nm)	25.64 mW/cm^2.

Skin reactions were scored by the Draize Scoring Scale 24 hours after each of the first 4 induction applications (first week). Reactions were not scored during the second and third week of induction. Four adjuvant injections were administered to the 4 corners of the application site (2 injections/week during the second and third weeks of induction). Each injection consisted of a 0.1 ml suspension of adjuvant complete Freund and physiological saline.

The first challenge began twelve days after the last induction irradiation, when the backs of the animals were shaved and depilated with Veet ®. Four days after this treatment, the animals were again treated with the test solution or the vehicle for 3 days by epidermal open exposure in the same manner as during the induction period. One hour after each application, the animals received a 3-minute sub-erythematogenic dose of UV-A, UV-B, or visible light from a lamp filtered with a Schott filter WG-280, 3 mm. Twenty-four hours after the challenge, skin reactions were evaluated using the Draize Scale. The authors did not state whether the animals were immobilized in a special animal holder during the irradiation process of the challenge periods or whether the skin surrounding the irradiated area was protected as it was during the induction.

The animals were rested for 14 days and the test solution or vehicle was applied again to the animals back for 3 days, followed by a 10-minute irradiation period using either UV-A or visible light (lamp filtered with a Schott filter WG 320, 3 mm), in the same manner as the first challenge. Skin reactions were again scored using the Draize Scoring Scale after 24 hours. Positive effects were counted if animals had any score higher at the end of the two challenge periods than at the end of the first induction week. Positive animals in the test group was compared with the number of animals in the control group treated with the vehicle (DAE433) alone. Although there was mention of the control group being treated with the vehicle alone, there was no discussion of whether the animals were treated in the same manner as the test group.

d. Clinical Observations

Dermal Sensitization: Skin reactions were scored by the Draize Scoring Scale 24 hours after each of the first 4 induction applications (first week only). Twenty-four hours after each of the 2 challenges, skin reactions were again scored.

Gross Signs of Toxicity: There was no description of other observations (clinical signs, etc.) provided.

Body Weights: Body weights were not measured.

e. Pathology

Necropsy: The report does not indicate that necropsies were performed.

Histopathological Examination: Histopathological examinations were not performed.

f. Statistical Evaluation

Fisher's exact test was used to compare the number of positive animals in the test group with the positive number of animals in the control group that were treated with the vehicle alone.

g. Critical Evaluation of the Protocol

Overall, the design and conduct of this skin photoallergenicity study was reasonable. However, there no details on the control animals. For example, it is not clear if the controls used in the rechallenge actually were naive controls. It is also not clear if the controls were handled in the same fashion as the animals that were administered the test material. Also, there was no information on the purity and stability of the test material, and there were no Quality Assurance or GLP Statements. In addition, the author did not state the basis for the selection of one dose level (0.1 ml of a 0.1% solution).

D. IN-DEPTH EXAMINATION OF RESULTS

1. Clinical Observations: There was no statistically significant difference observed between the test and control groups. Only 1 animal in the test group and 1 animal in the control group reacted 24 hours after the first challenge. The severity of the response was greater in the test animal (well defined erythema-- score of 2) than in the control animal (very slight erythema-- score of 1) and the response was the same severity in the test animal at both challenges. The same animal in the test

group also reacted during the second challenge, but no control animals had a positive response during the second challenge.

2. **Mortality:** No deaths occurred in the study.

3. **Body Weight Changes:** Body weights were not recorded.

4. **Pathology:** Necropsies and histopathological examinations were not performed.

E. ASSESSMENT

The study author concluded that (b) (4), as a 0.1% solution, did not induce erythemic reactions after dermal challenge followed by UV-A or UV-B irradiation and, therefore, is not a photoallergen. There was no statistically significant difference observed between the test and control groups and although 1 animal in the test group and 1 animal in the control group reacted 24 hours after the first challenge, the severity of the response was greater in the test animal (well defined erythema-- score of 2) than in the control animal (very slight erythema-- score of 1). Furthermore, the response was the same severity in the test animal at both challenges. There are several important gaps in the data reported, however. For instance, there was only one concentration (0.1 ml of a 0.1% solution) level of the test material applied at induction and at challenge. The basis for the selection of this concentration was not provided. No information was provided on the purity, stability, or storage conditions of the test material or on the stability or storage conditions of the test solution prepared. No GLP or Quality Assurance Statements were provided. These deficiencies limit a thorough assessment of the study results.

F. CONCLUSIONS

The author concluded that (b) (4) did not cause skin photoallergenicity in albino guinea pigs when applied as a 0.1% solution in 80% DAE433. Although there was a reaction in one test animal and one control animal, the severity of the response was slightly greater in the test animal and was observed after both challenges, whereas it was only present after the first challenge in the control animal. However, because of deficiencies in the study that include lack of GLP and Quality Assurance Statements, the lack of a basis for the selection of only 1 concentration of test material at induction and challenge, and lack of data on the purity, stability, and storage conditions of the test material, the confidence in the study is limited.

G. DATA VALIDATION CHECK

Data on individual animals were presented and validated.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patricia A. Bittner 8/21/98

Patte Bittner, M.S. Primary Reviewer Date

John Liccione 8/21/98

John Liccione, Ph.D. Secondary Reviewer Date

SKIN PHOTOTOXICITY STUDY

A. TITLE: Skin Phototoxicity Study in the Mouse of (b) (4) Dated April 14, 1980
Archive No. (b) (4)

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. GLP Statement: No GLP Statement was provided.

2. Quality Assurance Statement: No Quality Assurance Statement was provided.

3. Study Objective: The study was conducted to examine the phototoxic potential of (b) (4) in hairless mice by examining its potential to cause erythema or edema when applied topically followed by UV radiation.

4. Test Article: (b) (4) The chemical name was provided on page 000122 of the study report. The identification number was EN EP 102. No description of either the physical appearance of the test substance or the storage conditions was provided. The test material was received on February 21, 1980 and the study was initiated on April 14, 1980.

a. Purity: The purity of the test substance was not stated. The study authors did not state the identity, stability, or composition of the test article.

b. Stability: The stability was not reported.

5. Protocol

a. Animal Diet

Type: The animals received standard mouse pellets No. 890 Nafag rat food *ad libitum* and water.

Source: The source of the animal diet was Nafag, Gossau SG.

b. Test Animals

Species and Strain: SC:hairless HR/HR mice were used in this study. Three mice of each sex were used at each dose level.

Source: The mice were obtained from the breeder Bomholtgard. No further information was provided.

Age at Initiation of the Study: The age of the mice at study initiation was 2-2.5 months.

Weight: The initial body weights of the mice were 16-25 g.

Quarantine: The animals were acclimated for 10 days prior to the start of the experiment.

Animal Maintenance: Animals were housed individually in size 1 wire cages (type 2). The rats were housed at a room temperature of 24±1 °C, a relative humidity of 50 ± 5%, and a 14-hour light cycle day.

c. Experimental design

Dose Levels: The mice were treated with 0.3, 1, or 3% of the test substance. The test vehicle was a 50:50 mixture of acetone/ethanol. Positive controls were treated with 0.1% 8-methoxypsoralene, otherwise referred to as 8-MOP. Negative controls were treated with the vehicle only.

Number of Animals Sex per Group: Three animals/sex/dose were used.

Preparation of Test Article and Mode of Administration: The test material was administered dermally. The test substance was prepared as a suspension in acetone:ethanol (50:50). The suspension

was diluted to concentrations of 0.3, 1, and 3% using the vehicle. Aliquots of 0.05 ml of each concentration were applied evenly using a pipet onto the skin of both flanks of hairless mice. A separate group of animals was used for each concentration. Negative and positive controls were treated in the same way (one flank irradiated and one flank nonradiated).

Animals were placed in an animal holder about 5.6 cm in front of the UV-light source one-half hour after the application for a duration of 2 minutes. A 1-cm diameter area on one side of the mouse was exposed and the contralateral side was not exposed to light and served as the control. The light source was a Solar Ultraviolet Simulator with a 150 W Xenon burner, wavelength range was 320-400 n. The irradiation intensity at the focused area was 35 mW/cm^2.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed for skin reactions 24, 48, and 72 hours after the irradiation according to the Draize scoring system for primary irritation.

Body Weight: Body weight data were not recorded.

e. Gross Pathology

Necropsy: Not performed.

Histopathology Examination: Not performed.

f. Statistical Evaluation

No statistical analyses were performed.

g. Critical Appraisal of the Protocol

This study was designed as an acute dermal phototoxicity study. Adequate controls (both negative and positive) were used. It appears that the conduct of the study was appropriate to determine the photoxicity of the test substance as determined by primary irritation, but some critical information is lacking. Both GLP and Qualtiy Assurance statements were missing. No description of either the physical appearance of the test substance or the storage conditions was provided. No information was provided regarding the purity or

stability of the test material. A small number of animals were used (3 animals/sex/dose).

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** No local skin irritation was observed after administration of the test material either with or without irradiation. Moderate edema was observed in 6/6 animals 24 hours after application of the positive control followed by UV-irradiation, and necrosis was observed in 6/6 of these animals at 48 and 72 hours after treatment. None of the positive controls showed irritation when they were not irradiated. Neither the irradiated nor the nonradiated animals treated with the vehicle alone showed signs of irritation at any time point.

2. **Mortality:** No animals died after any treatment.

3. **Body Weight Changes:** No data were recorded.

4. **Gross Pathology**: Not performed.

E. ASSESSMENT

The design of this study was appropriate for an acute dermal phototoxicity study in the mouse. There were adequate controls, both positive and negative, built into the design of the study and three concentrations of the test material were used. There were several deficiencies in the reporting of the study, however, that hinder a full assessment of the data. No description of either the physical appearance of the test substance or the storage conditions was provided, no information was provided regarding the purity or stability of the test material, and small numbers of animals were used (3 animals/sex/dose). There were no GLP and Quality Assurance statements.

F. CONCLUSION

The study author concluded that (b) (4) does not cause skin phototoxicity in hairless mice when applied at concentrations of 0.3, 1, or 3% in acetone:ethanol (50:50). However, because inadequate information on the purity, stability, and storage conditions of the test material was provided, both GLP and Quality Assurance statements were missing, and the small number of animals studied, confidence in this study is limited.

G. EXECUTIVE SUMMARY

The phototoxic potential of (b) (4) was evaluated in an acute dermal study in

hairless mice. (b) (4) was administered once to the skin of hairless mice at concentrations of 0.3, 1, or 3% in acetone:ethanol (50:50). Thirty minutes after application of the test material, the mice were exposed to UV radiation for a 2-minute period. Animals were observed for primary dermal irritation reactions 24, 48, and 72 hours after the irradiation. Each animal served as its own control, using skin to which the test material was applied but not irradiated. Vehicle control animals were also used (irradiated flank and nonirradiated flank). Positive control animals, treated with 0.1% 8-methoxypsoralene, were also used. No adverse reactions occurred when the test material was applied to mice followed by UV irradiation or when the test material was applied but the area was not irradiated. Moderate edema, necrosis, and edema, were observed in positive controls on the irradiated skin, but not on the irradiated skin. The study author concluded that the test material did not cause phototoxicity in hairless mice. However, the lack of detail on the purity and stability of the test material, the small numbers of test animals used in the study, and a lack of both GLP and Quality Assurance statements limit confidence in the study.

H. DATA VALIDATION CHECK

Individual animal data and summary data were validated.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Patte Bittner, M.S. Primary Reviewer — Date 8/21/98

John Liccione, Ph.D. Secondary Reviewer — Date 8/21/98

SALMONELLA /MAMMALIAN-MICROSOME MUTAGENICITY TEST WITH (b) (4)

Title: Salmonella /Mammalian-Microsome Mutagenicity Test with (b) (4) (Test for mutagenic properties in bacteria); Study I.D. # (b) (4) . Pages 000139 to 147 of the petition.

Author/Laboratory: The authors were Dr. P. Arni and Dr. D. Muller (b) (4) Quality Assurance and GLP Statements were not provided.

Test Systems: This testing measured the induction of reverse mutagenesis in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537, with and without exogenous metabolic activation from the Aroclor 1254-induced S9 fraction of Sprague-Dawley rat livers. The age and sex of the rats was not specified. The strains were characterized as histidine-auxotrophic mutants. The report stated that cultures were prepared from frozen stocks; no further details were provided. It was also stated that the Standard Plate Test was performed. Citations pertaining to the methodology used in the study were provided in a reference section of the report.

Test Agent: The test substance was reported to be (b) (4) (Tinuvin 770). There was no further information regarding the purity and stability of the test substance, or how the substance was stored. The report stated that the test substance was dissolved in acetone.

Range-Finding: No separate range-finding study was reported. The highest amount of test substance used in the experiment was 2025 µg/plate, which is less than the maximum of 5000 µg/plate normally used in this assay system. However, the high concentration of 2025 µg/plate was said to have precipitated in soft agar.

Concentrations of Test Substance Tested: The cells were exposed to 0, 25, 75, 225, 675, and 2025 µg (b) (4) /0.1 ml. The authors stated that 0.1 ml of the test material solution was added to each plate.

Negative Control: The vehicle, acetone, was used as a control.

Positive Control: *No activation:* 2.5, 5.0, and 10.0 µg daunoblastin/plate for strain TA 98; 0.0625, 0.125, and 0.25 µg 4-nitroquinoline-N-oxide/plate for strain TA 100; 3.0 and 5.0 µg N-Methyl-N'-nitro-N-nitroso-guanidine/plate for strain TA 1535; and 25, 50, and 100 µg 9-aminoacridine hydrochloride/plate for strain TA 1537.

Activation: 10 and 25 µg cyclophosphamide/plate was used as the positive control with activation for strain TA 1535.

Exposure Time: Cultures containing the test agent were incubated for 48 hours at 37 °C on plates and in darkness.

Number of Replicates/Concentration: Three replicate plates were prepared for each strain/activation/concentration combination. In the positive control experiments two plates were used per strain and per concentration.

Number of Repeats: Only one experiment was performed with the test agent and all the positive controls (except for cyclophosphamide/TA 1535). The results of two experiments were presented for the cyclophosphamide/TA 1535.

Basis of Decision: A response was considered to be nonmutagenic if the colony count in relation to the negative control was not doubled at any concentration.

Results Claimed: (b) (4) did not induce mutations in *Salmonella* strains TA 98, TA 100, TA 1535, and TA 1537 at any of the concentrations tested in the absence or presence of exogenous metabolic activation. The highest concentration (2025 µg/plate) was reported to have precipitated in soft agar.

Assessment: Overall, the procedures used in carrying out these experiments were acceptable. However, there was no information on the purity of the test material, and there were no GLP or Quality Assurance statements. Details on the preparation of stock culture and storage were not provided; however, the report included citations in the reference section that dealt with these aspects of the study. In addition, data on individual plate counts were not presented. Only mean values were provided (but standard deviations were not included). Also, the data were not statistically analyzed. The high concentration of 2025 µg/plate was said to have precipitated in soft agar.

Based on the results of the assays, (b) (4) was non-mutagenic when tested in *Salmonella* strains TA 98, TA 100, TA1535, and TA 1537.

Summary: The substance tested in this experiment (b) (4) was non-mutagenic when tested in *Salmonella* strains TA 98, TA 100, TA 1535, and TA 1537. However, there were a number of reporting deficiencies in this study that limit the confidence in the study. These deficiencies include lack of information on the purity and stability of the test material, no GLP and Quality Assurance statements, the lack of data on individual plates, and no statistical analyses.

Data Validation: Only the arithmetic mean colony counts were presented. There were no standard deviations or individual plate counts. Therefore, the mean colony counts could not be validated against individual plate count data.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

SUBCHRONIC DUST INHALATION TOXICITY STUDY WITH TINUVIN®770 (b) (4) IN ALBINO RATS

A. TITLE

Subchronic Dust Inhalation Toxicity Study with TINUVIN®770 (b) (4)) in Albino Rats; Report No: IT (b) (4) (pages 000148 to 439 of the petition).

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential toxicity of Tinuvin® in the rat following inhalation exposure for 17 days.

4. **Test Article:** Tinuvin® 770 (b) (4)). The reviewer notes that the designation (b) (4) is not defined in the chemistry section of the petition. The test material was characterized as a white dust. No further information on the test material was provided.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: The study report stated that animals were fed a standard laboratory diet (Wayne LAB-BLOX for rats). No further information was provided.

Source: Allied Mills, Inc; Chicago, Ill.

Frequency of Administration: *ad libitum* except during exposures.

b. Test Animals

Species and Strain: albino rats (COBS)

Source: Charles River Breeding Laboratories, Inc., Wilmington, Mass.

Age at Initiation of the Study: Not reported.

Weight at Day 1 of the Study: males: 153 - 190 g; females: 161 - 189 g.

Quarantine: 10 days

Animal Maintenance: The report stated that all animals were housed individually in stainless steel cages. Temperature and humidity conditions were described for the experimental chambers used in the study (discussed below in the next section).

c. Experimental design

Basis or Selection of Doses: Not provided.

Concentration Levels: Target and actual chamber concentrations are summarized Table 1 below:

Table 1. Target and Actual Chamber Concentrations.

Group	Test Material	Chamber Concentration (mg/m^3)	
		Target	Actual
Control	Air	0	0.0
I	Tinuvin®770	3	4.1
II	Tinuvin®770	15	15.6
III	Tinuvin®770	75	73.3

Number of Animals per Sex per Group: 10

Test Material Dust Generation and Analysis:

The test material dust was ground and generated to respirable size (90% < 10 microns) by using blenders and settling cylinders. Clean, dry air was continually added to each blender and settling cylinder to keep the suspended respirable dust passing through the chamber. Rotameters were used to measure the air flows to the generating units. To achieve the desired dust concentrations, air flow rates into the blender and settling cylinder were varied.

The concentration of the test material, and particle size distributions, were measured daily at the beginning and end of each exposure period. The report stated that the concentrations of test material were determined by dividing the total weight of dust collected on a glass fiber filter by the total volume of air drawn through the filter during the sampling period. Particle size distributions were determined by using an Andersen Particle Fractioning Sampler.

Particle distribution (% respirable) varied between 87 to 95%.

Exposure Chambers:

The stainless steel and glass exposure chambers had a capacity of 500 liters. Chamber temperature and humidity were maintained at 26 to 27°C and 20 to 30%, respectively.

Length of Exposure: 6 hrs/day, 5 days/wk, for 17 days (males and females). There was a total of 17 exposures. The author stated that males were not exposed on day 15, while females were not exposed on day 16, so as to accommodate blood collection. The final exposure day was designated as day 18. At the end of day 18, one half of the number of animals in each group were sacrificed, while the other one half were observed (without exposure to the test material) during a recovery period lasting up to day 35.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were observed daily for mortality and abnormal behavioral reactions including anticholinergic effects, pupillary dilation, central nervous system depression and depression of body temperature.

Blood Pressure: Indirect systolic pressures was measured in 5 males and 5 females from all groups after day 1, 5, 10 and 15. Details of the procedure were provided in the study report.

Body Weight: Body weights were recorded prior to the first exposure and on

each exposure day thereafter.

Food Consumption: The study author reported that food consumption was determined weekly for each animal.

Water Consumption: Not recorded.

e. Clinical Laboratory Tests

Ophthalmology: Not performed.

Hematology: The study report stated that total and differential leukocyte counts, erythrocyte count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and prothrombin time were measured in 10 test and control animals per sex per group on day 21 and in 5 test and control animals per sex per group on day 35 of the study. No details on how the blood was collected from the animals were provided.

The reviewer notes that platelet counts were not included in the hematology examinations.

Blood Chemistry: The study report stated that clinical chemistry studies including fasting blood glucose, blood urea nitrogen concentration, serum alkaline phosphatase activity and serum glutamic pyruvic transaminase activity were performed on each control and test animal at the same times as the hematologic studies were done.

The reviewer notes that protein, albumin, globulin, bilirubin, sodium, potassium, calcium, chloride, phosphorus, creatinine, and serum glutamic-oxaloacetic transaminase were not included in the clinical chemistry analyses.

Urinalysis: The study report stated that urinalysis, including determinations for blood, ketones, glucose, protein, bilirubin, microscopic elements and pH, were conducted at the same time as the hematologic studies. Urine samples were collected from all animals in each group during the 24-hour period preceding the collection of blood samples.

f. Gross Pathology

Necropsy: The report stated that one-half of the animals from each group were sacrificed after the final exposure period (designated as day 18 in the

report). The remaining animals were observed up to day 35 and then sacrificed. Animals were sacrificed by exposure to carbon dioxide in a closed chamber immediately exsanguinated. The report stated that gross necropsies were performed and a complete set of representative tissues and organs was taken from each animal and preserved in 10% buffered formalin. The actual list of tissues and organs taken was not specified in the report.

Organ Weights: Adrenal glands, brain, gonads, heart, kidneys, liver, lungs, spleen and thyroid glands were weighed. Organ weights were expressed as absolute values, as organ-to-body weight ratios and as organ-to-brain weight ratios.

Histopathology Examination: Histopathology examination was performed on the following organs and tissues from each control and high-dose rat: adrenal glands, bone marrow, brain, ear, epididymis, esophagus, eye, gonads, gross lesions, heart, intestine (large and small), kidneys , lungs, lymph nodes (hilar, axillary, mesenteric and thoracic), mammary gland, nasal turbinates and adjacent bone, pancreas, pituitary, prostate, salivary glands, spinal cord, stomach, thymus, thyroid gland, trachea, urinary bladder and uterus.

The reviewer notes that the liver and spleen were not included in the histological examinations.

h. Statistical Evaluation

The study author stated that Analysis of Variance followed by "t" tests were conducted on absolute organ weights and on the organ -to-body weight and organ-to-brain weight ratios. The Tukey's multiple comparison test was applied to absolute organ weight data, and the Kruskal-Wallis test was applied to relative organ weights. Analysis of variance and the Tukey's multiple comparison test was used to analyze final body weight data.

j. Critical Appraisal of the Protocol

Although the title of the report includes the word "subchronic", the study design involved only a total of 17 inhalation exposures. Thus, this study is a short-term toxicity study. There were several deficiencies and limitations of the study, as outlined below:

a. Data on the purity and stability of the test material were not provided.

b. There were no GLP compliance or Quality Assurance statements.

c. A rationale for dose selection was not provided.

d. The age of the animals was not specified.

e. The incidence and frequency of clinical observations were not provided.

f. As discussed above, there were limitations in the hematology, clinical chemistry and pathological examinations.

g. The designation (b) (4) is not defined elsewhere in the petition.

In addition, no specific data pertaining to mean particle sizes were presented. However, particle size distributions showed that 87 - 95% of the particles were < 10 microns.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** The author stated that all test groups exhibited salivation, grooming, mild rhinitis, pale yellow discoloration of the fur and irritability during the experimental period. However, the reviewer notes that there was no summary tabulation of the incidence and frequency of these signs, nor were there any individual animal data showing clinical findings. The author reported that the magnitude of the clinical reactions increased with dosage, and that the only reactions noted in the high-dose level (group III) were hypoactivity and ptosis. In addition, one female from the high-dose group exhibited hyperpnea on day 7. Also, no anticholinergic effects were noted.

   The author stated that there was no significant difference in blood pressures between control and treated animals. The reviewer concurs.

2. **Mortality Rate:** The author stated that five rats died during the blood collection for the hematological studies. No further details were provided.

3. **Body Weight Changes:** Table 2, below, summarizes mean body weight data for this study. None of the changes in body weights were reported as statistically significant.

Table 2. Mean body weight in rats at day 18 and day 35[a].

Sex	Actual Chamber Concentration (mg/m^3)	Mean Body Weight (grams)	
		Day 18	Day 28
Male	0	332	388
	4.1	346 (+4.2%)	401 (+3.4%)
	15.6	307 (-7.5%)	340 (-12.4%)
	73.3	301 (-9.3%)	362 (-6.7%)
Female	0	238	255
	4.1	228 (-4.2%)	251 (-1.6%)
	15.6	234 (-1.7%)	245 (-4.0%)
	73.3	230 (-3.4%)	257 (+0.8%)

[a] Data obtained from Tables II and III of the study report (pages 000164 to 000182)

4. **Body Weight Gain Changes:** Mean body weight gains (at the end of exposure on day 18, and on day 28 of the observation period) were recalculated by the reviewer because of errors noted in the tabulation of body weight gain data in the study report. Results are presented in Table 3 below.

Table 3. Mean body weight gains in rats at day 18 and day 28[a].

Sex	Actual Chamber Concentration (mg/m^3)	Mean Body Weight Change (grams)	
		Day 18	Day 28
Male	0	161	212
	4.1	176 (+9.3%)	228 (+7.5%)
	15.6	140 (-13%)	176 (-17%)
	73.3	129 (-20%)	185 (-13%)
Female	0	82	102
	4.1	74 (-9.8%)	96 (-5.9%)
	15.6	81(-1.2%)	93 (-8.8)
	73.3	76 (-7.3%)	104 (+2.0%)

[a] Body weight gains calculated by the reviewer.

The author concluded that there were no significant differences between the test and untreated control weight changes. However, the reviewer recalculated the body weight gain data because of several errors noted in the author's original tabulation in the study report. The recalculation revealed that body weight gains were decreased (relative to controls) in the mid-dose males (13% decrease) and in the high-dose males (20% decrease) at day 18 (end of exposure period). The body weight gains in these males remained depressed at day 28 of the recovery period (17% decrease in the mid-dose males; 13% in the high-dose males). Slight decreases (<10%) in bodyweight gains were also observed in treated females at day 18, but the slight decreases were not dose-related.

5. Food Consumption:

The author of the report stated that there were no significant differences between control and test animals with regard to food consumption . The reviewer concurs that there were no apparent treatment-related effects on food consumption.

6. Clinical Laboratory Tests:

a. Ophthalmology: Not performed.

b. Hematology: The author stated that mean values for total leukocyte and erythrocyte counts, hemoglobin concentration and hematocrit of treated male and female rats were within the normal limits for rats in their laboratory at each time interval examined. The author also stated that mean values for prothrombin time of treated

male and female rats were normal when compared to those of control rats at each time interval examined. Also, no deviations from normal were observed in differential leukocyte counts of treated male and female rats. The reviewer concurs.

c. **Blood Chemistry:** The author stated that in general, mean values for fasting blood glucose, BUN, SAP and SGPT were within the normal limits for rats in their laboratory at each time interval examined. The reviewer's assessment is that there were no apparent effects of toxicological significance.

d. **Urinalysis:** The author stated that mean values for urine content of blood, ketones, glucose, protein and bilirubin, for pH and specific gravity and for microscopic constituents of urinary sediments of treated male and female rats were normal when compared to similar values of control rats at each time interval examined.

7. **Organ Weights**

The author stated that statistical evaluation of the organ weight data revealed no consistent or dose-related organ weight effects. The reviewer concurs.

8. **Gross Pathology**

There were no apparent treatment-related gross findings.

9. **Histopathology**

There was an increase in the incidence of minimal to mild focal subacute rhinitis of the nasal cavity mucosa in the high-dose males sacrificed at the end of the exposed period (day 18). Table 4 summarizes the incidence and severity of this effect in rats. The study pathologist stated that the rhinitis was characterized by infiltration of moderate numbers of lymphocytes and plasma cells and lesser numbers of neutrophils into the nasal mucosa . However, the incidence of this histologic change was similar in both controls and treated animals at the end of the recovery period (i.e., day 35). Based on these findings, the study pathologist concluded that the it is questionable that the focal subacute rhinitis observed at the end of the exposure period (i.e., day 18) was due to the test material. The reviewer also considers the findings equivocal. However, the reviewer notes that there was a slight increase in the severity of the histologic change in the mid-dose and high-dose males at day 35. The severity of the change in these animals, however, were classified as mild.

The reviewer also notes that the study author had stated that the magnitude of clinical observations (including mild rhinitis) increased with dosage, but the study report did not include a summary tabulation of the incidence and frequency of these signs, nor were clinical observation data provided for individual animals. Therefore, the

reviewer considers that the focal subacute rhinitis may be possibly related to treatment. In addition, the reviewer considers the study limited in that only a total of 17 exposures was examined, and the animals may have been able to tolerate exposure to a concentration greater than the high dose examined in the study.

Table 4. Incidence and severity of focal subacute rhinitis of the nasal cavity mucosa in rats following exposure to Tinuvin® [a,b].

Sex	Incidence and severity of focal subacute rhinitis in the nasal cavity of rats at concentrations (mg/mg3) of:			
	0	4.1	15.6	73.3
Male				
Day 18	-	1 (1.0)[c]	1 (1.0)	4 (1.7)
Day 35	3 (0.5)	2 (0.5)	4 (2.0)	4 (1.6)
Female				
Day 18	-	1 (0.5)	1 (P)	4 (1.2)
Day 35	3 (0.8)	3 (0.6)	5 (1.3)	3 (1.0)

[a] Data from Table XXXI (page 000245) and Table XXXII (page 000247) of the study report.
[b] The number of animals per sex per group examined was five.
[c] The numbers in parentheses are severity grades. The severity grades were defined in the study report as follows: 0.5 - trace; 1.0 - minimal; 2.0 - mild; 3.0 - moderate; 4.0 - marked; 5.0 - extreme; P - present, no grade.

E. ASSESSMENT

There are a number of deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol).

The magnitude of clinical signs of toxicity (i.e., salivation, grooming, mild rhinitis, pale yellow discoloration of the fur and irritability) noted in the test animals during the experimental period was reported to have increased with dosage However, there was no summary tabulation of the incidence and frequency of these signs, nor where there any individual animal data showing clinical findings. It was also reported that hypoactivity and ptosis were observed in only the high-dose level (group III) .

Mean body weight gains were decreased (> 10%) when compared to controls in the mid- and high-dose males at day 18 (at the end of the exposure period) and at day 28 (during the recovery period). Histological examinations revealed an increase in the incidence in focal subacute rhinitis of the nasal cavity mucosa in the high-dose males at day 18; severity of the change was also slightly increased. However, the incidence of this change in the high-dose males was comparable to controls at the end of the

recovery period (day 35). The reviewer is of the opinion that this histologic change may possibly be related to treatment, and that this assessment is supported by clinical observations. In this regard, the author reported that the magnitude of rhinitis (one of the clinical observations noted in the test animals) increased with dose level.

Despite the limitations of the study, the results may have utility for selection of dose levels for a 28-day or 90-day inhalation study. The reviewer believes the study supports a NOEL of 4.1 mg/m^3 in males based on decreased body weight gain.

F. CONCLUSION

Rats exposed by inhalation to 4.1, 15.6, or 73.3 mg/m^3 Tinuvin® 770 for a total of 17 exposures showed clinical signs of toxicity including salivation, grooming, mild rhinitis, pale yellow discoloration of the fur and irritability. The magnitude of the clinical reactions was reported to have increased with dosage, and that the only reactions noted only in the high-dose level (group III) were hypoactivity and ptosis. Mean body weight gains in the mid- and high-dose males were decreased (> 10%) relative to controls at the end of the exposure period (day 18) and at the end of the recovery period (day 35). Histological examination revealed an increase in the incidence of focal subacute rhinitis of the nasal cavity mucosa in the high-dose males at day 18. The incidence of this effect in the high-dose females, however, was similar to controls at the end of the recovery period. The NOEL is 4.1 mg/m^3 in males based on decreased body weight gains.

G. EXECUTIVE SUMMARY

Four groups of 10 rats of each sex were exposed by inhalation to 0, 4.1, 15.6, or 73.3 mg/m^3 Tinuvin® 770 for 6 hr/day, 5 days/wk for a total of 17 exposures. At the end of the exposure period (day 18), 5 rats/sex/group were sacrificed for pathological examination. The remaining animals were observed during a recovery period (ending day 35). Mean body weight gains were lower (>10%) than those of controls at the end of the exposure period and at the end of the recovery period in the mid- and high-dose males. The magnitude of clinical observations (i.e., salivation, grooming, mild rhinitis, pale yellow discoloration of the fur and irritability) was reported to have increased with dose level. An increased incidence of focal subacute rhinitis of the nasal cavity mucosa was observed in the high-dose males at day 18, but not at the end of the recovery period. There were no effects of toxicological significance on mortality, organ weights, food intake, hematology and clinical chemistry. The NOEL is 4.1 mg/m^3 in males based on decreased body weight gains.

H. DATA VALIDATION CHECK

For low- and mid-dose animals, approximately 20% of all summary toxicity data (mortality, clinical signs, body weight, hematology and blood chemistry, gross

necropsy and histopathology) were validated against individual animal data by inspection and recalculation. Because of errors noted in the study, 100% of all summary toxicity data for high-dose animals and controls were validated.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

(b) (4) : ORAL TOXICITY STUDY IN RATS (DAILY ADMINISTRATION FOR 4 WEEKS

A. TITLE

(b) (4) : Oral Toxicity Study in Rats (Daily Administration for 4 Weeks; Dated: August 24, 1972

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** No GLP statement was provided in the report. The study was performed prior to the establishment of current GLP regulations; however, the conduct of the study appears to have been in accordance with accepted GLP procedures.

2. **Quality Assurance Statement:** A quality assurance statement (signed but not dated) was provided in the report on page 000441.

3. **Study Objective:** The objective of the study was to determine the toxicity of the test article, (b) (4) , in the rat following oral gavage administration for 4 weeks.

4. **Test Article:** (b) (4) (no further identification provided) was prepared as a suspension in 0.5% sodium carboxymethyl cellulose fresh each day prior to administration. The test compound sample was taken from a batch dated February 17, 1972, about one week prior to initiation of the test.

   a. **Purity:** No statement of test article purity was provided in the report.

   b. **Stability:** No statement of test article stability was provided in the report.

5. **Protocol:**

a. **Animal Diet**

Type: Spiller's Laboratory Small Animals Diet (autoclaved) and tap water were provided *ad libitum*. No certificate of analysis was provided.

Source: Not provided.

Frequency of Administration: *ad libitum*

b. **Test Animals**

Species and Strain: CFY rats.

Source: Carworth Europe, Huntingdon, England.

Age at Initiation of the Study: Not specified.

Weight at the Initiation of the Study: males: 275 – 348 g; females: 180–240 g.

Quarantine: The report stated that the animals were acclimatized for 28 days.

Animal Maintenance: The report stated that the animals were housed in groups of five in wire mesh cages. Room temperature and relative humidity were maintained at 21 ± 2°C and 50 ± 5%, respectively. No information was provided on animal cage bedding and light/dark cycle.

c. **Experimental Design**

Basis of Selection of Doses: No justification for the dose selection was provided in the report.

Dose Levels: 0 (control; Group 1), 50 mg/kg/day (low dose, Group 2), 200 mg/kg/day (intermediate dose, Group 3), and 600 mg/kg/day (high dose, Group 4).

Number of Animals per Sex per Group: 10 males and 10 females per treatment group. Animals were randomly allocated to approximately equalize initial mean body weight among groups.

Preparation of Test Article and Mode of Administration: The test article was administered as a suspension in 0.5% sodium carboxymethyl cellulose. Suspensions were prepared fresh daily and immediately administered via oral gavage in volumes of 5 mL/kg.

Length of Exposure: 4 weeks.

d. Clinical Observations

Gross Signs of Toxicity: The report did not explicitly state the frequency of animal observation, but it was presumably at least once daily. Animals were examined for morbidity, mortality, and other gross signs of toxicity.

Body Weight: Animals were weighed prior to treatment on the first day of dosing, twice weekly thereafter, and before necropsy.

Food Consumption: The amount of food consumed by each cage of animals was determined weekly.

Water Consumption: Water consumption was monitored by inspection of the water bottles, but consumption was not quantified.

e. Clinical Laboratory Tests

Ophthalmology: Ophthalmoscopy was performed on all animals prior to treatment and after 4 weeks of treatment, using a Keeler indirect ophthalmoscope.

Hematology: Blood samples were collected by orbital sinus puncture from all rats after 4 weeks of treatment, prior to treatment on the day of sampling. The following parameters were measured: packed cell volume, hemoglobin concentration, red blood cell count, mean corpuscular hemoglobin concentration, mean cell volume, total and differential white cell count, platelet count, and thrombotest.

Blood Chemistry: Blood samples were collected from the orbital sinus of all rats after 4 weeks of treatment, prior to treatment on the day of sampling. The following parameters were measured: plasma urea, plasma glucose, serum alkaline phosphatase, and serum glutamic-pyruvic transaminase.

Urinalysis: Urine samples were collected from 5 males and 5 females from the control and high dose groups only after 3 weeks of treatment. The following parameters were monitored: pH, specific gravity, protein, reducing substances,

glucose, ketones, bile pigments, and urobilin.

f. Pathology

Necropsy: The report stated that at termination, animals were sacrificed by carbon dioxide asphyxiation. Macroscopic examination of the tissues and any abnormalities was performed.

Organ Weights: The following organs of the sacrificed rats were weighed: adrenals, brain, heart, kidneys, liver, and testes.

Histopathology Examination: The report stated that the following tissues were preserved in 4% buffered formaldehyde (except eyes, which were preserved in Davidson's fixative): adrenals, aorta, brain (medullary, cerebellar and cortical sections), cecum, colon, duodenum, eyes, femur, heart, ileum, jejunum, kidneys, mammary gland, liver, lungs, lymph nodes (cervical and mesenteric), esophagus, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, body fat, seminal vesicles, skeletal muscle, skin, spleen, stomach (glandular and non-glandular), testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, and all tissues showing macroscopic abnormality.

The report stated that histopathology was performed on the following tissues: adrenals, brain (medullary, cerebellar and cortical sections), cecum, duodenum, eyes, heart, ileum, kidneys, liver, lungs, lymph nodes (cervical and mesenteric), ovaries, pancreas, pituitary, spleen, stomach (glandular and non-glandular), testes, thymus, thyroid, urinary bladder, uterus, and all tissues showing macroscopic abnormality.

g. Statistical Evaluation

The report stated that Student's 't' test was performed to determine the significance of intergroup differences where a response to treatment was suggested.

h. Critical Appraisal of the Protocol

Overall, the design and conduct of the short-term oral study were acceptable. However, animals were housed 5/cage rather than individually, and no statement of the purity or stability of the test substance was provided. The incidence of clinical signs was not tabulated. Also, hematology and clinical chemistry were performed at only one sampling period at the end of the study and not prior to the study. This allowed for comparisons between treatment groups and controls, but

did not allow for comparisons between pre-dosing and post-dosing effects within treatment groups. Urinalysis was performed on only the control and high-dose groups at week 3, and on only five animals per sex per group. No information was provided on animal age, cage bedding and light/dark cycle. The report did not explicitly state the frequency of animal observation (twice daily is recommended).

Data were adequately reported. The number of animals of each sex per dose group and the protocols for assessing toxicological end points were appropriate for short-term toxicity testing.

IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** The report stated that animals in the high-dose group exhibited a reduction in grooming activity, with urine staining apparent on the fur, and salivation from between 15 and 25 minutes after dosing. Animals in this group, particularly females, became difficult to handle when dosing, although no further explanation was provided. Four males and seven females in the high-dose group exhibited signs of respiratory distress during weeks 3 and 4. Animals in the low- and mid-dose groups exhibited no abnormal clinical signs.

2. **Mortality Rate:** The report stated that one male in the control group died on day 17, having shown previous weight loss and pallor of the extremities. Enlargement of the spleen and liver were noted on macroscopic examination of this animal. Two females in the high-dose group died on day 8, with macroscopic examination revealing no abnormalities in one animal, and gaseous distension of the stomach and small intestine in the other.

3. **Body Weight Changes:** The report stated that the rate of body weight gain was reduced in the high-dose group, and to a lesser extent in the mid-dose group. Animals in the low-dose group showed no effect on body weight gain. Table 1, below, summarizes body weight and body weight gain data:

TABLE 1: Group Mean Body Weights and Body Weight Gains[a].

Dose (mg/kg/day)	Initial Weight (g)	Weight at 28 days	Weight Gain 0-28 days
Males			
0	318	464	146
50	314	469	155 (6%)
200	315	438	123 (-16%)
600	316	426	110** (-25%)
Females			
0	206	272	66
50	209	273	64 (-3%)
200	206	258	52* (-21%)
600	208	244	36*** (-45%)

[a]Data from page 000449A.
* P<0.05
**P<0.01

4. **Food Consumption**: The report stated that there were no marked effects on food consumption. However, because the animals were housed 5/cage, the data reported were per cage food consumption averaged over number of animals per cage. The authors stated that there was an overall reduction in the efficiency of food utilization in rats receiving 600 mg/kg/day, and to a lesser extent in those receiving 200 mg/kg/day.

5. **Clinical Laboratory Tests:**

a. **Ophthalmology:** The report stated that there were no ophthalmological changes noted in all groups.

b. **Hematology:** The report stated that there were no hematological changes that were considered to be related to treatment. The reviewer concurs.

c. **Blood Chemistry:** The report stated that there were no blood chemistry changes that were considered to be related to treatment.

d. **Urinalysis:** The report stated that all urinalysis results were considered to be within normal limits.

6. **Organ Weights:** The report stated that the adrenal glands in high-dose males were slightly heavier (statistically significant) than those in the males from the other groups. Since the adrenal glands from females exhibited no such change, and there were no corresponding histological changes, this was not considered by the study authors to be a significant finding. The reviewer agrees. All other organ weights exhibited no differences between treatment groups. No statistical test results on organ weight changes were provided.

7. **Gross Pathology:** The report stated that animals from the control and treatment groups exhibited minimal chronic respiratory disease generally characterized by the presence of subpleural foci. The incidence of this finding was comparable between control and treated animals, and is not regarded by the reviewer to be indicative of a dose-related effect.

   One animal in the low-dose group exhibited bilateral cortical scarring of the kidneys, while two animals in the mid-dose group and two animals in the high-dose group exhibited gaseous distension of the small intestine. The reviewer considers these findings of doubtful significance.

8. **Histopathology:** The report stated that non-specific vacuolation and occasional distension of hepatocytes were seen in the centrilobular areas of liver in both control and treated males. Small foci of mononuclear cell infiltration were seen in liver parenchyma of one male and two females in the control group, and in one male and two females in the high-dose group. In the kidneys, tubule formation, characterized by some distension, eosinophilic material, and minimal mononuclear cell infiltration of the renal cortex, was seen in two males in the control group and four males and one female in the high-dose group. One high-dose male exhibited small foci of dystrophic mineralization in the medulla. All of these changes were considered to be common lesions in laboratory rats and therefore not related to treatment. The reviewer agrees with the authors conclusions.

   Other histopathological changes considered to be unrelated to treatment were telangiectasis in the parathyroid in one control female, distended seminiferous tubules in one testis of one control male, plugs of eosinophilic material in the urinary bladder in two control males, minimal mononuclear cell infiltration in the ventricular myocardium in two control male and one high-dose female, and foci of extra-medullary hemopoiesis in the spleen in several control and treated animals.

ASSESSMENT

Overall, the design and conduct of the study were appropriate for a short-term oral

toxicity test. Limitations of the study are discussed above in "Critical Appraisal of the Protocol." The results of this study can be used to determine dose levels to be examined in a subchronic study.

F. CONCLUSION

Administration of the high dose (600 mg/kg/day) was associated with reduced grooming activity, salivation, and resistance to being handled. A dose-related reduction in body weight gain was noted at the mid dose (200 mg/kg/day) and high dose. Two deaths were noted in the high-dose group, and one death was noted in the control group. The NOAEL is 50 mg/kg/day.

G. EXECUTIVE SUMMARY

(b) (4) was administered via oral gavage to male and female CFY rats at dose levels of 0, 50, 200, and 600 mg/kg/day for 4 weeks. There were dose-related decreases in body weight gain in the mid- and high-dose groups. The high-dose group also exhibited 2 female deaths and an overall reduction in grooming activity, as well as increased salivation and resistance to handling. There were no effects of toxicological importance on hematology, blood chemistry, ophthalmology, urinalysis, food consumption, organ weights, gross necropsy, or histopathology.

DATA VALIDATION CHECK

Approximately 20% of all summary toxicity data (mortality, clinical signs, body weight, hematology and blood chemistry, urinalysis, gross necropsy, and histopathology) were validated against individual animal data and by recalculation.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

Steven G. Donkin, Ph.D. Primary Reviewer — Date: 8/20/98

John J. Ziccione, Ph.D. Secondary Reviewer — Date: 8/21/98

Toxicity to Rats - Dietary Administration for 13 Weeks

A. TITLE

Toxicity to Rats - Dietary Administration for 13 Weeks; Report No. (b) (4) (pages 000471 to 623 of the petition).

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential toxicity of Tinuvin 770 in the rat following oral exposure for 13 weeks.

4. **Test Article:** Tinuvin 770 (b) (4)). The reviewer notes that the designation (b) (4) is not described in the chemistry section of the petition. The test material was characterized as a white powder.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: Spratt's Laboratory Diet 2

   Source: Not reported

   Frequency of Administration: *ad libitum*

b. Test Animals

Species and Strain: Sprague-Dawley rats of the CD strain

Source: Charles River Laboratories (no further information was provided)

Age at Initiation of the Study: Not reported

Weight at Initiation of the Study: males: 150 - 210 g; females: 148 - 187 g.

Quarantine: 5 days

Animal Maintenance: The report stated that the rats were housed five to a cage (unless the number was reduced by mortality) in suspended cages with wire-mesh floors. Temperature was maintained at 21 ± 2°C; relative humidity was 50 ± 5% respectively. Lighting was controlled provide a 12 hour light and 12 hour dark cycle.

c. Experimental design

Basis or Selection of Doses: The basis for dose selection was not presented. However, the reviewer notes that a 4 week oral (gavage) study in rats treated with Tinuvin 770 was available for review. Reduced grooming activity, salivation, decreased body weight gain, and resistance to being handled was reported in animals treated with 600 mg/kg/day (the high dose). Two females treated with the high dose died. A slight reduction in body weight gains was reported in animals administered 200 mg/kg/day. No effects were observed at 50 mg/kg/day (low dose).

Dose Levels: 0 (control), 400 ppm (low-dose), 1300 ppm (mid-dose), and 4000 ppm (high-dose).

Number of Animals per Sex per Group: 25 males and 25 females for the control and high-dose groups. 20 males and 20 females for the low- and mid-dose groups. Animals were randomly allocated to each group on the basis of body weights.

Preparation of Test Article and Mode of Administration: A pre-mix containing 20000 ppm was prepared each week. This premix was used to prepare the test diets by dilution with further quantities of diet. Homogeneity of the diets was achieved by mixing for 10 minutes in a rotary double-cone

blender. The diets were stored until use in heat-sealed, opaque polythene bags. Controls received normal diet.
The author stated there was good correspondence between the concentrations found in the diets and the normal values following analyses of the diet by gas chromatography. However, no specific data showing the results of the dietary analyses were provided.

Length of Exposure: 13 weeks (males and females). During the recovery period of 4 weeks, 5 male and 5 female rats from the control and high-dose groups received normal (untreated) powered diet. The recovery period did not include low- or mid-dose animals.

d. Clinical Observations

Gross Signs of Toxicity: The author stated that animals were examined for mortality and gross signs of toxicity; however, the frequency of these observations was not specified.

Body Weight: Animals were weighed at pretest and at weekly intervals thereafter.

Feed Consumption: The quantity of food consumed by each cage of rats was recorded and the mean weekly intake calculated. Efficiency of food utilization was also determined.

Water Consumption: Water consumption was measured for each cage of the control and high-dose groups during weeks 5 and 12. Mean daily intake was calculated.

e. Clinical Laboratory Tests

Ophthalmology: Ocular examinations (using an indirect ophthalmoscope) were conducted prior to treatment and during weeks 4, 8 and 12 in male and female rats of the control and high-dose groups.

Hematology: Blood samples were obtained from 10 male and 10 female rats of the control and high-dose group during weeks 4, 8 and 12. Blood samples were taken from the orbital sinus after fasting overnight. The following parameters were measured: packed cell volume, hemoglobin, red cell count, mean corpuscular hemoglobin concentration, mean cell volume, total white blood cell count, differential white blood cell count, platelet count, and thromboplastin time.

Blood Chemistry: Blood samples were obtained from 10 male and 10 female

rats of the control and high-dose group during weeks 4, 8 and 12. Blood samples were taken from the orbital sinus after fasting overnight. The following parameters were measured: plasma urea, glucose, total serum proteins, serum protein electrophoresis, AG ratio, serum alkaline phosphatase, serum glutamic-pyruvic transaminase, sodium and potassium.

The reviewer notes that serum glutamic-oxaloacetic transaminase, and the levels of creatinine, bilirubin, chloride and phosphorus were not measured.

Urinalysis: Individual urine samples were collected from 10 males and 10 females from the control and high-dose groups during weeks 4, 8 and 12. Animals that were chosen for the urinalysis were fasted overnight. The following parameters were measured: pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigment, urobilin, and blood pigment. In addition, urine was examined for epithelial cells, polymorphonuclear leucocytes, mononuclear leucocytes, erythrocytes, organisms, casts, abnormal constituents and sperm.

f. Pathology

Necropsy: At the end of 13 weeks, 20 males and 20 females from each group were killed by carbon dioxide asphyxiation. The remaining 5 males and 5 females from the control and high-dose group were killed after a further 4 week observation period. Authors reported that the appearance of tissues was noted.

Organ Weights: The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, and thyroid. Organ weights were expressed as absolute values, as organ-to-body weight ratios, and as organ-to-brain weight ratios.

Histopathology Examination: The following organs and tissues from rats that died during the study, from control and high-dose animals sacrificed at 13 weeks, and from control and high-dose animals sacrificed at the end of the 4 week recovery period: adrenals, aorta, brain, caecum, duodenum, eye, heart, ileum, kidneys, liver, lungs, lymph nodes, ovaries, pancreas, pituitary, prostate, spleen, stomach, testes, thymus, thyroid, urinary bladder, and uterus. In addition, rib marrow smears were prepared.

g. Statistical Evaluation

The report stated that Student's 't' test was used to assess the significance of intergroup differences where the data suggested evidence of a response to

treatment.

h. Critical Appraisal of the Protocol

Overall, the design and conduct of the subchronic oral study was acceptable. The study included a recovery phase. However, there were several reporting deficiencies and limitations, as outlined below.

1.) There were no GLP or Quality Assurance statements.

2.) The age of the animals was not specified.

3.) The purity and stability of the test material were not reported.

4.) Animals were housed 5/cage.

5.) Data pertaining to dietary analyses were not presented.

6.) Several recommended clinical chemistry and histopathology parameters were not measured as discussed above.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** Authors stated that there were no visible signs of reaction to treatment with the test material.

2. **Mortality Rate:** The authors reported that one control male rat died during the removal of a blood sample during week 8. In addition, one female treated with 400 ppm was found dead in week 5 and one female treated with 1300 ppm was sacrificed during week 11 because of a suspected broken jaw. The authors stated that autopsy of these rats revealed no macroscopic change that could be related to treatment with the test material.

3. **Body Weight Changes:** Authors concluded that body weight gains of the high-dose males and the low-, mid- and high-dose females were lower than those of controls. In contrast, the body weight gain of the low- and mid-dose males were reported to be similar to those of controls. Table 1, below, summarizes mean body weight data at Week 13 and at Week 17 (end of the recovery period) for this study. Table 2, below, summarizes mean body weight gains at Week 13 and at Week 17 (end of the recovery period) for this study.

Table 1. Mean Body Weights at Week 13 and Week 17[a]

Sex	PPM TEST MATERIAL IN THE DIET			
	0	400	1300	4000
Male				
Week 13	527	506 (-4.0%)	521 (-1.1)	470 (-11%)
Week 17	573	-	-	534 (-7%)
Female				
Week 13	310	292 (-6%)	275 (-11.3%)	274 (-11.6%)
Week 17	310	-	-	305

[a] Data from table 2 of the report(page 000490)

Table 2. Mean Body Weight Gains at Week 13 and Week 17[a]

Sex	PPM TEST MATERIAL IN THE DIET			
	0	400	1300	4000
Male				
Wk.. 0-13	350	329 (-6%)	343 (-2%)	292***(-17%)
Wk. 14-17	48	-	-	60 (+25%)
Female				
Wk. 0-13	143	125**(-13%)	110***(-23%)	109***(-24%)
Wk. 14-17	16	-	-	34*(+112.5%)

[a] Data from table 2 of the report(page 000490)
*p<0.05 in comparison with control value
**p<0.01 in comparison with control value
***p<0.001 in comparison with control value

As seen in table 2, mean body weight gains were significantly lower (17%) in the high-dose males when compared to controls at week 13. Mean body weight gains in the high-dose males at the end of the recovery period were increased compared to controls. Mean body weight gains were significantly lower (13-24%) in the low-, mid-, and high-dose females when compared to controls at week 13. The high-dose females exhibited a greater body weight gain than the controls at the end of the recovery period.

4. **Food Consumption:** The authors reported that food consumption in the mid- and high-dose males and females were marginally lower than that of controls during the treatment period, although statistical significance was noted only in the high-dose females. The authors also reported that food consumption among the high-dose males remained at a similar level during the recovery period, while the high-

dose females showed a greater food intake than that of controls. Table 1 and appendix 2 of the report, and table 3 (below), summarize food consumption data.

Table 3. Group mean food consumption (g/rat/week) at selected weeks[a,b]

Week	PPM TEST MATERIAL IN THE DIET			
	0	400	1300	4000
	Males			
1	173	175	169	172
7	160	163	154	151
13	181	165	156	155
17	158	0	0	151
1-13	168	170 (101%)[b]	159 (95%)	156 (93%)
14-17	162	0	0	150** (92%)
	Females			
1	142	144	134	135
7	119	121	109	109
13	111	115	100	105
17	105	-	-	104
1-13	120	120 (100%)	111 (93%)	110* (92%)
14-17	101	-	-	107 (107%)

[a] Data from table 1 of the report (page 000489)
[b] The numbers in parentheses represent % of control values
*p<0.05 in comparison with control value
**p<0.01 in comparison with control value

As shown in table 3, group mean food consumption was significantly decreased in the high-dose females at weeks 1-13, and significantly decreased in the high-dose males at the end of the recovery period, weeks 14-17.

With regard to efficiency of food utilization, the authors reported that the high-dose males and low-, mid-, and high-dose females had an inferior efficiency of food utilization when compared with that of the controls during the treatment period. The authors also stated that the high-dose males and females had a

greater efficiency of food utilization when compared with that of the controls at the end of the recovery period. Data pertaining to the efficiency of food utilization were presented in table 3 of the study report.

Total mean test material intakes (weeks 1-13) in the low-, mid- and high-dose males were 26, 80 and 261 mg/kg/day, respectively. The intakes for females of the same dose groups were 29, 90, and 277 mg/kg/day. Data on test material intake were presented in table 4 of the study report.

5. Clinical Laboratory Tests:

a. Ophthalmology: The authors concluded that there were no treatment-related abnormalities of the eyes. The reviewer concurs.

b. Hematology: Authors stated that there were slight increases in red blood cell count, PCV and hemoglobin levels in the high-dose females at weeks 4, 8 and 12, when compared to controls. The increases were statistically significant. The authors concluded that these slight increases were within "normal" limits for the strain and age of rat used; however, historical control data were not presented in the report.

c. Blood Chemistry: Authors stated that statistically significant changes that were noted occasionally at measured intervals were within the normal limit, and not related to treatment. The reviewer concurs.

d. Urinalysis: Authors concluded that all parameters were comparable between control and treated animals. The reviewer concurs.

6. Organ Weights

The authors concluded there were no treatment-related organ weight changes (absolute or relative). The reviewer concurs.

7. Gross Pathology

The authors concluded that there were no treatment-related macroscopic findings at terminal sacrifice at the end of the treatment period (13 weeks) or at the end of the recovery period (17 week). The reviewer concurs.

8. Histopathology

The authors concluded that there were no treatment-related histological findings at terminal sacrifice at the end of the treatment period (13 weeks) or at the end of the recovery period (17 week). The reviewer concurs.

E. ASSESSMENT

There were several deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol).

Decreased (>10%) body weight gains were observed in the low-, mid-, and high-dose females; the decreases were statistically significant. A significant decrease in body weight gains was also seen in the high-dose males. Efficiency of food utilization was decreased in the low-, mid-, and high-dose females, and in the high-dose males during the 13 week treatment period. Body weight gains in the high-dose animals were higher than controls during the recovery period. There were no changes of biological significance in blood chemistry parameters, clinical signs, survival, and pathology.

The reviewer believes the study supports a LOAEL of 400 ppm (29 mg/kg/day) in females based on significant decreases (>10%) in body weight gains and reduced efficiency of food utilization. A NOAEL in females was not determined. The NOAEL in males was 1300 ppm (80 mg/kg/day) which is higher than the LOAEL in females. The LOAEL in males is 4000 ppm.

F. CONCLUSION

In this 13 week study, female rats fed diets containing 400 ppm, 1300 ppm or 4000 ppm Tinuvin 770 exhibited significant decreases (>10%) in body weight gains and reduced efficiency of food utilization. Males treated with 4000 ppm also showed decreased body weight gains and reduced efficiency of food utilization. No effects of toxicological importance on survival, hematology, clinical chemistry, and pathology were observed. The LOEL in females is 400 ppm (29 mg/kg/day) and the LOEL in males is 4000 ppm (261 mg/kg/day).

G. EXECUTIVE SUMMARY

Tinuvin 770 was administered via the diet to male and female Sprague-Dawley rats at dietary levels of 400, 1300, or 4000 ppm for 13 weeks. A recovery phase in which 5 males and 5 females from the control and high-dose groups were administered normal diets for 4 weeks was included in the study design. There were no effects of toxicological significance on survival, hematology, clinical chemistry, and pathology. However, significant decreases (>10%) in body weight gains (compared to controls) were observed in all treated females, and in the high-dose males during the 13 week treatment period. Also, efficiency of food utilization was reduced in the treated females and in the high-dose males. During the recovery period, body weight gains in the high-dose animals were greater than controls.

Based on significant decreases (>10%) in body weight gains and reduced efficiency of food utilization, the LOEL in females is 400 ppm. The LOEL in males is 4000 ppm.

H. DATA VALIDATION CHECK

Approximately 20% of all summary toxicity data (mortality, clinical signs, body weight, hematology and blood chemistry, gross necropsy and histopathology) were validated against individual animal data by inspection and recalculation.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

90 DAY DIETARY TOXICITY STUDY IN DOGS WITH (b) (4)

A. TITLE

90 Day Dietary Toxicity Study in dogs with Compound (b) (4); Dated: September 18, 1974 (pages 000624 to 788 of the petition).

B. STUDY CITATION

(b) (4)

C. STUDY SUMMARY

1. **GLP Statement:** Not reported.

2. **Quality Assurance Statement:** Not reported.

3. **Study Objective:** To determine the potential toxicity of Tinuvin 770 in the dog following oral exposure for 13 weeks.

4. **Test Article:** Tinuvin 770 ((b) (4)). The test material was characterized as a white powder.

   a. **Purity:** Not reported.

   b. **Stability:** Not reported.

5 **Protocol**

   a. **Animal Diet**

   Type: powdered Purina Dog meal was mixed with 15% dry malt (Diamalt OCD) and 10% water

   Source: Not reported

   Frequency of Administration: *ad libitum*

b. Test Animals

Species and Strain: Healthy pedigree Beagle dogs

Source: Dogs were bred and maintained on the premises. The report stated that all animals were fully immunized against canine distemper, infectious canine hepatitis and leptospirosis.

Age at Initiation of the Study: The report stated that the dogs were aged between 7 and 13.5 months at the start of the study.

Weight at Initiation of the Study: males: 10.2 - 15.1 kg; females: 8.7 -13.0 kg .

Quarantine: at least 2 weeks prior to the start of the study

Animal Maintenance: The report stated that the dogs were individually housed in metabolism cages. Temperature, humidity and lighting conditions were not reported.

c. Experimental design

Basis or Selection of Doses: Not provided.

Dose Levels: Dose levels are summarized below:

Group	Dietary level (ppm)
1 (control)	0
2 (low-dose)	800
3 (mid-dose)	2600
4 (high-dose)	8000/ 2600/ 5000*

*The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.

Number of Animals per Sex per Group: 5 males and 5 females for the control and high-dose groups. 4 males and 4 females for the low- and mid-dose groups. The report stated that the animals were randomly allocated to each group.

Preparation of Test Article and Mode of Administration: A 2 kg. pre-mix of the dog meal with appropriate amount of the test material was prepared and mixed for 1 hour. The pre-mix was then added to bulk diet and further mixed for 0.5 hours; water was added during this period. The resulting mixture was then pelleted and dried for 12 hours at a temperature not exceeding 45°C. Control diet was prepared in the same fashion except without test material.

The reviewer notes that there was no indication in the report as to whether the dietary mixtures were subject to analyses for concentration and homogeneity.

Length of Exposure: 13 weeks (males and females). During the observation period of 4 weeks, 1 male and 1 female dog from the control and high-dose groups received normal diet. The low- and mid-dose animals were not included in the study during the observation period.

d. Clinical Observations

Gross Signs of Toxicity: The report stated that animals were examined for clinical symptoms daily; however, the frequency of these observations was not specified. In addition, the report stated that a hearing examination was carried out on animals pre-test and during weeks 6, 9 and 13, and at week 17 for animals assigned to the recovery phase.

Body Weight: Animals were weighed at pretest and at weekly intervals thereafter.

Feed Consumption: The report stated that food consumption was recorded daily.

Water Consumption: Not measured.

e. Clinical Laboratory Tests

Ophthalmology: The report stated that an ophthalmic examination was carried out on animals pre-test and during weeks 6, 9 and 13. The animals assigned to the recovery phase of the study were also examined at week 17.

Hematology: The report stated that blood samples were obtained from all animals at pre-test and during weeks 5, 9 and 13. Samples were also taken from dogs assigned to the recovery phase (2 animals from the control and high-dose groups). The report did not provide details as to how the blood samples were obtained. The following parameters were measured: hemoglobin, erythrocytes, hematocrit, reticulocytes, inclusion bodies, thrombocytes, leucocytes (total and differential count), and prothrombin time.

Blood Chemistry: The report stated that blood samples were obtained from all animals at pre-test and during weeks 5, 9 and 13. Samples were also taken from dogs assigned to the recovery phase (2 animals from the control and high-dose groups). The report did not provide details as to how the blood samples were obtained. The following parameters were measured: sodium, potassium, glucose, urea, SGOT, SGPT, SAP, and serum proteins (total and electrophoresis).

The reviewer notes that levels of serum creatinine, bilirubin, chloride and phosphorus were not measured.

Urinalysis: The report stated that urine samples were obtained from all animals at pre-test and during weeks 5, 9 and 13. Samples were also taken from dogs assigned to the recovery phase (2 animals from the control and high-dose groups). The following parameters were measured: pH, specific gravity, protein, glucose, bilirubin, ketones, blood, and urine sediment.

f. Pathology

Necropsy: The report stated that an autopsy examination was carried out on all animals. Details of the examination were not provided.

Organ Weights: The following organs were weighed: adrenals, kidneys, brain, liver, gonads, heart, spleen, thyroids, thymus, prostate, uterus, pituitary. Organ weights were expressed as absolute values and as organ-to-brain weight ratios.

Histopathology Examination: The report stated that the following organs and tissues were preserved for histopathological examination: adrenals, brain, gonads, kidneys, lymph nodes, muscle, pituitary, spleen, thymus, aorta, colon, gross lesions, liver, pancreas, prostate, spinal cord, thyroids, bond marrow, eye, optic nerve, heart, lungs, mammary gland, peripheral nerve (sciatic), small intestine, stomach and urinary bladder.

g. Statistical Evaluation

The report stated that laboratory parameters and organ weight data were analyzed by the Mann Whitney U test.

The author(s) did not discuss the basis for applying only a nonparametric test to analyze the data.

h. Critical Appraisal of the Protocol

Overall, the design and conduct of the subchronic dietary study in the dogs was acceptable. The study included a recovery phase (limited to one male and one female from the control and high-dose groups). There were several reporting deficiencies and limitations as outlined below:

1.) There were no GLP or Quality Assurance statements.

2.) The purity and stability of the test material were not reported.

3.) Data pertaining to the concentration and homogeneity of the test agent in the dietary mixture were not provided.

4.) Temperature, humidity, and lighting conditions were not reported.

D. IN-DEPTH EXAMINATION OF THE STUDY RESULTS

1. **Clinical Observations:** The report stated that during the treatment period the majority of animals in all groups (including controls) suffered from a mild tracheitis for which no treatment was necessary. The author(s) did not consider the mild tracheitis to be related to treatment.

2. **Mortality Rate:** All animals survived the treatment period.

3. **Body Weight Changes:** Mean body weight and mean body weight gain data at selected intervals are summarized below in Table 1 and Table 2, respectively.

Table 1. Mean Body Weights (Kg) at selected intervals[a]

Sex	PPM TEST MATERIAL IN THE DIET			
	0	800	2600	8000/ 2600/ 5000[b]
Male				
Week 0	13.3	12.9	13.1	12.5
Week 3	13.6	13.0	13.0	11.0
Week 7	14.1	13.2	13.1	12.0
Week 10	14.4	13.4	13.1	12.1
Week 13	14.2	13.4	13.4	12.6
Female				
Week 0	11.0	10.8	10.6	10.3
Week 3	10.9	11.0	10.5	9.0
Week 7	11.3	11.1	10.7	9.9
Week 10	11.7	11.3	11.0	9.9
Week 13	11.8	11.5	11.6	9.9

[a] Data from tabulated data presented in the report (pages 000637)
[b] The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.

The author(s) stated that the dietary concentration of 8000 ppm was so unpalatable that dogs refused to eat. In addition, it was reported that the decreased food intake and body weight became so extreme that the survival of the animals was threatened. The author(s) also stated that food intake and body weights improved dramatically after the concentration was lowered to 2600 ppm at day 43; food intake was slightly decreased when the dietary concentration was increased to 5000 ppm at day 50 but improved over the course of the remainder of the treatment period.

Table 2. Mean Body Weight Gains (kg) at Week 13 [a] and during the Recovery Period

Sex	PPM TEST MATERIAL IN THE DIET			
	0	800	2600	8000/ 2600/ 5000[b]
Male				
Wk. 0-13	0.9	0.5 (-44%)	0.3 (-67%)	0.1 (-89%)
Wk. 14-17	0.1	-	-	0.5 (+400%)
Female				
Wk. 0-13	0.8	0.7 (-13%)	1.0 (+25%)	-0.4(-150%)
Wk. 14-17	-0.4	-	-	+0.3(+175%)

[a]Values calculated by the reviewer

[b]The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.

As seen in table 2, mean body weight gains were decreased (44-89%) in the low, mid-, and high-dose males when compared to controls at week 13. Mean body weight gain was also decreased (150%) in the high-dose females at the end of the 13 week study. Although body weight gain was also decreased (13%) in the low-dose females, mean body weight gain was increased (25%) in the mid-dose females.

During the recovery phase, the high-dose animals exhibited increased body weight gains when compared to controls.

4. **Food Consumption:** The author(s) reported that the dietary concentration of 8000 ppm was unpalatable and that the dogs refused to eat it, and consequently food intake decreased. Food intake was reported to improve dramatically when the dietary concentration was lowered to 2600 ppm on day 43, but decreased slightly when the dietary concentration was increased to 5000 ppm on day 50. However, at the 5000 ppm level, the food intake improved during the remainder of the period. Table 3, below, summarizes mean food consumption data at selected intervals.

Table 3. Mean food consumption (kg) at selected weeks[a,b]

Sex	PPM TEST MATERIAL IN THE DIET			
	0	800	2600	8000/ 2600/ 5000[b]
Male				
Week 0	2.94	2.73	2.55	2.48
Week 1	2.87	2.56	2.34	1.57
Week 3	2.72	2.92	2.22	1.31
Week 6	3.25	3.05	2.34	1.64
Week 7	3.05	2.98	2.35	3.19
Week 13	2.74	3.16	2.60	2.41
Week 17	3.21	-	-	2.31
Female				
Week 0	2.20	2.33	2.17	2.08
Week 1	2.50	2.41	2.03	1.38
Week 3	2.47	2.53	2.03	1.21
Week 6	2.30	2.39	2.36	1.29
Week7	2.47	2.41	2.31	2.86
Week 13	2.34	2.51	2.41	1.95
Week 17	1.54	-	-	1.66

[a] Data from tabulated data presented in the report (pages 000650)
[b] The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.

As shown in Table 3, the following changes in food consumption, when compared to controls, were observed during the study: (1) 8000 ppm males and females, week 1: mean food consumption decreased (45% for males and 44.8% for females). Mean food consumption remained decreased (23-47% in males; 44-55% in females) up to week 6 when compared to controls; (2) 2600 ppm males and females, week 7: mean food consumption increased over controls (5% in males; 16% in females); (3) 5000 ppm males and females , between weeks 8-13: mean food consumption was decreased (12-26% in males; 14-28% in females) when compared to controls. At the end of the recovery period, food consumption was decreased (-28%) in the one high-dose male, and was increased (8%) in the one high-dose female in comparison to controls.

The reviewer notes that mean food consumption was also decreased in the mid-dose animals during the treatment period. For the mid-dose males, mean food consumption was decreased from 5 to 28% , when compared to controls, during

week 1 through week 13. For females, mean food consumption was decreased from 5 to 24%. The decrease in food consumption suggests that the mid-dose diets may have been slightly unpalatable as well.

5. **Average Intake of Test Compound:** Table 4, below, summarizes average intake of test compound over 13 weeks.

Table 4. Average daily intake of test material over 13 weeks[a,b]

Dose Group (PPM Test Compound in Diet)	Average Test Material Intake (mg/kg/day)	
	Males	Females
0	0	0
800	26.7	26.7
2600	69.0	78.4
8000/ 2600/ 5000[b]	149.8	154.7

[a] Data from tabulated data presented in the report (pages 000636)
[b] The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.

6. **Clinical Laboratory Tests:**

   a. **Ophthalmology:** The report stated that during the study the majority of animals in all groups (including controls) "suffered" from a mild conjunctivitis for which no treatment was necessary. The author(s) stated that the condition was not related to the administration of the test material.

   b. **Hematology:** Author(s) stated that there was a slight decrease in red cell parameters (hemoglobin, erythrocyte and hematocrit) in the high-dose females at week 5. In addition, erythrocyte sedimentation rate was increased in a few individuals. The author(s) also stated that by week 13 the differences in the hematology changes were no longer significant and were considered to be due to the poor food intake during the first few weeks of the treatment period. The reviewer concurs. There were no significant differences in hematology parameters in the animals assigned to the recovery period. Table 5, below, summarizes the hematology changes in the females.

Table 5. Summary of hematology changes in female dogs[a,b,c]

Parameter/ week	Dietary level (ppm)			
	0	800	2600	8000/ 2600/ 5000[b]
RBC (10^6/mm^3)				
wk 0	6.56	6.55	7.02	6.75
wk 5	6.76	6.72	5.95	5.85*
wk 9	6.72	6.92	7.05	5.73*
wk 13	6.67	7.00	7.07	6.01
Hb (g/100 ml)				
wk 0	16.5	16.5	17.3	16.5
wk 5	16.6	16.2	15.3	14.8*
wk 9	16.5	16.5	16.5	14.9*
wk 13	16.6	16.9	16.9	15.2
HMT (%)				
wk 0	47	46	50	47
wk 5	48	47	45	44*
wk 9	48	48	48	43
wk 13	47	48	49	43
ESR (mm)				
wk 0	2	2	1	1
wk 5	1	5	4	17
wk 9	2	1	2	10*
wk 13	4	2	1	4

[a] Data from tabulated data presented in the report (pages 000660 to 000663)
[b] The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.
[c] Abbreviations: RBC = red blood cell; HMT = hematocrit; Hb = hemoglobin; ESR = erythrocyte sedimentation rate.
* $P < 0.05$

c. **Blood Chemistry:** Author(s) stated that the slight decreases in the levels of serum proteins in the mid-and high-dose females may have been due to the

poor food intake during the first few weeks of the study. The reviewer concurs. There were no significant differences in clinical chemistry parameters in the animals assigned to the recovery period. Table 6, below, summarizes serum protein levels in control and treated females.

Table 6. Serum protein levels in female dogs[a,b]

Serum protein levels/ week	Dietary level (ppm)			
	0	800	2600	8000/ 2600/ 5000[b]
Serum protein (g/100 ml)				
wk 0	5.70	5.32	5.63	5.33
wk 5	5.81	5.69	5.33	4.99**
wk 9	6.19	5.99	5.75*	5.40*
wk 13	6.44	6.15	5.87*	5.17**

[a] Data from tabulated data presented in the report (pages 000667 to 000700)
[b] The high-dose of 8000 ppm was changed to 2600 ppm at day 43 because of unpalatability of the diet. On day 50, the dose was changed to 5000 ppm.
*$p<0.05$
**$p<0.01$

d. Urinalysis: There were no changes in urinalysis parameters in treated animals when compared with controls during the 13 week treatment period or during the recovery period.

7. Organ Weights

Absolute and relative organ weights were comparable between control and treated animals.

8. Gross Pathology

The author(s) concluded that there were no changes at autopsy that were caused by the administration of the test material. The reviewer concurs.

9. Histopathology

The author(s) stated that the on histopathological examination the only change due to the administration of the test material was a minimal

hypertrophy of periportal hepatocytes in the high-dose males and females. The incidence of minimal hypertrophy of periportal hepatocytes in the high-dose animals was 2 out of 4 males, and 1 out of 4 females. One high-dose female showed slight hypertrophy of periportal hepatocytes. The author(s) considered the slight to mild hypertrophy as an adaptive phenomenon. The reviewer concurs.

The hypertrophy was not observed in animals assigned to the recovery group.

E. ASSESSMENT

There were several deficiencies and limitations of this study (as discussed above in the critical appraisal of the protocol).

Administration of the initial high-dose (8000 ppm) diets was unpalatable to the dogs, resulting in a serious decrease in food intake and body weight gains. Food intake and body weight gains improved after the dosage was adjusted. The average daily compound intake in the high-dose males and females over the 13 week period was 149.8 mg/kg/day and 154.7 mg/kg/day, respectively. The average daily compound intake in the mid-dose males and females was 69 mg/kg/day and 78.4 mg/kg/day, respectively. In the low-dose males and females, the average daily compound intake was 26.7 mg/kg/day. Based on food consumption data, the reviewer believes that the mid-dose diets may also have been slightly unpalatable to the dogs. Because of the palatability problem with the diets, it is difficult to assess a definitive LOAEL and NOAEL.

The reviewer agrees with the author(s) conclusion that the changes in hematology and clinical chemistry parameters may have been due to the poor food intake during the first part of the treatment period. The reviewer also agrees with the author(s) conclusion that the slight to minimal hypertrophy of hepatocytes reflects an adaptive phenomenon.

F. CONCLUSION

In this 13 week study, dogs fed diets containing 8000 ppm Tinuvin 770 exhibited decreased food intake and body weight gains as a result of the unpalatability of the diets. Food intake and body weight gains improved following the adjustment of the dosage. Slight decreases in red cell parameters (i.e., red blood cell count, hematocrit, hemoglobin) and serum protein levels were observed in the high-dose females; these changes may have been due to the poor food intake in these dogs during the early part of the study. Slight to minimal hypertrophy of periportal hepatocytes was noted

in the high-dose males and females, and is considered an adaptive response. Food consumption was also decreased in the mid-dose males and females during the treatment period.

G. EXECUTIVE SUMMARY

Groups of five male and female beagle dogs were fed diets containing Tinuvin 770 at concentrations of 0 (control group), 800, 2600, and 8000 ppm for 13 weeks. One male and one female from the control and high-dose groups were fed normal diets for an additional four weeks during a recovery period. Because the high dose (8000 ppm) diets were unpalatable to the dogs (resulting in serious decreases in food intake and body weight loss), the dietary concentration was adjusted to 2600 ppm (on day 43) and to 5000 ppm (on day 50). Food intake improved following the adjustment of the high dose, and there was a notable increase in body weight gains in the high-dose animals. Food consumption was also decreased in the mid-dose animals during the treatment period. The average daily compound intake in the high-dose males and females over the 13 week period was 149.8 mg/kg/day and 154.7 mg/kg/day, respectively. The average daily compound intake in the mid-dose males and females was 69 mg/kg/day and 78.4 mg/kg/day, respectively. In the low-dose males and females, the average daily compound intake was 26.7 mg/kg/day.

Slight decreases in red cell parameters (hemoglobin, red blood cell count, and hematocrit), and decreased serum protein levels, were observed in the high-dose females during the treatment period. The decreases in the red cell parameters and serum protein levels may have been due to the poor intake in these animals during the early part of the treatment period. Slight to minimal hypertrophy of periportal hepatocytes was noted in the high-dose males and females; this effect is considered an adaptive response.

H. DATA VALIDATION CHECK

Approximately 20% of all summary toxicity data (mortality, clinical signs, body weight, hematology and blood chemistry, gross necropsy and histopathology) were validated against individual animal data by inspection and recalculation.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98

PHARMACOLOGICAL EXPERIMENTS WITH TINUVIN 770

TITLE: Pharmacological Experiments with TINUVIN 770; Date: January 27, 1993
(Pages 000789 to 795 of the petition)

AUTHORS/LABORATORY: The authors were Dr. H. J. Weidelli, Dr. H. Rogg, Dr. M. Meier, and Dr. Leoluca Criscione. (b) (4) [redacted] There was no formal Quality Assurance or GLP compliance statement.

INTRODUCTION: Three pharmacological experiments were performed. The first experiment examined the binding of TINUVIN 770 to calcium-channels in isolated heart cell. The second experiment concerned the binding of TINUVIN 770 to calcium-channels in an isolated guinea pig heart preparation. In the third experiment, a perfused preparation (mesenteric arteries) was used to study the effect of TINUVIN 770 on KCL-induced vasoconstriction. Each of these experiments are discussed separately below.

1. Effect of Tinuvin 770 on ^{3}H-desmethoxyverapamil binding to guinea-pig heart membranes.

METHODS

Preparation of heart membranes.

Hearts were removed from guinea pigs that were sacrificed by cervical dislocation. The hearts were cleaned of connective tissue, and membranes prepared. Membranes were frozen until use.

Radioligand binding assay.

A competitive-binding assay was performed using ^{3}H-desmethoxyverapamil as a ligand. Membranes were incubated with the ligand for 60 min. At 25°C with radioligand and several competitive agents [TINUVIN 770, (b) (4) [redacted] (Tinuvin 770 fragment), verapamil, and diltiazem]. Following incubation, the samples were diluted with ice-cold buffer, and filtered. The filters were washed and radioactivity was measured by liquid scintillation counting. Binding in the presence of 10^{-5} M verapamil was defined as non-specific. Protein for each sample was determined.

Data analysis.

Dose-response curves were analyzed by non-linear regression.

CONCLUSION

Tinuvin 770 decreased the force and rate of contraction at a high concentration of 10 µmol/l, but not at lower concentrations. The effects are most likely related to the calcium-antagonist properties of Tinuvin 770. However, Tinuvin 770 was 10 times less active than diltiazem, indicating that the calcium-channel antagonistic properties of Tinuvin 770 are weak.

3. Vascular effects of Tinuvin 770 in the isolated, perfused mesenteric artery of the rat.

METHODS

Male Sprague-Dawley rats (Tif;RAlf Sisseln) were used. Animals were anesthetized and both carotid arteries were cut to allow exsanguination. The superior mesenteric artery was cannulated via the abdominal aorta, and the mesenteric arterial bed dissected free and mounted and perfused at a constant rate of 5 ml/min.

Perfusion pressure was measured using a transducer via a side arm of the arterial cannula, and recorded continuously. Noradrenaline (2µg) and KCl (150 µmol) were added as a bolus injection directly into the perfusion system by a peristaltic pump at intervals of 5 min alternately, in the presence or absence of cumulative concentrations of test compound (0.01, 0.1, 1, and 10 µM). A 0.5 mM solution of the compound was prepared in 20% DMSO and further diluted in 10% DMSO. Controls were infused with an appropriate dilution of solvent. The infusion of the solvent or the compound was done at a rate of 0.1 ml/min with a peristaltic pump. Each concentration was infused for 30 min. The value of the vasoconstrictor response for each agent obtained just before start of the compound or solvent was taken as the initial value and expressed as 100%.

RESULTS

A tabulation of the results was not provided in the report. However, the author stated that DMSO at the final concentration of 0.2% slightly inhibited the increase in perfusion pressure induced either by noradrenaline or KCl (6% and 9% respectively). The author also stated that DMSO (4%) inhibited noradrenaline and KCl effects by 30% and 23% respectively. In addition, the author stated that in comparison to noradrenaline or KCl, Tinuvin 770 was ineffective at concentrations between 0.01 and 1 µM, whereas at 10 µM it inhibited KCl-induced increase in perfusion pressure by 53%, without affecting noradrenaline-induced vasoconstriction.

ASSESSMENT

This study was performed to assess the potential calcium-antagonistic property of Tinuvin 770 which exhibited a high affinity to calcium channels in the receptor binding study (discussed above under "Pharmacological Experiments with Tinuvin 770"). An isolated, perfused mesenteric artery of the rat was utilized to determine the effects of the test material on noradrenaline-, and KCl-induced vasoconstriction. The methods used in the study were adequately described, and references included.

The results of the experiment showed that Tinuvin 770 was inactive at final concentrations between 0.01 and 1 μM. At the highest concentration tested (i.e., 10 μM) Tinuvin 770 exhibited a preferential inhibition of KCl-induced vasoconstriction, indicating a possible effect at the calcium channel. However, Tinuvin 770 was reported to be about 21- and 18-fold less active than verapamil and diltiazem, respectively. Verapamil and diltiazem are calcium antagonists.

CONCLUSION

Tinuvin 770 (10 μM) inhibited KCl-induced vasoconstriction, indicating a possible effect at the calcium channel. With respect to calcium antagonistic properties, Tinuvin 770 was about 21- and 18-fold less active than verapamil and diltiazem, respectively.

OVERALL ASSESSMENT OF THE PHARMACOLOGICAL EXPERIMENTS

Three pharmacological experiments were performed to assess the potential calcium antagonistic properties of Tinuvin 770. The first experiment involved an in vitro radioligand binding assay using isolated heart cell membranes prepared from guinea pigs. In the first experiment, Tinuvin 770 exhibited strong binding to the L-type calcium-channels. In order to assess the functional significance of these findings, two additional experiments were performed. The second experiment involved an examination of the effects of the test material on the rate and force of contraction in isolated guinea-pig atria. In the second experiment, Tinuvin 770 exhibited properties of a weak calcium-antagonist. The third experiment involved a perfused mesenteric artery preparation to study the effects of the test material on noradrenaline-, and KCl-induced vasoconstriction. In the third experiment, Tinuvin 770 exhibited a preferential inhibition of KCl-induced vasoconstriction, indicating a possible effect at the calcium channel. Tinuvin 770 was about 21- and 18-fold less active than the calcium antagonists verapamil and diltiazem, respectively. The results of the three experiments indicate that the high affinity binding of Tinuvin to L-type calcium-channels of isolated heart cell membranes is of minor biological significance in the intact heart.

Certification

I certify that the information presented in this review is true, accurate, and complete to the best of my knowledge.

John J. Liccione, Ph.D. Primary Reviewer — Date 8/21/98

David Gray, Ph.D. Secondary Reviewer — Date 8/21/98