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

JUN 22 1994

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

6(a)2

MEMORANDUM

SUBJECT: EPA Id# 067501. Piperonyl butoxide. Review of a series 82-4 subchronic inhalation toxicity study in rats.

TOX CHEM No.: 670
PC No.: 067501
Barcode No.: D182968
Submission No.: S426045

FROM: John Doherty 6/15/94
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: Alan Dixon/Bruce Sidwell
Product Manager #53
Special Review and Reregistration Division (7505C)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (7509C) 6/21/94

I. CONCLUSION

The series 82-4 subchronic inhalation toxicity study in rats with piperonyl butoxide was reviewed and classified as CORE MINIMUM. The study established NOEL and LELs of 0.074 and 0.155 mg/l based primarily on increased secretory activity. At higher doses (0.512 mg/l) there was increased liver weight.

The study did not establish a LEL for pathological changes in the respiratory tract as indicated by the presence of metaplasia/hyperplasia in the larynx. The higher dose levels indicated more extensive hyperplastic changes. The need for an additional series 82-4 subchronic inhalation toxicity study or a series 83-2 carcinogenicity study via the inhalation route of exposure have been referred to HED's Science Analysis Branch. They have been requested to evaluate the findings in this study

108 32

with piperonyl butoxide as well as other related information in order to establish an HED policy for inhalation toxicity studies not showing LELs particularly for hyperplastic, hypertrophy and/or metaplastic responses in the respiratory tract due to treatment.

6(a)2 status. No immediate regulatory action is required at this time. Regulatory action may be required pending the results of Science Analysis Branch's response to HED's referrals.

II. ACTION REQUESTED

The McKenna & Cuneo Law Offices consuls for the Piperonyl Butoxide Task Force has submitted a series 82-4 subchronic inhalation toxicity study with rats in response to the reregistration requirements for piperonyl butoxide. Refer to letter from John D. Conner, Jr. dated September 15, 1992. The study was reviewed by the Agency's contractor and the following comments apply.

III. Toxicology Branch Comments

1. The study was classified as CORE MINIMUM. A copy of the DER prepared by the Agency's contractor is attached. The study is further identified in Section IV below.

2. No NOEL was established for pathological findings in the larynx. A condition described as "pseudostratified ciliated/nonciliated columnar epithelium-squamous squamoid metaplasia/hyperplasia" was reported in 47% of the males and 93% of the females in the low dose group. Only one incident (7% of the female group) was reported in the controls.

3. HED is concerned with the presence of hyperplasia. As per discussion with Dr. Lucas Brennecke, hyperplasia is a common response in the mucosal glands in the larynx/pharynx to a aerosol that is an irritant. It is noted, however, that based on the acute toxicity studies, pyrethrum extract is not considered a dermal irritant.

The progression of the hyperplasia may but not definitely lead to neoplasia. Thus, TB-I is concerned with policy issues related to increases in hyperplasia especially when, such as in this study with piperonyl butoxide, the LEL is not established.

This problem will be referred to an HED's Science Analysis Branch for further discussion and evaluation. The issues presented were:

i. The need for a repeat series 82-4 subchronic inhalation toxicity study to establish the NOEL and LEL for hyperplasia and other lesions of the respiratory tract noted in the first study.

ii. The need for a series 83-2 carcinogenicity study via the inhalation route.

iii. How to use the endpoint of local toxicity of the respiratory tract for regulatory purposes.

iv. Other problems and issues that may arise and related to subchronic inhalation toxicity and risk assessment.

It is expected that resolution of these issues by the HED policy group will require up to six months.

4. Note: The Clements reviewer classified the study as SUPPLEMENTARY. TB-I, however, has reclassified this study as MINIMUM because the study demonstrated a NOEL and LEL for systemic effects. The limiting factor for this study which precludes a higher classification is that the study did not establish a NOEL for effects in the respiratory tract but HED needs to establish policy for this situation (refer to item 3 above.

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
82-4. Subchronic inhalation (3-month)- rats Bio/Dynamics, Study No.: 91-8333, September 14, 1992	Technical piperonyl butoxide Lot FEP-100 Task Force Blend II,, 90.78% purity	424771-01	Whole body inhalation of piperonyl butoxide in Sprague-Dawley rats for 6 hours/day for three months at 0, 0.015, 0.074, 0.155 and 0.512 mg/l (analytical concentration with MMADs of 1.6, 1.4, 1.9 and 2.0 microns for the low to high dose groups respectively. LEL (respiratory system effects) < 0.015 mg/l. Pseudostratified ciliated/nonciliated columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the larynx. At 0.152 mg/l: columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral diverticulum; and mucosal stratified squamous epithelium hyperplasia and hyperkeratosis. NOEL and LEL (systemic) = 0.074 and 0.155 mg/l. AT 0.155 mg/l: increased secretory activity. At 0.512 mg/l: increased absolute (23% males, 20% females) and relative (29% males, 24% females) liver weight and increased relative kidney weight (12% males, 10% females).	MINIMUM

FINAL

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DATA EVALUATION REPORT

Piperonyl Butoxide

Study Type: Subchronic Inhalation Toxicity in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9302 Lee Highway
Fairfax, VA 22031-1207

August 1993

Primary Reviewer Laura Kolb Date 11/22/93
Laura Kolb, MPH

Independent Reviewer William L McLellan for Date 11/22/93
John Liccione, Ph.D.

QA/QC Manager Sharon A Segal Date 11/22/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-36
Clement Number: 119
Project Officer: Caroline Gordon

5

82-4: Piperonyl Butoxide

011066 12/4/93

EPA Reviewer: John Redden, M.S.
Review Section 3, Tox. Branch I
Health Effects Division Signature: [signature] Date: 11/30/93

EPA Section Head: Marion Copley, DVM
Review Section 4, Tox. Branch I
Health Effects Division Signature: [signature] Date: 6/10/94

DATA EVALUATION REPORT

STUDY TYPE: 90-Day subchronic inhalation toxicity in rats

TEST MATERIAL: Piperonyl butoxide

Tox Chem. Number: 670

SYNONYMS: Butacide

P.C. Number: 067501

STUDY NUMBER: 91-8333

MRID Number: 424771-01

CAS NUMBER: 51-03-6

SPONSOR: Piperonyl Butoxide Task Force II
c/o McKenna and Cuneo
Washington, DC

TESTING FACILITY: Bio/dynamics, Inc.
East Millstone, NJ

TITLE OF REPORT: A Subchronic (3-Month) Inhalation Toxicity Study of Piperonyl Butoxide in the Rat via Whole Body Exposures

AUTHOR: Paul E. Newton, Ph.D., D.A.B.T.

REPORT ISSUED: September 14, 1992

CONCLUSIONS:

Dose levels: Whole body inhalation of Piperonyl Butoxide in Sprague-Dawley rats for 6 hours/day for three months at 0, 0.015, 0.074, 0.155, and 0.512 mg/l (analytical concentration) which had MMADs of 0, 1.6, 1.4, 1.9, and 2.0 microns for the low to high dose groups respectively.

LEL (respiratory system effects) < 0.015 mg/l. Pseudostratified ciliated/nonciliated columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the larynx. At 0.152 mg/l: columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral diverticulum; and mucosal stratified squamous epithelium hyperplasia and hyperkeratosis.

NOEL and LEL (systemic) = 0.074 and 0.155 mg/l. At 0.155 mg/l: increased secretory activity. At 0.512 mg/l: increased absolute (23% males, 20% females) and relative (29% males, 24% females) liver weight and increased relative kidney weight (12% males, 10% females).

6

Classification. ~~SUPPLEMENTARY~~ MINIMUM. The study did not establish a NOEL for "mucosa: pseudostratified ciliated/nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia" in the larynx. Refer to page 665 of the study. ~~The study may be upgraded if a clarification of the hyperplasia is provided with respect to its distinction from metaplasia and its actual frequency and intensity at the lowest test dose removes a concern for its presence~~. AT This Time This study does ~~not~~ satisfy the guideline requirement for a subchronic inhalation study (82-4) in rats. jml 6/10/94

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Piperonyl butoxide

Chemical formula: C19H3005

Lot number: FEP-100 Task Force Blend II

Purity: 90.78%; impurities were not identified.

Physical property: Clear, colorless viscous liquid. The Material Safety Data Sheet (MSDS) for Piperonyl Butoxide, Technical (Butacide) reports that the test material odor is aromatic, the vapor pressure is 1 mm at 168°C, the flashpoint is 310°F and it appears as a yellow-brown semi-viscous liquid.

Stability: Not reported

Vehicle: None.

2. Rationale for Dose Selection A previous acute inhalation study revealed no mortality or systemic toxicity at 5.9 mg/l in rats, therefore, exposure concentrations ranging from 0.015 - 0.512 mg/l were selected for this study with 0.512 mg/l representing the highest concentration achievable within the required particle size under these study conditions.

3. Test Article Analyses for Purity and Stability

No information was provided regarding analyses of test material for purity or stability. However, the sponsor has demonstrated stability under conditions of storage. Prestudy trials indicated that analytic and gravimetric measurements of chamber exposure levels were similar. Since the analytic and gravimetric values were roughly equivalent, four gravimetric measurements and one analytic measurement were made daily during the main study.

Samples of the test atmosphere were collected at approximately

7

90-minute intervals from the animal breathing zone on glass fiber filter paper. Piperonyl butoxide content was determined by gas chromatography with a flame ionization detector and a 30 meter DB-1 capillary column.

Exposure levels are presented in Table 1. The low concentration of piperonyl butoxide in the control chamber was attributed by the study authors to sample contamination.

4. Animals

Sprague Dawley Charles River (CD - Crl:(CD)BR) rats from the Charles River Breeding Laboratories were selected for the study (75 males at 311-357 g and 75 females at 202-262 g). The nine-week old rats were randomly distributed into groups so that body weight means for each group (5 groups: 15 rats/sex/group) were comparable.

The animals were housed in pairs in suspended stainless steel wire mesh cages during the first week of acclimation; thereafter, animals were housed individually. Food (Purine Mills Certified Rodent Laboratory Chow Brand Animal Diet #5002) and tap water were supplied *ad libitum* except during exposure. An approximate 12 hour light/dark cycle was maintained. Housing temperature was 16-24°C and housing humidity was 10-86%.

5. Exposure Conditions

Animals were individually housed in wire mesh, stainless steel cages within a 1000 liter glass and stainless steel exposure chamber. Food and water were withheld during dosing. Chamber temperature (17-29°C) and relative humidity (26-74%) were monitored continuously.

Exposures (6 hours/day, generally 5 days/week for 13 weeks) were conducted in a whole body Wahmann style exposure chamber with a total volume of 1000 liters. All animals received 65 exposures; some animals received additional exposures to accommodate the sacrifice schedule. Animal loading was less than 5% of the chamber and animals were rotated through the chamber.

Airflow rate ranged from 201-212 l/min; air change ranged from 4.7-5.0 min. (approximately 12 air changes per hour) and T99 (99% equilibrium time) ranged from 22-23 minutes. Target rates were a minimum flow rate of approximately 200 l/min, at least one air change/5 minutes (12 air changes/hour) and a T99 equilibrium time of 23 minutes. The chamber size and airflow rate were considered sufficient to maintain oxygen concentration above 19%. Chamber temperature, relative humidity, airflow rate, and static pressure were recorded every half hour during exposure.

The vehicle control and test atmospheres were generated using an atomizer supplied with room air or the test substance (as received) plus the house-line air supply. For Group I, room air was passed through the exposure chamber. For Groups II and III, the test material was drawn into a glass syringe which was then mounted on a syringe infusion pump. The test material was subsequently fed into

8

the air atomizing nozzle through Teflon® tubing. Air from the house-line supply was passed through a regulator and backpressure gauge via plastic tubing to a metering valve, it then passed through a flowmeter and backpressure gauge into the air inlet of the atomizer. The test atmosphere was then passed into the inlet turret of the exposure chamber. For Groups IV and V, the test material was placed into an Erlenmeyer flask and connected to a FMI fluid metering pump. The test material was then passed through Teflon® tubing to the liquid inlet of an air atomizing nozzle. Air from the house-line supply was passed through a regulator and backpressure gauge via plastic tubing to a metering valve, it then passed through a flowmeter and backpressure gauge into the air inlet of the atomizer. The test atmosphere was then passed into the inlet turret of the exposure chamber. Following exposure, animals remained in the chamber for 30 minutes to allow the chamber to clear; clean air was provided at the same airflow rate used during exposure.

Prior to study initiation, trial exposures were conducted using 6 ports to test compound distribution within the chamber. The aerosol was found to evenly distribute throughout the rat breathing zone. Values ranged from 0.014/0.013 mg/l - 0.017/0.015 mg/l for Group II to 0.589/0.535 mg/l - 0.663/0.601 mg/l for group V. During the study, four gravimetric measurements and one confirmatory analytical measurement of exposure were made each day. Exposure levels were determined gravimetrically at approximately 90-minute intervals; samples were drawn from the breathing zone of the exposure chamber through glass fiber filter paper mounted open-face in a filter holder. Gravimetric concentration was calculated by dividing the weight difference (in milligrams) of the filter paper before and after sample collection by the volume of air sampled. Exposure levels were determined analytically by gas chromatography (HP 5890II Gas Chromatograph with flame ionization detector) using gravimetric filter paper (one sample per exposure) extracted with diisobutyl phthalate in acetone; the quantity of test material was divided by the volume of air sampled.

The initial flow rate was 20 l/min for all groups, sample time ranged from 15 minutes (Groups I and II) to 1 minute (Group V), and total volume of the sample ranged from 300 liters (Groups I and II) to 20 liters (Group V). The nominal concentration (mg/L) was determined by measuring the flow of air (L/min) through the chamber and the volumetric flow (μl/min) of test substance in the chamber and dividing the volume of the test substance by the total volume of air passing through the chamber. The nominal concentrations used and the actual concentrations achieved in the breathing zones of the test animals are shown in Table 1.

For Group I, particle size distribution was measured once during each exposure for chamber and room air using a TSI Aerodynamic Particle Sizer. Samples were withdrawn at 5 L/min for 20 seconds. For Groups II-V, particle size distribution measurements were performed once during each exposure for chamber and room air using a Delcron DCI-6 cascade impactor. Calculations were based on the amount of material collected on the impactor stages using graphical analysis with an

assumed lognormal distribution. Results of particle size analysis are presented in Table 1.

The average mass median aerodynamic diameter of the aerosol particles fell between 1.4 and 2.0 µm, the average log-standard geometric deviation ranged from 2.3 to 2.8 µm, and the average number of particles ≤ 1 µm was 33, 37, 25 and 21% (mean 29%) for Groups II, III, IV and V, respectively. The trend for increasing particle size at the higher exposure levels was not considered to have affected the study conclusions. Particle size distributions are presented in Table 1.

6. Statistical Analyses

Parameters were analyzed statistically using parametric (ANOVA and Dunnett's) and nonparametric (Kruskal-Wallis and Dunn's Rank Sum) methods. Statistical tests for trend (standard regression techniques and Jonckheere's test) in the dose levels were also used. The test for equal variance (Bartlett's) was conducted at the 1%, 2-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

7. Animal Observations

Individual animal data are presented for mortality, physical observations, body weight, hematology, clinical chemistry, organ weights, histopathology, blind histopathology (liver and larynx), and necropsy.

(a) Mortality/moribundity

Animals were observed cage-side at least twice daily for mortality/ moribundity. Detailed physical examinations were made prior to testing and weekly thereafter. In the chamber, animals were observed daily for mortality from day 35.

No compound-related mortality was observed during the exposure period.

(b) Clinical observations

The animals were also observed once in-chamber during exposure (from Day 35 to termination) for clinical signs (see Table 2). The animals were also observed cage-side twice daily from Day 1 to termination for signs of toxicity (see Table 3). Weekly detailed physical observations were made immediately before exposure.

The in-chamber observations indicated an increase in the incidence of secretory signs at 0.155 and 0.512 mg/l. At 0.155 mg/l, the incidences of dried red material on facial area, dried brown material on facial area, and matted coat were increased. At 0.512 mg/l, the incidences of dried red nasal discharge, dried brown material on facial area, and matted coat were increased.

The cage-side observations also indicated an increase in secretory signs at 0.155 and 0.512 mg/l. For males, the increases in dried red nasal discharge, matted coat, and dried brown material on facial area at 0.155 and 0.512 mg/l appeared to be dose-related; mucoid nasal discharge, dried brown materials on extremities, yellow ano-genital staining, brown material on tail, brown material on extremities and brown ano-genital staining were found at 0.512 mg/l. For females, the increases in dried red nasal discharge, yellow ano-genital staining, dried brown material on facial area, and brown material on extremities at 0.155 and 0.512 mg/l appeared to be dose-related; increases in mucoid nasal discharge, matted coat, and brown material on tail were found at 0.512 mg/l.

These secretory signs were considered to be compound-related effects.

(c) Body weights/food consumption

Body weights--Individual body weights were determined twice pretest, weekly during treatment, and at termination (after fasting). No treatment-related effects on body weight were observed.

Food consumption--Individual food consumption values were determined weekly, starting one week prior to the first exposure. No treatment-related effects on food consumption were observed.

(d) Ophthalmoscopic examination

Eye examinations were conducted by ophthalmoscope prior to the first exposure and at the end of the test period. There were no indications of any ocular effects.

8. Clinical Pathology

Hematology and clinical chemistry evaluations were performed after the last exposure on blood samples obtained from all rats (15/sex/group) after fasting. Blood samples were obtained via venipuncture of the orbital sinus (retrobulbar venus plexus) under light anesthesia. The parameters marked (X) below were examined.

(a) Hematology

X	Activated partial thromboplastin time	X	Leukocyte differential count*
X	Hematocrit (HCT)*	X	Mean corpuscular HGB (MCH)
X	Hemoglobin (HGB)*		
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Erythrocyte morphology	X	Coagulation: prothrombin time (PT)
X	Platelet count*		
X	Reticulocyte count (RETIC)		

* = Recommended by Subdivision F (November 1984) Guidelines

There were no significant compound related hematological changes.

(b) Blood (clinical) chemistry

The parameters marked (X) below were examined.

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	Cholesterol
X Sodium*	X Globulin
	X Glucose*
Enzymes	X Total bilirubin*
	Direct bilirubin
X Alkaline phosphatase (ALP)	X Total protein*
Plasma Cholinesterase	Triglycerides
Red Blood Cell Cholinesterase	
Brain Cholinesterase	
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)	
Gamma glutamyltransferase (GGT)	

* Recommended by Subdivision F (November 1984) Guidelines

Slight differences in SGOT, SGPT, glucose, BUN, total protein, and albumin were noted in animals at 0.512 mg/l compared to controls. Serum levels of SGOT, SGPT, and glucose were decreased while BUN, total protein and albumin were increased. These differences were not statistically different in both sexes; however, a similar trend was noted in both sexes. The minor differences in the above parameters are not considered to be of sufficient magnitude or consistency to be considered definitive responses to treatment.

Other statistically significant differences in serum parameters that were noted between treatment and controls groups occurred sporadically and in only one sex and therefore, were not considered to be treatment-related. Representative results are shown in Table 4.

9. Sacrifice and Pathology

Necropsy examinations were conducted on all animals. Terminal sacrifices were performed on fasted animals over a three-day period (days 87-89).

Tissues from controls and rats exposed to the highest concentration that are marked with an X below were examined histologically and organs marked with XX were also weighed at necropsy. In addition, the lungs were examined in all groups.

12

011066

Guideline 82-4 indicates that histopathology should be performed on all target organs for all animals. Microscopic examination indicated that the liver and larynx were target organs; therefore, these tissues were examined for all animals in groups II-IV in addition to the control group and group V (high dose).

Respiratory	Cardiovascular/ Hematologic	Neurologic
X Nasal tissues*	X Heart*	XX Brain*
X Trachea*	X Aorta*	X Sciatic nerve
XX Lungs *	X Thymus*	X Pituitary*
	X Spleen*	Eyes*
Digestive System	X Bone Marrow*	Spinal Cord#
X Salivary glands*	X Lymph nodes*	
X Esophagus*		Glandular
X Stomach*	Urogenital	XX Adrenals*
X Duodenum*	XX Kidneys*	X Thyroid*
X Jejunum*	X Urinary Bladder*	X Parathyroids*
X Ileum*	XX Testes/ Epididymides*	Other
X Cecum*	X Uterus*	X Bone (sternum)*
X Pancreas*	XX Ovaries	X Mammary Gland#
X Colon*		X Tissues with gross lesions*
X Rectum*		X Larynx
XX Liver*		X Skin#
		Accessory Genital Organs#
		Thigh Musculature#
		Femur/articular surface#
		Extraorbital Lacrimal Glands#

* Recommended by Subdivision F (November 1984) Guidelines
\# Recommended by Subdivision F (November 1984) Guidelines if indicated by signs of toxicity or target organ involvement

(a) Macroscopic

There were no treatment-related gross changes.

(b) Organ weights and body weight ratios

The adrenals, brain, kidneys, liver, lungs, ovaries, and testes with epididymides were weighed for all animals. Organ weights, organ/body and organ/brain weight ratios were analyzed.

Statistically significant treatment related increases in relative kidney weights (11%, $p \leq 0.01$) and absolute and relative liver weights (25%, $p \leq 0.01$) were seen in Group V males and females at 0.512 mg/l. The relative (to body weight) liver weights were also significantly elevated ($p \leq 0.01$). Representative results are shown in Table 5.

13

(c) Microscopic Examination

Changes in the larynx that occurred in all groups (0-0.512 mg/l) included inflammatory cells, congestion, edema, dilated seromucous glands and lymphoid cell aggregates in the mucosa, eosinophilic material and inflammatory cells in the lumen.

The incidence of pseudostratified ciliated/nonciliated columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral seromucous glands of the larynx in both sexes was elevated in all dosed groups (0.015-0.512 mg/l) and the severity was slightly elevated at 0.512 mg/l (see Table 6). In addition, the incidences of columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral diverticulum were elevated in both sexes at 0.512 mg/l.

Subacute (chronic active)/chronic inflammation of the larynx was seen in all animals; the average severity of the lesion was slightly elevated at 0.512 mg/l. Mucosal stratified squamous epithelium hyperplasia and hyperkeratosis of the larynx in both sexes were seen only at 0.512 mg/l.

In the liver, vesiculation/vacuolation of hepatocellular cytoplasm (minimal to slight) was seen in most dosed animals, although severity was slightly more pronounced at 0.512 mg/l. (see Table 7). There was no consistent pattern of dose-related severity or incidence.

Other lesions occurred at comparable incidences and severity between control and dosed groups.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement, a Humane Treatment Statement, and a list of Quality Assurance Inspections were included.

B. DISCUSSION

Respiratory system effects included pseudostratified ciliated/nonciliated columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral seromucous glands of the larynx in all dosed groups (0.015-0.512 mg/l). Columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral diverticulum, mucosal stratified squamous epithelium hyperplasia, and hyperkeratosis were present at 0.512 mg/l.

A NOEL for respiratory system effects was not established; the LEL was 0.015 mg/l based on an increased incidence of pseudostratified ciliated/ nonciliated columnar epithelium-squamous/squamoid metaplasia/hyperplasia in the ventral seromucous glands of the larynx in all dosed groups.

Systemic effects included an increase in secretory activity at 0.155 and 0.512 mg/l. Increased absolute (23% males, 20% females) and relative (29% males, 24% females) liver weight and increased relative kidney weight (12% males, 10% females) were observed at 0.512 mg/l.

The NOEL and LEL for systemic toxicity were 0.074 mg/l and 0.155 mg/l, respectively, based on increased secretory activity at 0.155 mg/l. The reviewers agree with the study author that the NOEL and LEL for systemic toxicity (excluding clinical signs) were 0.155 mg/l and 0.512 mg/l, respectively, based on increased absolute and relative liver weight and increase relative kidney weight at 0.512 mg/l.

Protocol Deviations: Exposure temperature (17-29°C) and humidity (26-74%) were outside the desired ranges (22+/-2°C and 40-60%, respectively). Housing temperature (16-24°C) and humidity (10-86%) were outside the desired ranges also. Food consumption intervals for weeks 1B and 13 were 4 days in length, all others were seven. These protocol deviations were not considered to have effected the study outcome.

Classification. ~~SUPPLEMENTARY~~ MINIMUM. The study did not establish a NOEL for "mucosa: pseudostratified ciliated/nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia" in the larynx. Refer to page 665 of the study. ~~The study may be upgraded if a clarification of the hyperplasia is provided with respect to its distinction from metaplasia and its actual frequency and intensity at the lowest test dose removes a concern for its presence.~~ ml 6/10/94

AT This Time ml
This study does ~~not~~ satisfy the guideline requirement for a subchronic inhalation study (82-4) in rats.

TABLE 1. Characteristics of Exposure Atmospheres for a Subchronic Inhalation Study with Piperonyl Butoxide using Sprague Dawley Rats [a]

	Group I-Control	Group II	Group III	Group IV	Group V
Target Concentration (mg/l)	0	0.015	0.070	0.150	0.500
Nominal Concentration (mg/l)	-	0.047	0.230	0.593	2.625
Gravimetric Concentration[b] (mg/l)	2E-5±5E-5	0.015±0.001	0.071±0.004	0.150±0.013	0.499±0.042
Analytic Concentration[b] (mg/l)	33E-7±3E-5	0.015±0.002	0.074±0.005	0.155±0.013	0.512±0.062
Average Mass Median Aerodynamic Diameter(µm) (Mean 1.7)	-	1.6	1.4	1.9	2.0
Average Geometric Standard Deviation (Mean 2.6)	-	2.8	2.6	2.5	2.3
Average % of Particles ≤ 1 µm (Mean 29)	-	33	37	25	21
Average % of Particles ≤ 10 µm (Mean 97)	-	96	98	96	97
Mean Chamber Temperature	24	24	24	24	25
Minimum	20	20	20	20	21
Maximum	27	27	27	27	28
Mean Relative Humidity %	52	51	52	53	52
Minimum	37	33	42	44	31
Maximum	64	65	62	68	65

[a]Data were extracted from Section III (Results and Discusssion) and Appendix B of the study report.
[b]Values represent the mean and standard deviation.

011066

TABLE 2. In-Chamber Clinical Signs Exhibited by Rats (Males and Females Combined, N=15 per sex, per group) Exposed to Piperonyl Butoxide for Three Months[a,b]

	Incidence (%) at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Dried red nasal discharge					
35 days	0	0	0	0	0
40 days	0	0	0	0	10-30
45 day	0	0	0	0	10-30
50 days	0	0	0	0	0
55 days	0	0	0	0	10-30
60 days	0	0	0	0	0
65 days	0	0	0	0	0
Dried red material on facial area					
35 days	0	0	0	0	0
40 days	0	0	0	0	0
45 days	0	0	0	0	0
50 days	0	0	0	10-30	0
55 days	0	0	0	10-30	0
60 days	0	0	0	10-30	0
65 days	0	0	0	10-30	0
Dried brown material on facial area					
35 days	0	0	0	0	40-60
40 days	0	0	0	40-60	40-60
45 days	0	0	0	10-30	10-30
50 days	0	0	0	0	40-60
55 days	0	0	0	0	40-60
60 days	0	0	0	0	40-60
65 days	0	0	0	0	40-60

TABLE 2 (continued).

	Incidence (%) at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Matted coat					
35 days	0	0	0	0	40-60
40 days	0	0	0	0	70-90
45 days	0	0	0	10-30	70-90
50 days	0	0	0	10-30	40-60
55 days	0	0	0	10-30	40-60
60 days	0	0	0	10-30	40-60
65 days	0	0	0	10-30	40-60

[a]Data were extracted from Appendix D of the study report
[b]The numbers in the table represent the percentage of animals with the specified clinical sign

NOTE: In-chamber observations were done only on days 35-67.

TABLE 3. In-Life Clinical Signs (at Selected Intervals) Exhibited by Rats (N = 15 per sex, per group) Exposed to Piperonyl Butoxide for Three Months[a,b]

Males	Incidence at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Excess Lacrimation					
week 1	0	0	0	0	0
week 6	1	0	0	0	1
week 13	0	0	0	0	0
Dried Red Nasal Discharge					
week 1	0	1	0	2	4
week 6	1	5	8	13	14
week 13	0	6	6	11	15
Red Nasal Discharge					
week 1	0	0	1	1	0
week 6	0	0	1	0	3
week 13	0	0	0	3	1
Mucoid Nasal Discharge					
week 1	0	0	0	0	4
week 6	0	0	0	0	2
week 13	0	0	0	1	0
Matted Coat					
week 1	0	0	0	0	9
week 6	0	0	0	3	15
week 13	0	0	0	6	15

TABLE 3 (continued).

Males	Incidence at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Dried Brown Material on Facial Area					
week 1	0	0	0	0	1
week 6	0	2	4	6	7
week 13	0	1	2	3	10
Dried Brown Material on Extremities					
week 1	0	0	0	0	0
week 6	0	0	0	0	1
week 13	0	0	0	0	1
Yellow Ano-genital Staining					
week 1	0	0	0	0	0
week 6	0	0	0	0	2
week 13	0	0	0	0	5
Dried Red Material on Facial Area					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	0	0	0
Brown Material on Tail					
week 1	0	0	0	0	0
week 6	0	0	0	0	4
week 13	0	0	0	0	7
Dried Black Material on Facial Area					
week 1	0	0	0	0	0
week 6	1	1	0	0	0
week 13	1	0	0	0	0

TABLE 3 (continued).

Males	Incidence at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Brown Material on Fur					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	0	1	0
Brown Material on Extremities					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	0	0	3
Brown Ano-genital Staining					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	0	0	1

TABLE 3 (continued).

Females	Incidence at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Excess Lacrimation					
week 1	0	0	0	0	0
week 6	0	0	0	0	1
week 13	0	0	0	1	0
Dried Red Nasal Discharge					
week 1	0	0	0	3	0
week 6	3	2	9	7	13
week 13	3	8	14	14	15
Red Nasal Discharge					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	1	0	0
Mucoid Nasal Discharge					
week 1	0	0	0	0	2
week 6	0	0	0	0	0
week 13	0	0	0	2	1
Matted Coat					
week 1	0	0	0	0	10
week 6	0	0	0	3	15
week 13	0	0	0	6	15
Dried Brown Material on Facial Area					
week 1	0	4	10	6	14
week 6	2	8	14	14	15
week 13	3	8	14	13	15

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TABLE 3 (continued).

Females	Incidence at each Concentration (mg/l)				
	0	0.015	0.074	0.155	0.512
Yellow Ano-genital Staining					
week 1	0	0	0	0	0
week 6	0	0	0	1	4
week 13	0	0	1	3	14
Dried Red material on Facial Area					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	0	0	0	0
Brown Material on Tail					
week 1	0	0	0	0	0
week 6	0	0	2	1	8
week 13	0	0	1	2	12
Dried Black Material on Facial Area					
week 1	0	0	0	0	0
week 6	0	0	0	0	0
week 13	0	1	0	2	0
Brown Material on Extremities					
week 1	0	0	0	0	0
week 6	0	0	2	1	1
week 13	0	0	2	4	10

[a]Data were extracted from Appendix D of the study report

TABLE 4. Mean Clinical Chemistry Values at Terminal Sacrifice for Rats Exposed to Piperonyl Butoxide for Three Months[a,b]

Exposure Group	SGOT IU/L	SGPT IU/L	ALK PHOS IU/L	BUN mg/dl	GLU mg/dl	TOTAL PROT g/dl	ALB g/dl	NA+ mEg/l
				Males				
Air Control	62±15	29±6	65±14	13±2	165±49	6.5±0.4	4.0±0.4	146±2
15 mg/m3	60±13	31±5	77±12*	12.4±1.4	166±32	6.6±0.3	4.1±0.3	146±1
70 mg/m^3	57±6	28±4	65±16	12.9±1.5	160±23	6.6±0.3	4.0±0.2	147±1
150 mg/m^3	53±9	27±4	64±12	13.2±1.2	146±24	6.7±0.4	4.2±0.2	146±2
500 mg/m^3	51±9*	25±3*	62±8	14.9±1.4**	149±31	7.1±0.4**	4.4±0.2**	147±2
				Females				
Air Control	53±10	29±11	40±11	13.3±1.9	162±27	7.2±0.5	4.8±0.4	147±2
15 mg/m3	53±10	28±6	38±7	13.8±3.2	158±31	7.0±0.5	4.6±0.4	148±2*
70 mg/m^3	48±6	27±7	44±13	14.8±4.6	159±30	7.2±0.4	4.7±0 3	148±2
150 mg/m^3	60±25	33±19	41±9	13.2±2.1	151±30	7.4±0.4	4.7±0.4	149±2*
500 mg/m^3	47±9	21±4*	37±11	14.6±1.6	133±16*	7.6±0.5	5.0±0.3	147±2

[a]Data were extracted from Apendix I of the study report.
[b]Mean standard deviation

*Significantly different from air control; p≤0.05.
**Significantly different from air control; p≤0.01.

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TABLE 5. Absolute and Relative Organ Weights of Rats Exposed to Piperonyl Butoxide for Three Months[a,b]

Exposure Group	Absolute Organ Weight (grams)		Organ to Body Weight Ratio x 1000	
	Liver	Kidneys	Liver	Kidneys
		Males		
Air Control	14.83 ± 1.92	4.10 ± 0.409	2.63 ± 0.17	7.30 ± 0.69
15 mg/m³	14.84 ± 2.90	4.09 ± 0.492	2.66 ± 0.27	7.38 ± 0.60
70 mg/m³	14.76 ± 1.66	4.03 ± 0.432	2.73 ± 0.16	7.45 ± 0.53
150 mg/m³	15.78 ± 1.90*	4.11 ± 0.322	2.83 ± 0.21*	7.39 ± 0.60
500 mg/m³	18.20 ± 1.45**	4.39 ± 0.440	3.39 ± 0.21**	8.20 ± 0.74**
		Females		
Air Control	9.12 ± 1.44	2.37 ± 0.306	2.76 ± 0.18	7.22 ± 0.75
15 mg/m³	9.08 ± 1.36	2.5 ± 0.231	2.73 ± 0.29	7.55 ± 0.93
70 mg/m³	9.23 ± 1.00	2.38 ± 0.217	2.79 ± 0.19	7.19 ± 0.52
150 mg/m³	9.58 ± 1.02	2.42 ± 0.209	3.01 ± 0.25*	7.60 ± 0.69
500 mg/m³	10.90 ± 1.46	2.52 ± 0.236	3.43 ± 0.31**	7.97 ± 0.70*

[a]Data were extracted from Appendix J of the study report.
[b]Mean ± standard deviation

* Significantly different from air control; $p \leq 0.05$
** Significantly different from air control; $p \leq 0.01$

TABLE 6. Incidence of Lesions in the Larynx from Blind Histopathology on Rats Exposed to Piperonyl Butoxide for Three Months[a]

	Lesions - Ventral Diverticulum Incidence at each Concentration (mg/l)				
Males	0	0.015	0.074	0.155	0.512
Number Examined	15	15	15	15	15
Mucosa					
Pseudostratified ciliated/ nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	0	0	0	0	11
Stratified squamous epithelium-hyperplasia	0	0	0	0	1
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	1
Subacute (chronic active)/ chronic inflammation	15	14	15	13	15
Granulomatous inflammation/ granuloma	0	0	1	0	0
Ventral diverticulum					
Columnar epithelium-squamous/squamoid metaplasia/hyperplasia	0	0	0	0	12

TABLE 6 (continued).

Males	Lesions - Ventral Seromucous Glands Incidence at each Concentration (mg/l) 0	0.015	0.074	0.155	0.512
Number Examined	14	15	15	15	15
Mucosa					
Pseudostratified ciliated/ nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	0	7	14	14	15
Stratified squamous epithelium-hyperplasia	0	0	0	0	1
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	1
Subacute (chronic active)/ chronic inflammation	14	15	14	14	15
Granulomatous inflammation/ granuloma	1	0	2	0	1

TABLE 6 (continued).

Expanded Incidence of Lesions (Ventral Seromucous Glands and Ventral Diverticulum) and Severity at each Concentration (mg/l)

Males	0	0.015	0.074	0.155	0.512
Number Examined	15	15	15	15	15
Mucosa					
Pseudostratified ciliated/nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	0	7	14	14	15
Severity 1 - Minimal	0	7	14	13	1
Severity 2 - Slight	0	0	0	1	14
Stratified squamous epithelium-hyperplasia	0	0	0	0	1
Severity 1 - Minimal	0	0	0	0	1
Severity 2 - Slight	0	0	0	0	0
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	1
Severity 1 - Minimal	0	0	0	0	1
Severity 2 - Slight	0	0	0	0	0
Subacute (chronic active)/ chronic inflammation	15	15	15	15	15
Severity 1 - Minimal	1	6	3	3	0
Severity 2 - Slight	12	9	12	12	7
Severity 3 - Moderate	2	0	0	0	8
Average Severity	2.07	1.60	1.80	1.80	2.53
Granulomatous inflammation/ granuloma	1	0	2	0	1
Severity 1 - Minimal	0	0	1	0	0
Severity 2 - Slight	1	0	0	0	1
Severity 3 - Moderate	0	0	1	0	0
Ventral diverticulum					
Columnar epithelium-squamous/squamoid metaplasia/hyperplasia	0	0	0	0	12
Severity 1 - Minimal	0	0	0	0	12

TABLE 6 (continued).

Females	Lesions - Ventral Seromucous Glands Incidence at each Concentration (mg/l) 0	0.015	0.074	0.155	0.512
Number Examined	15	14	15	15	15
Mucosa					
Pseudostratified ciliated/ nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	1	13	14	15	15
Stratified squamous epithelium-hyperplasia	0	0	0	0	3
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	3
Subacute (chronic active)/ chronic inflammation	15	14	15	15	15
Granulomatous inflammation/ granuloma	2	2	0	6	6

TABLE 6 (continued).

Females	Lesions - Ventral Diverticulum Incidence at each Concentration (mg/l) 0	0.015	0.074	0.155	0.512
Number Examined	15	15	15	15	15
Mucosa					
Pseudostratified ciliated/ nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	0	0	0	0	13
Stratified squamous epithelium-hyperplasia	0	0	0	0	2
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	2
Subacute (chronic active)/ chronic inflammation	15	15	12	13	15
Granulomatous inflammation/ granuloma	2	0	0	0	2
Ventral diverticulum					
Columnar epithelium-squamous/squamoid metaplasia/hyperplasia	0	1	0	0	13

Table 6 (continued).

Expanded Incidence of Lesions (Ventral Seromucous Glands and Ventral Diverticulum) and Severity at each Concentration (mg/l)

Females

	0	0.015	0.074	0.155	0.512
Number Examined	15	15	15	15	15
Mucosa					
Pseudostratified ciliated/nonciliated columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	1	13	14	15	15
Severity 1 - Minimal	1	13	14	14	7
Severity 2 - Slight	0	0	0	1	8
Stratified squamous epithelium-hyperplasia	0	0	0	0	3
Severity 1 - Minimal	0	0	0	0	1
Severity 2 - Slight	0	0	0	0	2
Stratified squamous epithelium-hyperkeratosis	0	0	0	0	3
Severity 1 - Minimal	0	0	0	0	1
Severity 2 - Slight	0	0	0	0	2
Subacute (chronic active)/ chronic inflammation	15	15	15	15	15
Severity 1 - Minimal	0	3	4	1	0
Severity 2 - Slight	13	12	11	13	7
Severity 3 - Moderate	2	0	0	1	8
Average Severity	2.13	1.80	1.73	2.0	2.53
Granulomatous inflamm./granuloma	2	2	0	6	6
Severity 1 - Minimal	0	0	0	3	1
Severity 2 - Slight	2	2	0	2	3
Severity 3 - Moderate	0	0	0	1	2
Ventral diverticulum					
Columnar epithelium-squamous/ squamoid metaplasia/hyperplasia	0	1	0	0	13
Severity 1 - Minimal	0	1	0	0	13

[a]Data were extracted from Table VI and Appendix K of the study report, averages were calculated by the reviewers

[b]Severity: 1-minimal, 2-slight, 3-moderate, 4-marked, 5-severe

TABLE 7. Blind Histopathology Results - Severity of Hepatocellular Cytoplasm: Vesiculated/Vacuolated in Rats Exposed to Piperonyl Butoxide for Three Months[a,b]

Concentration mg/l	Number of Animals Examined	Incidence of Vesiculation/ Vacuolation	Average Lesion Severity
Males			
0	15	15	1.27
0.015	15	15	1.27
0.074	15	15	1.20
0.0155	15	15	1.07
0.512	15	15	1.60
Females			
0	14	14	1.21
0.015	15	15	1.33
0.074	15	14	1.14
0.0155	14	14	1.36
0.512	15	15	1.47

[a]Data were extracted from Appendix K of the study report, averages were calculated by the reviewers.
[b]Severity: 1-minimal, 2-slight, 3-moderate, 4-marked, 5-severe