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

004106

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Acute, Subacute, Mutagenicity, and Teratology Studies With Folpet.
Accession Nos. 253165, 253166, 253167; CASWELL #464.

TO: Henry M. Jacoby (21)
Registration Division (TS-767)

FROM: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

D. S. Saunders Jr. 11-23-84

THRU: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)
and
William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

LDC 11/23/84

Chemical: Folpet (Phaltan, Folpan; N-[trichloromethyl]thiophthalimide).

Action Requested

Review the studies listed below that were submitted in support of the registration of Folpet. All studies were done with the technical grade of Folpet, with the exception of the acute and subchronic inhalation studies, which were done with the 50% WP.

Study type	Study No.
1) Acute oral LD_{50}- rats	S-2151
2) Acute dermal LD_{50}- rabbits	S-2152
3) Primary eye irritation- rabbits	S-2074
4) Primary skin irritation- rabbits	S-2075
5) Acute inhalation- rats	S-648
6) Dermal sensitization- guinea pigs	SOCO 63/II:69
7) Teratology- rabbits	303-002
8) In vivo cytogenetics- rats	MRI-225-CCC-83-32
9) Reverse mutation- Salmonella	S-1261
10) Subacute inhalation- rats	CHR-2/747:5

In addition to the studies listed above, a proposed label was submitted for review.

(con't)

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Discussion/Recommendations

It is recommended that Folpet be considered for Special Review. A positive mouse oncogenicity study has been submitted and reviewed (memo of 7-19-83 W. Teeters to H. Jacoby). A dose-related increase in the incidence of malignant duodenal adenocarcinoma, a rare type of tumor in mice, was demonstrated in that study. In the present submission, positive teratogenic findings of hydrocephalus in rabbits were reported with only minimal maternal toxicity. Also included in the present submission was a reverse mutation study in bacteria which demonstrated that Folpet was a direct-acting mutagen (i.e. no metabolic activation required for mutagenicity). These findings are consistent with a proposed mechanism of toxicity for Folpet, and its structural analogue Captan, which suggests that under alkaline conditions (such as are encountered in the intestine) thiophosgene, a highly reactive metabolite, is liberated. Reactive metabolites that bind covalently to tissue macromolecules have been implicated as a mechanism of toxicity for a number of toxic chemicals.

In order to get a rough estimate of the teratogenic risk of Folpet, we have calculated a Margin of Safety (MOS) for some common foods using tolerances currently esatbiished for Folpet on these foods. It should be noted that there are tolerances for many more foods than those listed below. Serving sizes were obtained from the "Family Food Buying" guide, Home Economics Research Report #37, USDA (1977). Some common foods with established tolerances for Folpet were selected from the list below, and combined to form a hypothetical dietary intake in a single day. Estimates are based on an average body weight of 60 kg for a pregnant female.

Food	Tolerance (ppm)	Grams/ serving	Human dose (ug/kg/day)	Margin of Safety
Onion	50	67.3	56.0	178.6
Celery	50	170.0	141.7	70.6
Lettuce	50	79.0	65.8	152.0
Tomato	25	227.0	94.6	105.7
Grape	25	90.8	37.8	264.6
Avocado	25	103.3	43.0	232.6
Apple	25	151.3	63.1	158.5
Raisins	25	141.7	69.0	144.9
Cucumber	15	162.3	40.6	246.3
Squash	15	106.8	26.7	374.5
Oranges	15	259.4	64.9	154.1
Grapefruit	15	227.0	56.8	176.1

If a person were to eat a single serving of onion, celery, lettuce, tomato, cucumber (e.g. a salad), grapefruit, and apple in one day, the total dose of Folpet consumed would be 518.6 ug/kg/day. Based on the rabbit teratology NOEL of 10 mg/kg/day, the MOS of such a diet would be 10/0.52 = 19.3. Other combinations are, of course, possible. However, it is clear that the large tolerances currently established for Folpet on a number of common foods are not supported by available toxicolgical data.

(con't)

An oncogenic risk assessment for Folpet is in progress but will not be completed until around Jan. 1, 1985. Based upon preliminary risk calculations, this reviewer has strong concerns as to the oncogenic potential of this compound. Further, if one considers the positive teratogenic and mutagenic findings for Folpet, its proposed mechanism of toxicity, and the substantial residue tolerances currently established for this chemical, Folpet appears to be a strong candidate for Special Review.

DER Summary

1) The acute oral study (#S-2151) was classified as Core-Minimum data. The LD_{50} for male rats was calculated by the investigators to be 43.8 g/kg, and for females was 19.5 g/kg. These values correspond to Toxicity Category IV (>5.0 g/kg).

2) The acute dermal study (#S-2152) was classified as Core-Guideline data. The dermal LD_{50} in rabbits was determined to be >5.0 g/kg, the only dose tested. This value corresponds to Toxicity Category III (2.0 - 20.0 g/kg).

3) The eye irritation study (#S-2074) demonstrated that reversible corneal opacity occurred in unrinsed eyes, with a maximum Draize score of 16 at 72 hours. Rinsed eyes exhibited no corneal opacity, and the Draize score at 72 hours was 0.67. All effects were reversible by 14 days. These findings correspond to Toxicity Category II. This study was classified as Core-Guideline data.

4) The primary skin irritation study (#S-2075) was classified as Core-Guideline data. The Primary Irritation Score (PIS) was 0 for abraded or intact skin, which corresponds to Toxicity Category IV.

5) The acute inhalation study (#S-648) was classified as Core-Supplementary data. No measure of particle size was reported, and only one dose level was tested. Agency guidelines allow for single-dose limit testing of 5 mg/L for a four-hour exposure period, in contrast to this study in which a single dose of 13.6 mg/L for 1 hour was used. No calculation of LC_{50} was possible from these data.

6) The skin sensitization study (#SOCO 63/II:69) was classified as Core-Guideline data. Folpet was determined to be a skin sensitizer, and cross-reactivity with Captan was demonstrated.

7) The rabbit teratology study (#303-002) was classified as Core-Minimum data. A dose-related increase in the incidence of fetuses with hydrocephalus and related skull malformations was noted in litters from the mid and high dose dams. These dams exhibited only minimal maternal toxicity. The incidences of malformations were higher than concurrent study controls or historical control data supplied by the investigating laboratory. The teratogenic/fetotoxic NOEL was 10 mg/kg, and the teratogenic LEL was 20 mg/kg. The maternal LEL was established as 20 mg/kg based on alterations in food consumption and body weight gain during gestation. No other toxic signs or lesions were noted in treated dams. The maternal NOEL was 10 mg/kg.

(con't)

8) The in vivo cytogenetics study (#MRI-225-CCC-83-32) was classified as Unacceptable. Although no chromosomal abnormalities were demonstrated, the doses used in the study were not sufficient to produce any toxic effects in the target tissue, the bone marrow. The high dose in this study was 2.0 g/kg, in contrast to the range-finding study in which rats survived a single oral dose as high as 16 g/kg, or the oral LD_{50} of 20-40 g/kg in rats.

9) The reverse mutation study in Salmonella (#S-1261) was classified as Acceptable. The data clearly demonstrate that Folpet is a direct-acting mutagen. However, because no assessment of the effect of metabolic activation was provided in this study, a gene mutation study in which the effect of metabolic activation is assessed will still be required. Therefore, this study does not completely satisfy the minimum data requirement for gene mutation testing.

10) The subacute inhalation study (#CHR-2/74725) was classified as Core-Supplementary data. Rats were allowed a 12-day recovery period before necropsy, only one dose level was used, histopathological examination was inadequate, no serum chemistry or urinalysis determinations were done, no food consumption data and no individual animal data for physical signs were submitted. No treatment-related lesions were noted at the single dose of 0.048 mg/l. for 5 days/week for three weeks.

11) The proposed label is consistent with established guidelines for acute hazards. However, in consideration of potential chronic hazards associated with this compound, Toxicology Branch defers further comment on the proposed label until completion of further review.

HED/TOX:DSS:DISK 6:11/21/84

Study Title: The Acute Oral Toxicity of Chevron Folpet Technical (SX-1356) in Adult Male and Female Rats.

Accession No.: 253165

Study No.: S-2151

Sponsor/Contracting Lab.: Chevron Chemical Co./Chevron Environmental Health Center, Richmond, California.

Report Date/Submitted: 3-4-83/5-2-84

Test Material- Folpet technical, a pale yellow powder, code SX-1346, 91.2% a.i.

Test Animal- Male and female Sprague-Dawley derived rats (Simonsen Labs., Gilroy, CA.); 5/sex/group.

Doses Tested- 0, 5, 6.5, 8.5, 11.2, 14.8, 20.0, 26.5 g/kg (gavage, 22.0 or 52.6 ml/kg).

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

1) The submitted study is actually composed of two separate studies: one initiated on 8-19-82, with doses of 5.0 to 8.5 g/kg in a volume of 22.0 ml/kg, and a second study initiated on 1-13-83, with doses of 11.2 to 26.3 g/kg in a volume of 52.6 ml/kg. In principle, dose-response studies should be conducted such that all doses are given to the same population of animals under identical conditions of treatment. This was not the case in the present study. However, given the low degree of acute toxicity of the test article, this deficiency does not alter the interpretation of the study.

Results

A. Mortality- The effect of the test article on animal survival is presented in table 1. The oral LD_{50} for males was calculated by the investigators to be 43.8 g/kg (95% c.i. = 3.5 to 556 g/kg), and the LD_{50} for females was calculated to be 19.5 g/kg (95% c.i. = 7.5 to 51 g/kg).

Because of the large 95% confidence interval for the male LD_{50} value, the male and female LD_{50} values were recalculated by Dr. Herbert Lacayo of the Toxicology Branch Statistics Team using the SAS package. By this method, the male LD_{50} was calculated to be 30.76 g/kg, with a 95% c.i. of 19.5 to 7.42×10^{11} g/kg, a nonsensical result due to the non-linear response of the males. For females, the LD_{50} was calculated by Dr. Lacayo to be 19.50 g/kg, with a 95% c.i. of 12.6 to 205.1 g/kg.

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Table 1. Effect of Oral Folpet on Animal Survival to 14 Days[a]

Experiment[b]	Dose g/kg	Mortality Males	Females
1	0	0/5	0/5
2	0	0/5	0/5
1	5.0	0/5	0/5
1	6.5	0/5	1/5
1	8.5	0/5	0/5
2	11.2	1/5	2/5
2	14.8	0/5	3/5
2	20.0	3/5	3/5
2	26.3	1/5	2/5

[a]data excerpted from tables 3 and 4 of submitted study.

[b]experiment 1 initiated 8-19-82; experiment 2 initiated 1-13-83.

B. Physical Signs and Body Weights- Signs noted in animals given doses of 11.2 g/kg or greater included decreased motor activity, diarrhea, reduced food intake, and ocular discharge.

An apparent dose-related decrease in body weight gain was noted. However, because the test animals came from two separate populations with unequal body weights at study initiation (experiment 1 initial B.W. = 274 ± 4 grams, experiment 2 initial B.W. = 227 ± 4 grams), and the volumes of administration were different in the two experiments the apparent dose-related effect of the test article on weight gain is not scientifically valid.

C. Pathologic Findings- No significant treatment-related effects were noted on gross or microscopic examination.

Conclusion

The oral LD_{50} of technical Folpet in rats was calculated (by the investigators) as follows:

Males: 43.8 g/kg (3.5-556 g/kg)
Females: 19.5 g/kg (7.5-51 g/kg)

These values correspond to toxicity catagory IV (>5.0 g/kg).

Classification: Core-Minimum Study conducted in two separate phases.

Acute Oral LD50

S-2151

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IV. MATERIALS AND METHODS

A. Materials

1. Test Material: The CHEVRON Folpet Technical used in this study was a pale yellow powder, coded SX-1346. It was supplied by Chevron Chemical Company, Ortho Division, Richmond, California, and was received on May 21, 1982.

2. Vehicle: The vehicle used was 1% carboxymethyl cellulose (CMC) in distilled water. This vehicle is known to meet all EPA specifications for testing [40 CFR 163.80-3, (b)(3)(iv)].

3. Animals: Young adult male (208-295 gms) and female (198-248 gms) Sprague-Dawley derived rats, supplied by Simonsen Laboratories, Gilroy, California, were used in this study. They were allowed a conditioning period of 16-24 days prior to dosing in our laboratory. The males were 57-89 days old and the females were 84-89 days old at the time of dosing. The animals were housed individually in wire-bottom cages in an air-conditioned room maintained at approximately 21°C, and the relative humidity ranged from 51-78%. The animals were on a 12-hour light/dark cycle. The animals had free access to Purina Laboratory Rodent Chow #5001 and water except during the overnight period prior to dosing when only water was available.

B. Methods

General procedures for animal husbandry, identification and randomization and retention of samples and raw data are presented in Appendix A.

Single doses ranging from 5.0 g/kg to 26.3 g/kg of the test material diluted in 1% carboxymethyl cellulose in distilled water at concentrations ranging from 227 mg/ml to 500 mg/ml were administered intragastrically to groups of five fasted rats of each sex approximately 6-9 hours after the onset of the light cycle. Five fasted rats of each sex were dosed with 22.0-52.6 ml/kg of 1% carboxymethyl cellulose in distilled water and served as the controls. The animals were observed frequently on the day of dosing and at least once each morning and late afternoon for 14 days after treatment, except on weekends, when the animals were observed once daily. The LD50, slope and 95% confidence limits were determined using the method of Berkson (1).

The animals were weighed prior to dosing and at 7 and 14 days after treatment. The body weights of the treated animals in groups with complete survival were compared to controls using a Student t-test.

Animals that died during the study and all survivors sacrificed following the 14-day observation period were examined for gross pathological changes. The following organs and tissues were examined: skin, spleen, pancreas, stomach, small and large intestine, liver, adrenals, kidneys, gonads, uterus or seminal vesicles, bladder, heart, thymus, salivary glands, lungs, trachea, thyroid, and fat.

The study was initiated on August 19, 1982, and completed on January 27, 1983.

V. RESULTS

The mean (+S.D.) volume and weight of the test material administered to each group of animals are given in Tables 1 and 2. The LD50 and 95% confidence limits were 43.8 (3.5-556) g/kg for males (Table 3) and 19.5 (7.5-51) g/kg for females (Table 4). The slope and 95% confidence limits were 4.7 (0.27-81) for males and 3.5 (0.62-19) for females. At the highest dose level, the incidence of mortality leveled off, suggesting that the extent of absorption due to the low solubility of the test material in aqueous solutions rather than the dose was a limiting factor.

Signs of toxicity observed during the study that were attributed to treatment with the test material were: decreased motor activity, reduced food intake, weakness, ocular discharge, nasal discharge, dyspnea, scruffiness, discolored fur, chewed feet and toes, collapse, and death. The mean body weight of the males dosed at 6.5 g/kg was significantly less ($p \leq 0.01$) than that of controls at 7 and 14 days after dosing. There were no other significant differences in mean body weights between groups (Tables 5 and 6). At necropsy, slightly grainy livers and kidneys were observed in some animals. Histopathological examination of these tissues revealed no microscopic changes that could be attributed to treatment with the test material (Appendix B).

VI. REFERENCES

(1) Berkson, J., Tables for use in estimating the normal distribution function by normit analysis. Biometrika, 44: 411-435, 1957.

Study Title: The Acute Dermal Toxicity of Chevron Folpet Technical (SX-1346) in Adult Male and Female Rabbits.

Accession No.: 253165

Study No.: S-2152

Sponsor/Contracting Lab.: Chevron Chemical Co./Chevron Environmental Health Center, Richmond, California.

Report Date/Submitted: 10-11-82/5-2-84

Test Material- Folpet technical, a pale yellow powder, code SX-1346, 91.2% a.i.

Test Animal- Male and female New Zealand white rabbits (Nitabell Rabbitry, Hayward, CA.); 5/sex/group.

Doses Tested- 5 g/kg (dermal), only dose tested.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

None.

Results

No deaths or other signs of toxicity were reported. Body weight gain was equal among control and treated animals. Hyperkeratosis, non-suppurative dermatitis, and acanthosis (thickened skin) were noted in treated females only upon histological examination of treated skin.

Conclusion

The dermal LD_{50} of technical Folpet in albino rabbits was >5.0 g/kg, the only dose tested. This value corresponds to toxicity catagory III (2.0 - 20.0 g/kg).

Classification: Core-Guideline

Acute Dermal LD50 S-1970

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IV. MATERIALS AND METHODS

A. Materials

1. Test Material: The CHEVRON Folpet Technical used in this study was a pale yellow powder, coded SX-1346. It was supplied by Chevron Chemical Company, Ortho Division, Richmond, California, and was received on May 21, 1982.

2. Animals: Young adult male (2.16 to 2.61 kg) and female (1.99 to 2.59 kg) New Zealand White rabbits, 10 to 12 weeks old were used in this study. The animals were obtained from Nitabell Rabbitry, Hayward, California, and used after a conditioning period of 15 days in our laboratory. The rabbits were individually housed in wire-bottom cages in air-conditioned rooms where the temperature was maintained at approximately 21°C, and the relative humidity ranged from 65-77%. The animals were on a 12-hour light/dark cycle. The rabbits were given a daily ration (approximately 115 grams) of Purina Laboratory Rabbit Chow #5321 and had free access to water.

B. Methods

General procedures for animal husbandry, identification and randomization and retention of samples and raw data are presented in Appendix A.

The fur on the trunks of five male and five female rabbits was clipped the day prior to testing. On the day of testing, the exposed skin was abraded with a hypodermic needle. Five grams of the test material per kilogram of body weight were mixed 1:1 (w/v) with physiological saline and applied to the trunk of each animal. The material was held in contact with the animal's skin by a plastic sheet wrapped around the animal's trunk, and paper towels were wrapped over the plastic sheet to prevent tearing. The mean weights (±S.D.) of test material administered were 12.2 (0.63) g (males) and 11.6 (0.95) g (females). The animals were dosed approximately 9-11 hours after the onset of the light cycle. Five male and five female clipped and abraded rabbits, treated with 5 ml/kg of physiological saline were wrapped as described above and served as the controls. After a 24-hour exposure period, the wrappings and any remaining material were removed from the animals. Collars were placed on the animals for six days to prevent oral ingestion of test material. The animals were observed frequently on the day of dosing and at least once in the morning and late afternoon for 14 days after treatment, except on weekends, when they were observed once a day.

The animals were weighed prior to dosing and at 7 and 14 days after treatment. The weights of the treated animals were compared to the weights of the control animals using a Student t-test.

After the observation period, the animals were sacrificed and examined for gross pathological changes. The following organs and tissues were examined: skin, spleen, pancreas, stomach, small and large intestine, liver, adrenals, kidneys, gonads, uterus or seminal vesicles, bladder, heart, thymus, salivary glands, lungs, trachea, thyroid, and fat. Sections of skin from each animal and any other abnormal appearing tissue were submitted for histopathological evaluation.

The study was initiated on July 14, 1982, and completed on July 28, 1982.

V. RESULTS

No deaths occurred during the study, and no signs of toxicity were observed. There were no significant differences between body weights of treated and control groups (Table 1). At necropsy, no gross pathological changes attributable to the test material were observed. Histopathological evaluation revealed mild hyperkeratosis, mild non-suppurative dermatitis, and mild acanthosis only in treated females (Appendix B). No other histopathological changes were observed.

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Study Title: The Eye Irritation Potential of Phaltan Technical (PN 2623).

Accession No.: 253165

Study No.: S-2074

Sponsor/Contracting Lab.: Chevron Chemical Co./Chevron Environmental Health Center, Richmond California.

Report Date/Submitted: 8-3-82/5-2-84

Test Material- Phaltan [Folpet] technical (PN 2623), a cream-colored powder, code SX-1346, 91.2% a.i.

Test Animal- Male New Zealand White rabbits (Nita Rabbitry, Hayward, CA.); 9 total, 6 unrinsed, 3 rinsed.

Doses Tested- 100 mg/eye of un ' test material.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

None.

Results

Complete corneal opacity with iritis was observed in the eye of 1/6 rabbits in the absence of rinsing. Both signs were present at 7 days but absent at 10 days. Iritis only, of 72 hours duration, was noted in the eye of one other rabbit in the absence of rinsing. The maximum mean Draize score of 16 (range 2-55) for treated animals without rinsing was noted at 72 hours. Redness, chemosis, and discharge were noted in all treated, unrinsed eyes within 1 hour of treatment. All effects were absent by 14 days after treatment.

No corneal opacity was noted in the eyes of rabbits that were rinsed after treatment with the test article. Redness, chemosis and discharge were noted in the conjunctivae of these animals within 1 hour of treatment. Eyes appeared normal in 2/3 animals at 24 hours, however discharge was present in 1/3 animals for 96 hours after treatment.

Conclusion

Draize score at 72 hours (unrinsed): 16 (range 2-55), reversible by 14 days.
Draize score at 72 hours (rinsed): 0.67 (range 0-2), reversible by 14 days.

These values correspond to Toxicity Category II.

Classification: Core-Guideline

IV. MATERIALS AND METHODS

A. Materials

1. Test Material: The PHALTAN Technical (PN 2623) used in this study was a cream colored powder, coded SX-1346. It was supplied by Chevron Chemical Company, Ortho Division, Richmond, California, and was received on December 8, 1981.

2. Animals: Male New Zealand White rabbits, 13-15 weeks old and free of ocular defects were used in this study. The animals were obtained from Nitabell Rabbitry, Hayward, California, and used after a conditioning period of 39 days in our laboratory. The rabbits were individually housed in wire-bottom cages in an air-conditioned room where the temperature was approximately 21°C and the relative humidity ranged from 49-73%. The animals were on a 12-hour light/dark cycle. The rabbits were given a daily ration (approximately 115 grams) of Purina Laboratory Rabbit Chow #5321 and had free access to water.

B. Methods

General procedures for animal husbandry and identification and retention of samples and raw data are presented in Appendix A.

One-hundred milligrams of the test material was placed in the conjunctival sac of one eye in each of nine rabbits. The untreated eye served as the control. Three of the rabbits were further treated by rinsing the control and treated eye for one minute at a rate of 250 milliliters per minute with distilled water 30 seconds after treatment. All the eyes were examined and graded for ocular reaction at one hour and at 1, 2, 3, 4, 7, 10, and 14 days, using the method of Draize et al. (1), which is given in Appendix B.

The study was initiated April 5, 1982, and completed April 19, 1982.

V. RESULTS

A. Treated-Unrinsed Eyes (Table 1):

Complete corneal opacity was observed in one eye, and iritis in two eyes within 72 hours after treatment. Moderate to severe conjunctival irritation was observed in most eyes during this period. All eyes appeared normal by 14 days after treatment.

B. Treated-Rinsed Eyes (Tables 2 and 3):

No corneal opacity or iritis were observed. Only slight conjunctival irritation was observed one hour after treatment and all eyes were clear by 24 hours following treatment.

Study Title: The Four-Hour Skin Irritation Potential of Phaltan Technical (PN 2623).

Accession No.: 253165

Study No.: S-2075

Sponsor/Contracting Lab.: Chevron Chemical Co./Chevron Environmental Health Center, Richmond California.

Report Date/Submitted: 8-3-82/5-2-84

Test Material- Phaltan [Folpet] technical (PN 2623), a cream-colored powder, code SX-1346, 91.2% a.i.

Test Animal- Female New Zealand White rabbits (Nitabell Rabbitry, Hayward, CA.); 6 animals total.

Doses Tested- 500 mg/test site (4/rabbit, 2 abraded, 2 intact); 4 hour exposure.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

None.

Results

No irritation of either normal or abraded skin was noted at 1, 24, 48, or 72 hours after exposure.

The Primary Irritation Score (PIS) was 0.

Conclusion

Not a skin irritant. PIS = 0.

Classification: Core-Guideline

1° Skin irritation

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IV. MATERIALS

A. Materials

1. Test Material: The PHALTAN Technical (PN 2623) used in this study was a cream colored powder, coded SX-1346. It was supplied by the Chevron Chemical Company, Ortho Division, Richmond, California, and was received on April 12, 1982.

2. Animals: Young adult New Zealand White female rabbits, aged 14-16 weeks, were used in this study. The animals were obtained from the Nitabell Rabbitry, Hayward, California, and used after a conditioning period of eight days in our laboratory. They were individually housed in wire-bottom cages in an air-conditioned room where the temperature was maintained at approximately 21°C, and the relative humidity ranged from 49-64%. The animals were on a 12-hour light/dark cycle. The rabbits were given a daily ration (approximately 115 grams) of Purina Laboratory Rabbit Chow #5321 and had free access to water.

B. Methods

General procedures for animal husbandry and identification and retention of samples and raw data are presented in Appendix A.

The fur on the backs of six rabbits was clipped the day prior to testing. One-half gram of the test material was applied to each of two intact and two abraded sites on the back of each rabbit. The epidermal abrasions (cross-hatching) were made with a hypodermic needle penetrating the stratum corneum over areas of one square inch. After application the treated area was covered with a gauze patch which was moistened with 0.5 ml of physiological saline and secured by adhesive tape. The trunk of each animal was loosely wrapped in a plastic sheet and paper towels were wrapped around the plastic sheet to prevent tearing. A collar was also placed on each animal to protect the wrappings during the exposure period. After a four-hour exposure period, the wrappings were removed and the skin wiped to remove any remaining test material. Irritation was scored at 4, 24, 48, and 72 hours and at 7 days after the end of the four-hour treatment, using the scoring method (Table 1) of Draize et al. (1).

The study was initiated April 14, 1982, and completed April 21, 1982.

V. RESULTS

The test material caused no skin irritation (Table 2).

The primary irritation score was 0 (Table 3).

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Study Title: The Acute Inhalation Toxicity of Phaltan 50

Accession No.: 253165

Study No.: S-648

Sponsor/Contracting Lab.: Chevron Chemical Co./Standard Oil Company of California, Safety Division, San Francisco.

Report Date/Submitted: 7-15-74/5-2-84

Test Material- Phaltan 50 [Folpet], code SX-599, a beige powder, 49.5% a.i.

Test Animal- Male and female rats, strain and supplier not disclosed; 5/sex/group.

Doses Tested- 13.6 mg/L, 1 hour exposure (only dose tested).

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

1) No determination of particle size (mean aerodynamic diameter).

2) Insufficient number of doses for calculation of LC_{50}.

Results

One female rat died following exposure to 13.6 mg/L air for one hour. No other mortalities were reported. Treated animals did not gain as much body weight as controls during the first week after exposure, however they recovered so that at the end of 14 days treated animals weighed the same as controls. Two of five treated males had rales, and one female had diarrhea of two days duration after exposure. No other significant effects were noted.

Conclusion

The acute inhalation LC_{50} was determined to be >13.6 mg/L in a 1-hour exposure. This value corresponds to toxicity catagory III.

Classification: Core-Supplementary No determination of particle size, inadequate number of doses for calculation of LC_{50}. Guidelines allow limit testing of 5 mg/L for 4-hour exposures.

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IV. MATERIALS AND METHODS

A. Materials

1. Test Material: The PHALTAN 50 was supplied by the Chevron Chemical Company, Ortho Division, Richmond, California. It was a beige powder and was coded SX 599. The product was micronized.

2. Animals: Adult male (280-299 grams) and female (249-280 grams) rats were used in this study. They were housed individually in hanging wire-bottom cages in an air-conditioned room where the temperature was maintained at approximately 70°F. Food and water were available ad libitum except during the exposure period.

B. Methods

An airborne dust of PHALTAN 50 was generated from a two-liter round-bottom flask into a cylindrical 21-liter exposure chamber. The test material in the flask was continuously agitated by a power-driven glass stirring rod. Compressed air was metered into the flask at a rate of 20 liters per minute.

The concentration of dust in the chamber was gravimetrically determined 15 times during the 60-minute exposure. Samples were alternately taken from three sampling ports spaced evenly along the length of the bottom of the chamber. One-minute samples of the dust were collected at a rate of 6 l/min on 0.8 micron Millipore® filters.

Five rats of each sex were exposed to the dust at an average concentration of 13.6 mg/l (±SE 1.00). The exposed animals were restrained in clear glass tapered tubes, the tapered ends of which fit into openings spaced evenly along the length of each side of the exposure chamber. Only the animals' noses projected into the exposure chamber. After the exposure the rats were returned to their cages and observed for 14 days. Five unexposed rats of each sex were held for 14 days and served as controls. The exposed and control rats were weighed pre-exposure, immediately following exposure (exposed animals only), and 1, 7 and 14 days post-exposure. Following the observation period, the rats were sacrificed with Beuthanasia®, exsanguinated and autopsied. Any gross pathological changes were recorded. The following organs and tissues were examined: thymus, heart, lungs, liver, kidneys, adrenal glands, spleen, gonads, gastro-intestinal tract, bladder, pancreas, salivary glands, body fat, skeletal muscle, eyes, teeth and skin.

V. RESULTS

Only one death occurred among animals exposed to 13.6 mg (±SE 1.00) of PHALTAN 50 per liter of air. One female died during the overnight period following exposure. The animal's lungs were edematous and "liver-like."

004106

Study Title: The Skin Sensitization Potential of Difolatan II

Accession No.: 253165

Study No.: SOCO 63/II:69

Sponsor/Contracting Lab.: Chevron Chemical Co./Standard Oil Company of California, Safety Division, San Francisco.

Report Date/Submitted: 3-3-69/5-2-84

Test Material- Phaltan [Folpet] technical, code SX 121, 91.5% a.i.

Test Animal- Male albino guinea pigs (Don B Labs., Chatsworth, CA.); 10/group.

Doses Tested- 0.3 ml/animal of 0.1% acetone solution of test material; 6 hour exposure, 3x/wk for 3 wks, challenged 14 days later.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following points:

None.

The protocol is actually a study of the cross-reactivity of Phaltan and some structural analogues (e.g. Captan, Difolatan and others). However, since the skin sensitization potential of Phaltan is measured, the study is adequate for that purpose.

Results

No irritation of the skin was noted after the initial application of the test article. The reaction at the application site after the final sensitizing dose was judged as "slight", with scores of 1 or 2. The positive control, 2,4-dinitrochlorobenzene (DNCB) caused a "marked" reaction after the final sensitization dose or challenge, with scores of 2-6.

Upon challenge, animals sensitized to technical Phaltan exhibited a "weak" response, with a score of 2+ for sensitization (table 1, photocopied from submitted study). Cross-reactivity of these animals was seen with technical Captan, which produced a "questionable" (1+) response. Animals sensitized with technical Captan or Difolatan and then challenged by exposure to technical Phaltan also exhibited sensitization reactions of 3+ (moderate) and 1+ (questionable), respectively.

Conclusion

Phaltan technical is a skin sensitizer. Animals sensitized to Phaltan also reacted to challenge with technical Captan. Animals sensitized to technical Captan or Difolatan reacted to Phaltan.

Classification: Core-Guideline

004106

COMPOUND		DEGREE OF SENSITIZATION
Technical DIFOLATAN	SX 173	3+
Purified DIFOLATAN	SX 190	3+
Technical CAPTAN	SX 115	3+
Purified CAPTAN	SX 194	3+
Technical PHALTAN	SX 121	1+ - 2+
RE 5933-6	SX 192	3+
RE 5871-5	SX 193	4+
RE 8456-4	SX 195	2+
Dinitrochlorobenzene		4+
Acetone		0

Code:
0 No sensitization
1+ questionable
2+ weak
3+ moderate
4+ strong

The results of the cross-challenge are summarized in the following table:

CHALLENGE COMPOUND	SENSITIZING COMPOUND							
	Technical DIFOLATAN	Purified DIFOLATAN	Technical CAPTAN	Purified CAPTAN	Technical PHALTAN	RE 5933-6	RE 5871-5	RE 8456-4
Technical DIFOLATAN SX 173	3+		3+		0	3+	3+	3+
Purified DIFOLATAN SX 190								
Technical CAPTAN SX 115	0		3+		1+	0	2+	2+
Purified CAPTAN SX 194		2+		3+				
Technical PHALTAN SX 121	1+		3+		2+			
RE 5433-6 SX 192						3+		
RE 5871-5 SX 193		4+		3+			4+	
RE 8456-4 SX 195		2+						3+
DIFOLATAN 80W dry powder		0						
DIFOLATAN 80W water wet powder		0		0				
Technical DIFOLATAN dry powder SX 173		0						

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004106

IV. MATERIALS AND METHODS

A. Materials

1. Animals: One-hundred male random bred albino guinea pigs, obtained from Don B Laboratories, Chatsworth, California, were used for this study. They were housed in wire bottom cages. The room in which they were kept was air-conditioned and maintained at 70° ± 2°F. Free access to food (Wayne Guinea Pig Diet) and water was allowed at all times.

2. Compounds: All materials tested were obtained from Chevron Chemical Company, Ortho Division, Richmond, California. Reagent grade acetone was used as a solvent. The following materials were used to sensitize and challenge the animals:

Material	Ortho Identification No.
Technical DIFOLATAN	SX 173
Purified DIFOLATAN	SX 190
Technical CAPTAN	SX 115
Purified CAPTAN	SX 194
Technical PHALTAN	SX 121
RE 5933-6	SX 192
RE 5871-5	SX 193
RE 8456-4	SX 195
2,4-dinitrochlorobenzene (Positive control)	
Acetone (Solvent control)	

B. Methods

The guinea pigs were prepared by clipping all of the hair from the trunk.

The animals were divided into groups of 10 each. Sensitization was attempted according to the following modification of the method described by Buehler (1).

The skin was abraded by scratching gently with a sharp hypodermic needle. 0.1% acetone solutions of the fungicides and 0.05% acetone solution of the dinitrochlorobenzene (DNCB) were used. Three-tenths of one milliliter of solution were placed on a polyethylene backed patch of Webril® and the patch placed over the abraded skin and the animal wrapped with Blenderm® tape. Six hours later, the patches were removed.

This procedure was repeated three times a week for three weeks. Fourteen days after the last application, a patch of the appropriate compound was placed on intact skin remote from the original site of application. Skin irritation was determined by the Draize method (2) 24 hours after the first application and 24 hours after the challenge application.

Seven days after the challenge, the following cross-challenges were done:

Compound Used For Sensitization	Cross Challenges
SX 115	SX 115, SX 173, SX 121
SX 121	SX 121, SX 173, SX 115
SX 173	SX 173, SX 115, SX 121
SX 190	SX 173, DIFOLATAN 80W dry, DIFOLATAN 80W wet, SX 194, SX 193, SX 195
SX 192	SX 192, SX 173, SX 115
SX 193	SX 193, SX 173, SX 115
SX 194	SX 194, SX 193, DIFOLATAN 80W wet
SX 195	SX 195, SX 173, SX 115

V. RESULTS

A. Sensitization

Twenty-four hours after the first exposure patch, no more than barely discernable erythema was seen for any of the treated groups. There was no erythema seen in the solvent control (acetone) group at this time and only barely discernable erythema in two of 10 animals in the positive control (0.05% DNCB) group. Table 1 gives the Draize scores.

Twenty-four hours after the last sensitizing dose, animals from all treated and positive control groups showed increased reaction which varied from a small increase for the Technical PHALTAN (SX 121) group to marked for the RE 5871-5 (SX 193) and DNCB treated groups. There was no significant increase in

004106

Study Title: Teratology Study in Rabbits with Folpet Technical

Accession No.: 253165

Study No.: 303-002

Sponsor/Contracting Lab.: Chevron Chemical Co./Argus Research Laboratories, Inc.

Report Date/Submitted: 2-15-84/5-2-84

Test Material- Folpet technical, an off-white powder, code no. SX-1388, 88.6% a.i.

Test Animal- Female New Zealand White (DLI:NZW) rabbits (Dutchland Labs., Denver, PA.); 20/group.

Doses Tested- 0, 10, 20, 60 mg/kg (gavage, 5ml/kg); days 6-28 of gestation.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and the following point(s) were noted:

1) Animals were treated with the test article over days 6-28 of presumed gestation. This treatment schedule is consistent with agency guidelines, however some investigators recommend dosing over days 6-18 in rabbits.

Results

A. Physical Signs and Mortality- No significant physical signs were noted that could be considered treatment-related. Observations noted in all animals without apparent dose-dependency included alopecia and soft stool. Anorexia and lacrimation were also noted in some of the test groups, however a dose-effect relationship was not apparent.

Two animals died during the experiment. Animal #7918 of the 10.0 mg/kg (low dose) group died of an apparent intubation error. Animal #7966 of the 60.0 mg/kg (high dose) group died on day 27 of gestation (day 21 of treatment). This death was considered by the investigators to be treatment-related. Relevant findings included yellow, transparent fluid in the peritoneal cavity and ulceration with bleeding in the stomach. In the opinion of this reviewer, this death could have also been due to intubation error: Folpet is not a primary skin irritant, and necropsy data were not submitted to document an effect of the test compound on the stomachs of other treated animals.

B. Body Weight and Food Consumption- (1) Maternal Weight Gain- The test compound had a variable effect on maternal weight gain. All treated animals lost weight on the average in the initial period of treatment, days 6-9 of gestation. Over days 9-12, treated animals gained more weight on the average than did controls. Over days 12-18, animals in low and mid dose groups gained less weight than control. The average body weight of high dose animals actually decreased over this period (days 12-18). Over days 24-29, animals of the low and high dose groups gained weight relative to control while animals of the mid dose group lost weight. When considered over days 6-29 or 0-29, significant decreases in weight gain were observed for the mid and high dose groups.

Table 1. Effect of Folpet on Maternal Weight Change[a]

Days	Dose (mg/kg) 0	10	20	60
6-9	0.02±0.04	-0.01±0.05	-0.02±0.06*	-0.07±0.07**
9-12	0.00±0.06	0.16±0.04	0.28±0.04	0.02±0.04
12-18	0.08±0.07	0.07±0.07	0.02±0.10*	-0.05±0.12**
18-24	0.06±0.11	0.04±0.10	0.02±0.12	0.02±0.14
24-29	0.02±0.13	0.07±0.07	-0.06±0.12	0.08±0.09
6-29	0.19±0.21	0.19±0.11	-0.02±0.34*	0.04±0.22
0-29	0.28±0.24	0.26±0.14	0.07±0.35*	0.11±0.24*

[a]data excerpted from submitted study. Values are average change in weight in kg ± std. dev.

*p<0.05, **p<0.01

(2) Food Consumption- A dose-related decrease in food consumption was noted (table 2). Food consumption was decreased by 30-50% (relative to control) in the high dose group (60.0 mg/kg) during treatment (days 6-28) however this decrease was judged statistically significant only on days 6-22. Animals of the mid dose group (20.0 mg/kg) also ate about 15-50% less food (relative to control) beginning with day 12 of gestation. However, by days 24-29, mid dose animals ate less food than either control or high dose animals.

Table 2. Effect of Folpet on Maternal Food Consumption[a]

Day	Dose (mg/kg) 0	10	20	60
5	166.8±15.9	164.9±19.9	174.7± 8.6	162.8±18.2
10	162.0±17.8	154.1±27.5	143.7±32.7	120.3±39.9**
15	150.8±33.2	149.8±41.4	117.6±65.9	76.1±43.9**
20	144.2±42.5	131.1±46.3	120.1±57.0	82.3±43.6**
25	97.0±60.7	102.3±53.4	69.8±62.8	71.2±43.4
28	101.7±60.4	103.5±49.9	51.6±41.7*	81.8±48.7

[a]data and statistics excerpted from submitted study. Values are average food consumption (grams/day) ± std. dev.

*p<0.05, **p<0.01

(3) Food efficiency- The amount of maternal body weight gain relative to food intake was highly variable in treated animals (table 3). All treated animals lost weight over days 6-9, and consequently had negative food efficiency values, whereas over days 10-12 a rebound occurred and treated animals had greater apparent food efficiency than did control animals. In contrast, efficiency in control does was relatively constant over the treatment period. The observed variable effects were therefore clearly treatment-related. Overall (days 6-29), apparent food efficiency in the control and low dose groups was similar (6.3% and 6.4%), whereas this value was altered in mid and high dose animals over days 6-29.

Table 3. Food Efficiency (%)[a]

	Dose (mg/kg)			
Days	0	10	20	60
6-9	4.04	-2.11	-4.42	-22.50
10-12	0.00	34.78	67.68	6.30
13-18	8.90	7.93	2.80	-11.82
19-24	7.69	5.55	3.37	4.08
25-29	5.29	16.49	-25.78	26.65
6-29	6.27	6.42	-0.83	2.17

[a]data excerpted from submitted study. Values are (g body weight gain/g food intake) x 100, calculated by reviewer from average weight gain and food consumption data.

C. Reproductive Data- No toxicologically significant effects of the test article on litter size, resorptions, or implantations were noted (table 4a). An apparent decrease in the % pregnant females was noted in the high dose group. It is unlikely that the test article could have an effect on the pregnancy rate since dosing commenced after implantation (i.e. day 6 of presumed gestation). These findings may be related to the practice of artificial insemination, which has been shown to be related to increased pre-implantation loss compared to naturally-mated does. Two does aborted, one each from the low and high dose groups, and one doe from the control group delivered naturally on day 28 of gestation. These does and their fetuses are not included in the summary (table 4a).

Table 4a. Reproductive Effects of Folpet[a]

Parameter	Dose (mg/kg) 0	10.0	20.0	60.0
No. pregnant	19/20	16/20	16/20	14/20
died	0	1	0	1
aborted	0	1	0	1
delivered	1	0	0	1
No. litters examined by caesarean (day 29)	18	14	16	11
Corpora Lutea	9.7 ± 2.4	10.8 ± 2.2	11.8 ± 2.5	11.5 ± 1.8
Implantations	6.8 ± 3.3	5.8 ± 3.1	7.8 ± 2.3	6.9 ± 2.9
Litter Size	5.3 ± 3.4	5.2 ± 3.1	7.2 ± 2.1	5.8 ± 2.8
Resorptions	1.5 ± 1.9	0.6 ± 1.1	0.6 ± 0.8	1.1 ± 1.6
No. does with any resorptions (%)	12 (66.7)	5 (35.7)	7 (43.8)	5 (45.4)
No. does with total resorption (%)	2 (11.1)	0 -	0 -	0 -

[a]data excerpted from submitted study.

D. Litter Data- Only one fetus was not alive after caesarean sectioning, and was from a doe of the high dose group (table 4b). This fetus also had hydrocephalus, which was noted with increased frequency in the mid and high dose groups. Other parameters, such as fetal body weights and the percent of males, were not altered in a statistically significant manner, however the data suggest a possible treatment-related decrease in the body weights of fetuses from the mid and high dose groups.

Table 4b. Litter Effects of Folpet[a]

Parameter	Dose (mg/kg) 0	10.0	20.0	60.0
Live fetuses/litter	5.3 ± 3.4	5.2 ± 3.1	7.2 ± 2.1	5.7 ± 2.8
Dead fetuses/litters	0/18	0/14	0/16	1/11
Mean fetal body weight -male	46.0 ± 7.8	47.7 ± 6.8	42.1 ± 8.3	44.9 ± 9.2
-female	48.1 ± 7.6	47.1 ± 5.9	40.3 ± 8.0*	42.2 ± 7.6
-both	46.8 ± 7.1	48.0 ± 6.8	41.6 ± 7.8	44.6 ± 9.1
% male fetuses	46.9	50.7	48.7	49.2

[a]data and statistics excerpted from submitted study; *$p \leq 0.05$.

E. Fetal Malformations- (1) External- One fetus from the mid dose and three fetuses from two litters of the high dose group had domed head (includes one fetus from the doe that died on day 27, table 5). One fetus from the low dose group had missing front digits. No other external malformations were noted.

The increased incidence of domed skull appears to be treatment-related, and correlates with the incidence of hydrocephalus. The historical control fetal and litter incidences of this external malformation were reported by the investigators as 5/2160 (0.2%) and 5/285 (1.8%), respectively. These values compare to the fetal and litter incidences noted in high dose animals from the present study of 4.1% and 16.7%, respectively.

(2) Soft tissue- One fetus from the mid dose group had hydrocephalus ("severe dilation of the lateral ventricles") and cleft palate (table 5). Three fetuses from two litters of the high dose group necropsied at term, and a fourth fetus from the doe that died on day 27 (#7966) also had hydrocephalus. In addition, two of these fetuses from one litter had lungs that did not float and stomachs that contained dark semisolid material. The malformed fetus from the dead doe also had a distended stomach which contained dark semisolid material. The investigators did not tabulate the malformations noted in fetuses from the high dose dead doe (#7966) with the summary tabulations for other fetuses. In the opinion of this reviewer, fetuses from the dead doe should be considered with the other high dose fetuses: development should be completed by day 27, and the fetuses were in adequate condition for evaluation and diagnosis of their status.

The increased incidence of hydrocephalus in the high dose group was apparently treatment-related. Both fetal and litter incidences of this malformation were increased in an apparent dose-related fashion. Historical control data for this malformation, supplied by the investigating laboratory, indicated that the spontaneous fetal and litter incidences of hydrocephalus were 3/2160 (0.1%) and 3/285 (1.0%), respectively. These control values are significantly lower than the fetal and litter incidences noted in the high dose group of the present study of 5.5% and 25.0%, respectively.

(3) Skeletal- A number of skeletal variations and malformations were noted. The only alterations of toxicological significance were related to development of the skull. Specifically, enlarged or irregularly-shaped fontanelles appeared to occur in a dose-related manner, and were noted in all fetuses which also had hydrocephalus (table 5). Parietals and frontals with holes were also noted in the mid and high dose groups. These effects, however, occurred with greatest frequency in the mid dose group and were apparently not dose-related. The historical fetal and litter incidences of irregularly-shaped fontanelle were 2/2162 (0.1%) and 2/285 (0.7%), respectively, compared to fetal and litter incidences of 6.8% and 25.0%, respectively, in high dose fetuses from the present study. The incidences of incomplete ossification of parietals and frontals were also higher in mid and high dose fetuses than the historical controls, however the lack of a dose-effect relationship reduces the significance of this finding.

Other skeletal anomalies observed included angulated hyoid alae, split or thickened ribs, and assymetric or fused sternebrae. None of these variations occurred in a treatment-related manner.

No effect of treatment was noted on the number of skeletal ossification sites in fetuses.

Table 5. Selected Fetal Malformations and Variations[a]

	Dose (mg/kg)			
	0	10.0	20.0	60.0
No. litters examined	16	14	16	12†
No. fetuses examined	107	73	115	73†
-live	96	73	115	70†
-dead	0	0	0	2†
-late resorptions	1	0	0	0
-delivered	10	0	0	1
Malformations- External (#fetuses/#litters)				
Domed head			1/1[b] (0.9/6.3)*	3/2[def]† (4.1/16.7)
Missing digit		1/1 (1.4/7.1)		
Soft Tissue				
Hydrocephalus			1/1[b] (0.9/6.3)	4/3[defg]† (5.5/25.0)
Cleft palate			1/1[b] (0.9/6.3)	
Skeletal				
Skull -parietals contain holes			8/2[bc] (7.0/12.5)	2/2[eg]† (2.7/16.7)
-frontals contain holes			2/2[bc] (1.7/12.5)	
-irregularly shaped fontanelle			1/1[b] (0.9/6.3)	5/3[defg]† (6.8/25.0)

[a]data excerpted from submitted study.

[b]these variations were noted in the same fetus.

[c]these variations were noted in the same fetus.

[d]these variations were noted in the same fetus.

[e]these variations were noted in the same fetus.

[f]these variations were noted in the same fetus.

[f]these variations were noted in the same fetus.

†numbers include fetuses examined from rabbit 7966, died on day 27 of gestation.

*percent affected fetuses/percent affected litters.

Conclusion

Treatment with Folpet caused an increase in the incidence of hydrocephalus and related skull malformations, i.e. domed skull and irregularly-shaped fontanelles, at doses of Folpet that produced minimal maternal toxicity. These increases were significant if compared to concurrent study controls or to historical control data supplied by the investigating laboratory.

Evidence for maternal toxicity consisted of decreases in food consumption and body weight gain in the mid (20 mg/kg) and high (60 mg/kg) dose groups. These effects were variable and not strictly dose-dependent since mid dose animals gained less weight than high dose animals, although weight gain in either group was less than control. Food efficiency calculations suggested an effect of the test article in the mid and high dose groups, as low dose and control group food efficiencies and weight gains were equal. No significant clinical signs were noted as a result of treatment. The death of one high dose doe (#7966, day 27) from an ulcerated stomach may have been related to intubation error or may have been a toxic effect of the test article. Necropsy data were not submitted to document an effect of the test compound on the stomach. Therefore, the maternal toxicity demonstrated by these data is considered to be minimal.

No significant effects of the test article were noted on litter size, resorptions, sex ratio, or the number of skeletal ossification sites. The data were suggestive of a decrease in fetal body weight, however the effect was not statistically significant.

Classification: Core-Minimum

Positive for teratogenic potential.

Teratogenic LEL = 20 mg/kg dose-related increase in hydrocephalus and related skull malformations.

Fetotoxic and Teratogenic NOEL = 10 mg/kg

Maternal LEL = 20 mg/kg decreases in body weight gain and food consumption.

Maternal NOEL = 10 mg/kg

Folpet toxicology review

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004106

Study Title: In Vivo Cytogenetics Study in Rats [with] Folpet Technical (SX-1388).

Accession No.: 253165

Study No.: MRI-225-CCC-83-32

Sponsor/Contracting Lab.: Chevron Chemical Co./EG&G-Mason Research Institute Worcester, Massachusetts

Report Date/Submitted: 10-28-83/5-2-84

Test Material- Folpet, an off-white powder, code SX-1388, 88.6% a.i.

Test Animal- Male and female Sprague-Dawley rats (Charles River Breeding Labs., Kingston, N.Y.); 4/sex/group.

Doses Tested- 150, 500, 1500, 2000 mg/kg (gavage, 10 ml/kg), single dose.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

1) The doses used were not supported by the results of the range-finding study.

Results

A. Range Finding Study- Two rats of each sex were treated by gavage with single doses of Folpet of 0, 5, 7, 11, or 16 g/kg, and observed for 14 days subsequently. A dose-related decrease in weight gain was noted, however all animals gained weight over the observation period. No deaths were noted. Treatment-related clinical signs included diarrhea, red exudate from the eyes and nose, and suppressed appetite.

B. Chromosome Analyses- Treatment of rats with oral doses of Folpet from 150 mg/kg to 2 g/kg did not produce any increase in chromosomal aberrations, or have a significant effect on the mitotic index, compared to control (tables 1 and 2). Injection with cyclophosphamide (25 mg/kg), caused an increase in chromosomal aberrations, an indication that the test system could respond to a known clastogen.

No measurement of cell viability was included. Therefore, it was not possible to determine if the test compound reached the target organ, the bone marrow, in sufficient concentration to produce a toxic effect.

Conclusion

Under the conditions of the study, Folpet failed to cause an increase in chromosomal aberrations of bone marrow cells.

Classification: Unacceptable No evidence that the test article reached the bone marrow in sufficient concentration to produce a toxic effect; results of the range-finding study suggest that doses higher than 2 g/kg could have been used.

Table 1. Effect of Folpet on Chromosome Aberrations - Male Rats[a]

Test Substance	Number with 42 Chromosomes	Chromatid Gaps	Chromatid Breaks	Markers: Rings	Markers: Deletions	Markers: Fragments	Markers: Exchanges	Mitotic Index
6 Hours								
dH_2O	48.75±0.96[b]	2.00±0.00						4.35±1.14
CMC	48.50±1.73	2.75±1.50	0.50±0.58					3.20±1.92
Folpet								
150 mg/kg	49.00±1.41	2.50±0.58						3.75±0.30
500 mg/kg	49.50±1.00	2.00±1.41				0.25±0.50		2.60±1.63
1.5 g/kg	48.00±2.31	2.50±1.00	0.75±1.50			0.25±0.50		3.40±1.69
2.0 g/kg	48.00±1.41	3.50±2.38	0.25±0.50					4.20±0.69
24 Hours								
dH_2O	48.25±1.71	2.50±1.00	0.25±0.50	0.25±0.50	0.25±0.50			4.60±2.47
CP	19.75±6.34	8.75±2.22*	7.75±4.11*	1.25±1.26	1.00±1.15	9.75±7.41	16.25±5.74	2.20±0.49
CMC	48.25±2.36	1.75±0.50	0.25±0.50			0.50±0.58		3.05±1.86
Folpet								
150 mg/kg	46.50±1.29	2.25±0.96	0.25±0.50					1.95±1.00
500 mg/kg	48.00±2.45	2.00±0.00	0.25±0.50	0.25±0.50	0.25±0.50			3.55±2.92
1.5 g/kg	49.00±1.41	2.25±0.50	0.25±0.50		0.50±0.58	0.25±0.50		4.15±1.57
2.0 g/kg	49.00±1.15	3.00±0.00	1.00±1.15					4.75±1.02
48 Hours								
dH_2O	48.25±0.96	0.25±0.50					0.25±0.50	3.10±1.98
CMC	49.25±0.96	2.25±2.63	0.25±0.50					5.50±1.25
Folpet								
150 mg/kg	48.25±0.50	1.50±1.91	0.25±0.50			0.25±0.50		5.30±2.38
500 mg/kg	46.75±2.06	1.50±1.73						2.90±2.38
1.5 g/kg	47.50±1.73	1.75±0.50				0.25±0.50		2.55±1.93
2.0 g/kg	48.25±1.26	2.25±2.63	0.50±0.58			0.25±0.50		2.05±1.14

[a]data and statistics excerpted from submitted study. Abbreviations and doses: dH_2O- distilled water, 3 ml/kg. CP- cyclophosphamide, 25 mg/kg; CMC- carboxymethyl cellulose, 10 ml/kg.

[b]values are mean ± std. dev. of number of cells (out of 50) in which each finding was noted.

*$p<0.01$

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Table 2. Effect of Folpet on Chromosome Aberrations - Female Rats[a]

Test Substance	Number with 42 Chromosomes	Chromatid Gaps	Chromatid Breaks	Markers: Rings	Markers: Deletions	Markers: Fragments	Markers: Exchanges	Mitotic Index
				6 Hours				
dH_2O	48.00±1.41[b]	2.75±1.26						5.45±1.69
CMC	48.25±0.50	2.00±0.82	1.00±0.00					5.60±1.12
Folpet								
150 mg/kg	48.00±1.41	2.00±0.82						4.65±1.11
500 mg/kg	49.25±0.96	3.00±2.00	0.25±0.50	0.25±0.50		0.50±1.00		6.25±1.45
1.5 g/kg	48.75±1.26	2.75±0.96	0.25±0.50					6.75±0.60
2.0 g/kg	49.00±0.00	1.75±1.50			0.25±0.50			4.85±1.04
				24 Hours				
dH_2O	47.25±2.06	2.00±1.41	0.50±0.58					2.95±2.27
CP	15.67±2.89	8.33±2.08*	12.33±3.21*	0.67±0.58	2.00±1.00	9.33±3.51	10.00±7.00	1.74±0.42
CMC	48.50±1.29	3.25±1.50				0.25±0.50		4.35±1.15
Folpet								
150 mg/kg	49.00±1.41	1.50±0.58						4.60±1.83
500 mg/kg	47.50±2.08	2.75±1.26						4.35±2.61
1.5 g/kg	48.00±1.41	3.75±2.75	0.25±0.50					4.85±2.15
2.0 g/kg	48.00±1.83	2.75±1.26			0.50±0.58	0.50±1.00		2.80±2.01
				48 Hours				
dH_2O	49.75±0.50	2.75±1.50			0.25±0.50	0.50±0.58		4.90±1.01
CMC	48.50±0.58	3.25±2.22						4.35±1.98
Folpet								
150 mg/kg	49.75±0.50	1.25±1.26	0.25±0.50		0.75±0.96			4.55±0.81
500 mg/kg	48.00±1.83	2.25±1.89	0.50±1.00	0.25±0.50		0.50±1.00		4.65±2.49
1.5 g/kg	46.50±1.73	1.50±1.00			0.25±0.50		0.25±0.50	2.10±2.48
2.0 g/kg	49.50±0.58	2.75±2.06	0.25±0.50			0.25±0.50		5.10±1.06

[a]data and statistics excerpted from submitted study. Abbreviations and doses: dH_2O- distilled water, 3 ml/kg. CP- cyclophosphamide, 25 mg/kg; CMC- carboxymethyl cellulose, 10 ml/kg.

[b]values are mean ± std. dev. of number of cells (out of 50) in which each finding was noted.

*$p<0.01$

Folpet toxicology review

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Study Title: The Potential of Technical Phaltan (Calhio) and Technical Phaltan (Port De Bouc) to Mutate TA 100, A Histidine-Deficient Strain of Salmonella Typhimurium.

Accession No.: 253165

Study No.: S-1261

Sponsor/Contracting Lab.: Chevron Chemical Co./Chevron Safety and Health Div., San Francisco, CA.

Report Date/Submitted: 7-26-78/5-2-84

Test Material- Phaltan [Folpet] technical (Calhio), code SX-946, 93.7% a.i.; and Phaltan [Folpet] technical (Port De Bouc), code SX-980, 90.2% a.i.

Test Animal- Salmonella Typhimurium bacteria, strain TA-100 (B. N. Ames, Univ. of Calif. at Berkeley).

Doses Tested- 0.1, 1.0, 10.0, 100.0 ug/plate; 0.1 ml/2.0 ml agar.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

1) No assessment of the effect of metabolic activation.

2) No assessment of cell toxicity.

Results

Incubation of a histidine-deficient strain (TA 100) of Salmonella typhimurium with concentrations of Folpet from 0.1 to 100 ug/plate caused a dose-related increase in the incidence of mutation at doses of 1 ug/plate and higher. Two separate sources of technical Phaltan (Folpet) were assayed- Calhio and Port De Bouc. Both materials were mutagenic in this system, and their potencies were approximately equal.

Incubation of bacteria with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), the positive control, also caused a large increase in the number of mutant colonies observed compared to the vehicle control. These data indicated that the test system could respond to a known direct-acting mutagen.

These data are presented in table 1 (photocopied from submitted study).

Conclusion

Under the conditions of this study, Folpet was shown to be a direct-acting mutagen, causing a dose-related increase in the number of mutant colonies in the absence of metabolic activation.

Classification: Acceptable Relevant toxicological data is provided by this study. However, because the effect of metabolic activation was not assessed, a study in which the effect of activation is measured is still required to satisfy the minimum data requirement for gene mutation testing.

TABLE 1: TA 100 PLATE INCORPORATION TESTS WITHOUT A LIVER MICROSOMAL SYSTEM.

Compound	Dilution in DMSO µg/ml	Amount Compound Per Plate µg	Number of Mutant Colonies Per Plate
Technical Phaltan	1000	100	>800 >800
	100	10	529 >500
	10	1.0	216 214
	1.0	0.1	134 147
Technical Phaltan (Port De Bouc)	1000	100	>800 >800
	100	10	637 >600
	10	1.0	256 256
	1.0	0.1	153 153
Dimethylsulfoxide	Neat	0.1 ml	137 131 131 130 132 139
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	20	2.0	>1000 >1000

IV. MATERIALS AND METHODS

A. Materials

1. Test Material: The following test materials were used in this study: Technical Phaltan (Calhio), an off-white solid, coded SX-946, and Technical Phaltan (Port de Bouc), an off-white solid, coded SX-980. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was purchased from the Aldrich Chemical Company, Inc. D. Biotin, DL-Histidine and dimethylsulfoxide (DMSO) were obtained from the J. T. Baker Chemical Company.

2. Bacterial Strains: The strain used in this test was TA 100. It is a mutant of Salmonella typhimurium LT-2. (1) The strain was originally received from B. N. Ames, Biochemistry Department, University of California, Berkeley, California. It is maintained as a frozen permanent by freezing 0.8 ml of a freshly grown nutrient broth culture with 0.07 ml of DMSO in a glass screw-capped vial.

B. Methods

The basic test system used is that of B. N. Ames et al. (1) Histidine-deficient strains of Salmonella are grown on media that contains only minimal amounts of histidine. Only bacteria that can mutate back to the wild-type strain, which is capable of histidine synthesis, will grow into a colony after a two-day incubation at 37°C. The number of colonies per plate is an index of the mutation rate.

Test compounds can be spotted directly onto plates of bacteria or incorporated into the agar. Some chemicals require metabolic activation before they are mutagenic. These can be detected by adding a small amount of microsomal enzymes derived from rat liver to the media.

Mutagenecis Plate Incorporation Assay Without Liver Microsomal Activating System: Bacterial tester strain cultures were grown by inoculating nutrient broth with a tester strain and incubating overnight in a water bath at a temperature of 37°C. Pour plates were made by adding 0.1 ml of the solution to be tested and then 0.1 ml of the grown culture (approximately 3×10^7 bacteria) to 2 ml of molten (45°C) top agar (0.6% Bacto agar, 0.5% NaCl) containing a trace (4.5×10^{-5} mmoles/ml) of histidine and excess (4.5×10^{-5} mmoles/ml) biotin. The compound and bacteria containing top agar was mixed and then uniformly distributed over the surface of a minimal agar plate (1.5% agar in Vogel-Bonner Medium E with 2% glucose). Other plates were prepared in a similar manner with DMSO and appropriate positive controls. The plates were incubated for two to three days at 37°C and the revertant colonies counted. Also the presence and condition of the bacterial lawn were noted.

Study Title: Subacute Inhalation Toxicity to the Rat of Phaltan 50 W.P.

Accession No.: 253157

Study No.: CHR-2/74725

Sponsor/Contracting Lab.: Chevron Chemical Co./Huntingdon Research Centre, Huntingdon, Cambridgeshire, U.K.

Report Date/Submitted: 3-24-75/5-2-84

Test Material- Phaltan [Folpet] 50 W.P., 51.3% a.i.

Test Animal- Male and female Sprague-Dawley rats (Grade IV, Charles River Ltd., Mansion, Kent, U.K.), 10/sex/group.

Doses Tested- 0.170 mg/L (targeted), 0.048 ± 0.006 (actual); 4 h/d, 5 d/wk x 3 wks.

Methods

A photocopy of the submitted methods is appended. The protocol used in this study was reviewed and found to be deficient in the following point(s):

1) After the 3 week exposure period, rats were allowed a 12 day recovery period before necropsy. No animals were necropsied immedately after the termination of treatment.

2) Only one dose level was used.

3) Inadequate histopathology. Tissues not examined: spinal cord, eye, salivary gland, thymus, trachea, esophagus, stomach, intestine, pancreas, prostate, uterus, lymph node, bone and marrow, skeletal muscle, skin, sciatic nerve, mammary gland.

4) No serum chemistry or urinalysis determinations.

5) No food consumption data.

6) No individual animal data for physical signs.

Results

A. Exposure Conditions- The targeted concentration of the test article was 0.170 mg of Phaltan 50 dust per liter of air. Colorimetric analysis of chamber samples indicated that the actual concentration of the test article was 0.048 ± 0.006 mg/L. The analysis of particle size distribution indicated that 99% of the generated dust particles were in the range of 1-5 um, and therefore innalable.

B. Clinical Signs and Mortality- No data for clinical signs was submitted. The report narrative stated that "there were no signs of irritation" in treated animals and that "behaviour of all animals was in every way normal".

No deaths were noted.

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C. Body Weights and Food Consumption- The body weight gain of treated males was slightly less than control during the treatment phase, however the difference was not judged statistically significant (table 1). Female rats also weighed less than control during treatment, but the difference in average body weight between treated and control females was present at the start of treatment. By the end of the recovery period, the average body weights of treatment groups were similar to controls.

No food consumption data was submitted. The final report narrative stated that "food and water consumption of the test rats showed no deviation from normal".

Table 1. Body Weight Data[a]

	Male		Female	
Day	Control	Treated	Control	Treated
-3	185.6± 7.3	190.3±10.7	183.7± 5.4	172.7± 6.8
1*	222.7±10.1	223.2±10.2	198.9± 5.8	185.7± 6.0
19*	361.2±20.3	350.7±23.6	251.5±17.3	241.5±12.1
31**	434.8±28.0	432.2±33.9	272.9±17.4	270.6±12.3

[a]data excerpted from submitted study.

*treatment between days 1 and 19.

**post-treatment phase between days 19 and 31.

D. Hematology- No toxicologically significant alterations in these parameters were noted. A statistically significant ($p < 0.05$) difference in mean corpuscular hemoglobin concentration (MCHC) between treated males (29.8 ± 0.45%) and controls (28.8 ± 0.45%) was observed, however this small change is not considered biologically significant in the absence of any other effects.

E. Necropsy Data- (1) Gross observations- No treatment-related changes were noted upon macroscopic examination. Two of 10 treated male rats and 1/10 control males had hydronephrosis (kidney), however this finding is not considered significant by this reviewer.

(2) Organ weights- No toxicologically significant alterations in organ weights were noted. A statistically significant increase in the brain/body weight ratio was noted in treated males. However, absolute organ weights were similar in control and treated males, and the effect on relative organ weight was likely due to the difference in body weights tabulated for control and treated rats. A significant discrepancy was noted by the reviewer in comparison of body weights recorded for day 31 (the day of termination, according to the reported methods), and body weights recorded with organ weight data. These values should have been identical, however differences in the means for these data were apparent, and in some cases the body weights of individual rats differed by as much as 140g. No explanation for this discrepancy was offered.

E.(3) Microscopic examination- No toxicologically significant effects on organ histopathology were noted. Changes observed in control and treated animals included peribronchial lymphoid hyperplasia and chronic inflammatory cells in the liver. Chronic myocarditis was noted in 2/10 treated males vs. 0/5 controls, however since unequal numbers of animals were examined the significance of this finding is unclear.

Conclusion

Under the conditions of this study, no significant treatment-related findings were noted. However, the number of tissues examined microscopically did not meet minimum data requirements, and no serum chemistry or urinalysis studies were conducted. Also, the practice of allowing animals a 12 day recovery period after the 3 week treatment phase is not considered valid by this reviewer. Therefore, although no treatment-related effects were noted, these data are not adequate to identify potential toxic effects that may result from inhalation of the test compound. Further, the discrepancy noted in body weight tabulations raises questions as to the reliability of these data.

The data are not adequate for the establishment of an LEL or NOEL.

Classification: Core-Supplementary Animals necropsied 12 days after final exposure; only one dose level; inadequate histopathological examination of tissues; no serum chemistry or urinalysis data; no food consumption data; no individual animal data for physical signs; significant discrepancy in organ weight data.

Folpet toxicology review

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