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

FEB 17 1981

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg.#264-GUE; 264-GUR; PP#0F2413, OH5275; Larvin, Thiodicarb; Petition proposing tolerances in or on cottonseed/hulls and soybeans/hulls and straw for residues of thiodicarb, Dimethyl N,N'-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate] CASWELL#900AA; Accession#099581-96; 099598

FROM: William Dykstra WMD
Toxicology Branch, HED (TS-769)

TO: Jay Ellenberger (12)
Registration Division (TS-767)
and
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Recommendations:

1) The inhalation LC_{50} of Larvin 500 Thiodicarb Insecticide has not been adequately determined. There were no deaths at gravimetric concentrations of 0.0221 mg/L and 0.0836 mg/L (Toxicity Category I) in the Hazelton study (400-618; February 8, 1980). The study needs to be repeated at higher gravimetric concentrations.

2) In the reproduction study (Carnegie-Mellon 42-65; July 2, 1979), pathology reports of the F3a pups at dosage levels of 3.0, 1.0 and 0.5 mg/kg/day and the F2a parental male and female rats at dosage levels of 3.0, 1.0 and 0.5 mg/kg/day are required to be submitted.

3) In the chronic oncogenicity feeding study in mice, the registrant is required to submit comprehensive tables by sex of all primary neoplasms in male and female mice examined from the study, which represents the total of each of the primary neoplasms from the 18-month sacrifice, final sacrifice and mice that died or were euthanized when moribund. Statistical analysis of the data of the comprehensive tables is required.

4) In the chronic toxicity/oncogenicity feeding study in rats, no tabular presentation of the microscopic findings of the 241 animals found dead or euthanized moribund has been submitted. Tabular presentation of the microscopic findings of tissues from these animals is required. In addition, comprehensive tables by sex of histologic findings of all rats examined in the study are required. Statistical analysis of the data of the comprehensive tables is required.

5. In the chronic toxicity/oncogenicity feeding study in rats, the table (Table 3) of primary tumors in rats killed at various intervals (p. 14914, Volume 15) does not include the primary tumors of the 241 rats which died or were killed moribund. Comprehensive tables by sex of all primary neoplasms of all rats examined in the study are required to be submitted. Statistical analysis of the data of the comprehensive tables is required.

6. In the chronic toxicity/oncogenicity feeding study in rats, the animals selected for cholinesterase (plasma and RBC) determination were placed on control diet for 48 hours and before sacrifice for brain cholinesterase were placed on control diet for 24 hours.

   These procedures of placing the rats on control diets for 24 or 48 hours before sampling for cholinesterase inhibition prevents the determination of a NOEL for cholinesterase inhibition. A separate 90-day rat feeding study is required at dosage of 10, 3, 1, and 0.5 or less (plus controls) mg/kg/day of UC-51762 to determine the NOEL for plasma, RBC and brain cholinesterase. The samples taken for determinations of cholinesterase levels shall be from rats which are neither fasted or placed on control diets for any period of time.

7. In the chronic toxicity/oncogenicity feeding study in rats, no statistical comparisons of organ weights were made between the UC-51762 treated and control groups sacrificed at 24 months. The registrant is required to report the calculated statistical comparisons of organ weights at 24 months for treated and control groups.

8. In the chronic toxicity/oncogenicity feeding study in rats, the addendum to the pathology report (vol. 12, pp. 17275-17295) states that neoplastic nodules were found in 11 rat livers during the examination of 4 additional pieces of the 351 male rats remaining at final sacrifice compared with 24 livers with neoplastic nodules in the initial study (Table 5, Vol. 15, pp. 14930). Oral communication with Dr. Edward H. Fowler, pathologist for the study, indicated that 9 of the 11 nodules were found in addition to the initial 24 neoplastic nodules. This aspect of the addendum pathology report is not clear from the report and is required to be submitted in writing together with a new statistical evaluation of all neoplastic nodules.

9. An extended subchronic oral feeding study (6-months or longer) in the dog, which measures cholinesterase inhibition as stated in #6, and also measure the NOEL for cholinesterase inhibition and systemic toxicity is required.

10. The registration of Larvin 75 WP is supported. The registration of Larvin 500 is not supported. The permanent tolerances are not supported.

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A. Section F: Proposed Tolerance for the Pesticide Chemical

The petitioner proposes that the following Pesticide and Feed Additive tolerances be established for the combined residues of thiodicarb, Dimethyl N.N'-[thiobis[(methylimino) carbonyloxy]]bis[ethanimidothioate] in or on:

Pesticide Tolerances

cottonseed	0.4 ppm
soybeans (seed)	0.1 ppm
soybean straw	0.2 ppm

Feed Additive Tolerances

cottonseed hulls	0.8 ppm
soybean hulls	0.4 ppm

B. Related Petitions: 9G2152

C. Established Tolerances: None

"CONFIDENTIAL"

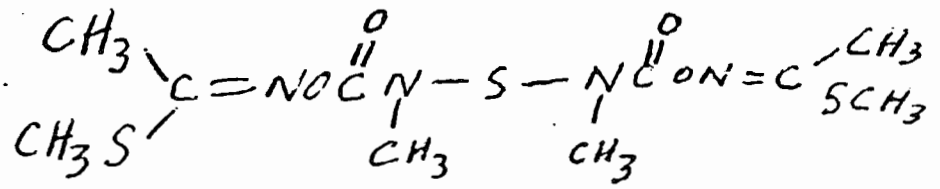
D.1. Product Name: Larvin Thiodicarb Insecticide 95% Technical

Company Code Number: UC-51762

Ingredients	Percent Weight
Thiodicarb (technical)	96.0
[redacted]	
	100.0

All except thiodicarb (technical) are impurities.

Structural Formula:



INERT INGREDIENT INFORMATION IS NOT INCLUDED

D.2. Product Name: Larvin Thiodicarb Insecticide 75 WP

Company Code Number: UC-51762 75 WP

Ingredients	Percent Weight	Chemical Name
Thiodicarb	78.1	
[redacted]	[redacted]	[redacted]
	100.0	

Inerts cleared under 180.1001.

D.3. Product Name: Larvin 500 Thiodicarb Insecticide

Company Code Number: UC-51762 4 Flowable

Ingredients	Percent Weight	Chemical Name
[redacted]	[redacted]	[redacted]
Thiodicarb	45.8	
[redacted]	[redacted]	[redacted]

Inerts cleared under 180.1001.

Review:

1. Document 1. Acute Oral Toxicity Study in male and female rats with Larvin Insecticide Technical (UC-51762) (Hazelton Project No. 400-613, January 21, 1980).

   Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

   Results: LD_{50} = 398 (256-620) mg/kg; males
   LD_{50} = 248 (120-511) mg/kg; females
   LD_{50} = 325 (204-516) mg/kg; both sexes

   Toxicity Category II: Warning

   Classification: Core-Minimum Data

2. Document 2. UC-51762 technical; Range Finding Toxicity Studies (Carnegie Mellon Institute of Research; Project Report 42-19; February 26, 1979)

   Peroral. Compound administered by stomach intubation to Wistar-derived male rats, 90-120 grams in weight and 3 to 4 weeks of age.

   1. LD_{50} = 190 (125-291) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Signs of Toxicity
320	4/4	0,0,0,0	-	Tremors, death
160	1/4	0	98 to 106 gm.	Tremors, unsteady gait
80	0/4	-	103 to 117 gm.	Tremors, unsteady gait

   Gross Pathology: In victims, lungs with petechiae, stomachs transparent, distended, fluid-filled; kidney sections slightly congested; intestines opague, fluid-filled. In survivors, nothing remarkable.

   Toxicity Category II: Warning

   Classification: Supplementary Data

   (a) Detailed individual results not provided.

2. Peroral, Single Dose to Rats

LD_{50} = 141 (86.6 to 231) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Signs of Toxicity
200	4/4	0,0,0,0	-	tremors, death
100	0/4	-	99 to 126 gm.	tremors

Gross Pathology: In victims, lungs with petechiae; stomachs transparent, distineded, fluid-filled; medullae of kidneys pink, intestines injected, opaque. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadeqaute number of doses.

3. Peroral, Single dose to Rats

LD_{50} = 238 (156-363) mg/kg; 1 ml = 1 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Sign of Toxicity
400	4/4	0,0,0,0	-	tremors, death
200	1/4	0	89 to 93 grams	tremors, death
100	0/4	-	97 to 124 grams	slight tremors

Gross Pathology: In victims, lungs with petechiae, stomach transparent, fluid-filled; intestines transparent or opaque in sections, fluid-filled. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

4. Peroral, Single dose to Rats

LD_{50} = 141 (81.2-246) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Deaths	Weight Change	Signs of Toxicity
400	4/4	0,0,0,0		tremors, death
200	3/4	0,0,0	70 grams	tremors, death
100	1/4	0	87-166 gm.	tremors, death
50	0/4	-	104-119 gm.	tremors

Gross Pathology: In victims, lungs with petechiae; stomachs transparent, fluid-filled; medullae of kidneys pink; intestines opaque or transparent in sections, fluid-filled. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Individual detailed results not provided.

5. Peroral, Single dose to Rats

LD_{50} = 141 (74.6-268) mg/kg; 1 ml = 10 suspension in 0.25% agar plus 0.1% Tween 80.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Signs of Toxicity
400	4/4	0,0,0,0	-	tremors, death
200	2/4	0,0	87-100 gm.	tremors, death
100	2/4	0,1	92-107 gm.	tremors, death
50	0/4	-	101-124 gm.	slight tremors

Gross Pathology: In victims, lungs with petechiae; stomachs transparent, fluid-filled; adrenals pink. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

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6. Peroral, Single dose to Rabbits

LD_{50} > 400 mg/kg; dosed as a dry solid

The dose for each rabbit was weighed into a No. 0 gelatin capsule which was placed in the back of the rabbits mouth. The rabbit was then allowed to chew and swallow the capsule.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Sign of Toxicity
400	1/4	0	-3-320 gm.	-
200	1/4	14	86-230 gm.	-

Gross Pathology: Nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadequate number of doses.

7. Peroral, Single dose to Rabbits

LD_{50} = 566 (347-924) mg/kg; 1 ml = 50 mg suspension (poor) in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Sign of Toxicity
800	4/4	0,0,0,8	-	Coordination loss, death
400	0/4	-	-20-460 gm.	-
200	0/4	-	66-83 gm.	-

Gross Pathology: Nothing remarkable.

Toxicity Category III: Caution

Classification: Supplementary Data

(a) Inadequate number of doses.

8. Skin Penetration, Single dose to Rabbits

LD_{50} > 3200 mg/kg; 1 ml = 200 mg suspension in corn oil applied to intact skin for 24 hours under an impervious cuff.

Dosage mg/kg	Dead/ Dosed	Days of Death	Weight Change	Skin Irrit.	Signs of Toxicity
3200	0/6	-	-238-157 gm.	-	diarrhea

Gross Pathology: Intestines liquid + gas-filled.

Toxicity Category III: Caution

Classification: Supplementary Data

(a) Abraded skin not tested.

9. Skin Penetration, Single 4-hr. contact to Rats

LD_{50} = 2540 (1120-5750) mg/kg; 1 ml = 250 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days of Death	Weight Change	Skin Irrit.	Signs of Toxicity
6400	3/4	1,1,3	83 gm.	-	-
3200	1/4	1	94-104 gm.	-	tremors, death
1600	3/4	0,1,1	98 gm.	-	tremors, death
800	0/4	-	110-122 gm.	-	tremors, death

Gross Pathology: In victims, lungs with petechiae; livers mottled red and pink; stomachs transparent, gas-filled; medullae of kidneys red; intestines transparent or opaque in sections, gas-filled; adrenals red or pink; in survivors, nothing remarkable.

Toxicity Category III" Caution

Classification: Supplementary Data

(a) Inadequate number of doses tested.

10. Inhalation, Single, by Rats

Sample delivered as 1.0% (w/w) solution in dimethyl-sulfoxide.

Time	Conc.	Dead/Dosed	Weight Change	Signs
6 hr.	200 mg/m^3 (.200 mg/L)	0/6	70 to 93 gm	tremors

Classification: Supplementary Data

(a) Inadequate number of doses.

3. Document 3. Oral LD_{50} in Rats with UC-51762 technical; (CDC Research Inc., Study No. CDC-UC-011-79; 10/2/79) Five groups of 4 male Charles River CD Sprague-Dawley rats received oral doses of 320, 400, 500, 630 and 800 mg/kg. Observation for 14 days.

Results: Biphasic LD_{50} reported.

LD_{50} = 410 (331-508) mg/kg
LD_{50} = 800 (559-1144) mg/kg

Toxic Signs: Salivation, body tremors, depression, lacrimation and chromodacryorrhea were seen at most dose levels.

Body Weight: Survivors gained weight.

Necropsy: Gastric mucosal erosins and a distended stomach and intestines in some animals.

Classification: Supplementary Data

(a) LD_{50} not accurately calculated.

4. Document 4. Acute Oral Toxicity Study in male and female Rats with Larvin-500 Insecticide Formulated (Hazelton Project No. 400-613; Dec. 13, 1979).

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2125.

Results: LD_{50} = 298 (193-404) mg/kg; males
LD_{50} = 142 (100-202) mg/kg; females
LD_{50} = 192 (150-245) mg/kg; both sexes

Toxicity Category II: Warning

Classification: Core-Minimum Data

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5. Document 5. UC-51762 4F Formulation: Range Finding Toxicity Studies (Carnegie Mellon Research Institute, Project Report 41-146, October 27, 1978)

a. Peroral, Single dose to Rats

LD_{50} = .177 (.0443-.706) ml/kg

Dosage ml/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
4.00	2/2	0,0	-	tremors, pilo-erection, death
1.00	4/4	0,0,0,0	-	tremors, pilo-erection, death
0.50	4/4	0,0,0,0	-	tremors, pilo-erection, death
0.25	2/4	0,1	114-119	tremors, pilo-erection, death
0.125	2/4	0,0	114-124	tremors, death

Gross Pathology: In victims, petechiae of the lungs; stomachs contain some fluid; medullae of kidneys pink; intestines injected. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadequate number of doses tested.

b. Skin Penetration, Single 4-hour contact to Rats

LD_{50} > 32.0 ml/kg

Male rats, 201-238 gms, were immobilized during a 4-hour contact period with the compound retained under impervious sheeting on the clipped, intact skin of the trunk.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Skin Irrit.	Signs of Toxicity
32.0	0/4	-	84-106	-	tremors
16.0	0/4	-	87-115	-	yellow fur

Gross Pathology: Fur yellow, lobular patterns of livers visible.

Toxicity Category IV: Caution

Classification: Supplementary Data

(a) Abraded skin not tested.

c. Inhalation, Single, by Rats

Sample delivered as suspensions in distilled water (DW).

LC_{50} = 2.110 (.724-6.13) mg/L

Time	Conc.	Dead/Dosed	Death	Weight Change	Toxic Signs
5.75 hr.	7.84 mg/L	6/6	in exposure	-	tremors, death
6.0 hr.	1.36 mg/L	2/6	in exposure	68-88	tremors, death

Gross Pathology: In victims, lungs dark red with many small darker foci; stomachs and intestines gas-filled.
In survivors, nothing remarkable.

Toxicity Category III: Caution

Classification: Supplementary Data

(a) Inadequate numbers of dosages.

d. Skin Irritation, Rabbit, uncovered

Results: No irritation on 5 rabbits.

Classification: Supplementary Data

(a) No occlusion and no abrasion.
(b) No detailed report of results

e. Eye Irritation, Rabbit

Results: No corneal injury on 5 eyes from an excess, 0.5 ml per eye.

Classification: Supplementary Data

(a) Inadequate number of rabbits.

(b) No detailed report of results.

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6. Document 6. Acute Oral LD_{50} Study in R. with Larvin-75 WP (Hazelton Project No. 400-619; April 1, 1980)

Groups of 5M + 5F Sprague-Dawley rats received single oral doses of 100, 147, 215, 316 and 464 mg/kg. Observation was for 14 days.

Results: LD_{50} = 241 (184-316) mg/kg; males
LD_{50} = 128 (95-173) mg/kg; females
LD_{50} = 175 (140-220) mg/kg; both sexes

Toxic Signs: Salivation, depression, red stains on nose and/or eyes, soft feces, ataxia, prostation.

Body Weight: Survivors gained weight.

Necropsy: Compound-like material in stomach, yellowish fluid in stomach and intestines, dark coloration of liver.

Toxicity Category II: Warning

Classification: Core-Guideline

7. Document 7. UC-51762 75-WP; Range Finding Toxicity Studies (Carnegie-Mellon Institute, Project Report#41-156, November 17, 1978)

a. Peroral, Single dose to Rats

LD_{50} = 63.0 (27.8-143) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
200	4/4	0,0,0,0	-	tremors, death
100	3/4	0,0,0	130 gm.	tremors, death
50	1/4	0	125-129 gm.	tremors, death
25	1/4	0	130-143 gm.	tremors, death

Gross Pathology: In victims, petechiae of the lungs; stomachs transparent, distended, fluid-filled; kidney sections slightly congested; adrenals slightly congested; in sursivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

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b. Peroral, Single dose to rats

LD_{50} = 141 (81.2-246) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
400	4/4	0,0,0,0	-	tremors, death
200	3/4	0,0,0	103 gm.	tremors, death
100	1/4	0	115-127 gms.	tremors, death
50	0/4	-	101-125 gms.	tremors

Gross Pathology: In victims, petechiae of lungs; stomachs transparent, distended, fluid-filled; arenals pink. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

c. Peroral, Single dose to Rats

LD_{50} = 119 (80.4-176) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
400	4/4	0,0,0,0	-	tremors, death
200	4/4	0,0,0,0	-	tremors, death
100	1/4	0	108-125 gm.	tremors, death
50	0/4	-	109-124 gm.	tremors, death

Gross Pathology: In victims, petechiae of the lungs; stomachs transparent, distended, gas-filled; intestines transparent or opaque in sections, fluid-filled. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual reults not provided.

d. Peroral, Single dose to Rats

LD_{50} = 100 (63.6-157) mg/kg; 1 ml = 10 mg suspension in corn oil.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
400	4/4	0,0,0,0	-	tremors, death
200	4/4	0,0,0,0	-	tremors, death
100	2/4	0,0	99-105 gm.	tremors, death
50	0/4	-	116-129 gm.	tremors, death

Gross Pathology: In victims, petechiae of the lungs; stomachs transparent, fluid-filled; medullae of kidneys pink; intestines transparent or opaque in sections, injected, fluid-filled; adrenals pink. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

e. Peroral, Single dose to Rats

LD_{50} = 100 (38.5-260) mg/kg; 1 ml = 10 mg suspension in 0.25% agar plus 0.1% Tween 80.

Dosage mg/kg	Dead/ Dosed	Days to Death	Weight Change	Toxic Signs
400	4/4	0,0,0,0	-	tremors, death
200	3/4	0,0,0	109 gm.	tremors, death
100	2/4	0,0	93-114 gm.	tremors, death
50	1/4	0	97-120 gm.	tremors, death

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Toxicity Category II: Warning

Classification: Supplementary Data

(a) Detailed individual results not provided.

f. Skin Penetration, Single dose to Rabbits

LD_{50} = 6.40 (1.79-22.9) gm/kg; 1 ml = 0.4 gm suspension in distilled water.

Dosage gm/kg	Dead/ Dosed	Days to Death	Weight Change	Skin Irrit.	Toxic Signs
6.4	2/4	2,3	87-165 gm.	-	tremors, death
3.2	0/4	-	-50-427 gm.	-	-

Gross Pathology: In victims, liver mottled dark red; kidneys tan; intestines gas and liquid-filled. In survivors, intestines gas-filled.

Toxicity Category III: Caution

Classification: Supplementary Data

(a) Inadequate number of doses.

(b) Abraded skin not tested.

g. Skin Penetration, Single 4-hr. contact to Rats

LD_{50} = 2.26 (0.142-36.1) gm/kg; 1 ml = 0.1 gm in corn oil.

Male albino rats, 167-223 gms, were immobilized during a 4-hr. contact period with the compound retained under impervious sheeting on the clipped, intact skin of the trunk.

Dosage gm/kg	Dead/ Dosed	Days to Death	Weight Change	Skin Irrit.	Toxic Signs
3.20	2/4	1,1	55-60 gm.	-	tremors
1.60	2/4	1,3	50-66 gm.	-	tremors
0.80	1/4	1	90-107 gm.	-	tremors
0.40	0/4	-	75-86 gm.	-	-

Gross Pathology: In victims, lungs mottled red and pink; livers with visible lobular patterns; stomachs gas-filled; medullae of kidneys pink; intestines yellow; adrenals pink. In survivors, livers with visible lobular pattern.

Classification: Supplementary Data

(a) Inadequate number of doses.
(b) Abraded skin not tested.

h. Inhalation, Single, by female Rats

Dust exposure at room temperature.

Time	Measured Concentration	Dead/ Dosed	Death in Days	Weight Change	Toxic Signs
4 hr.	325 mg/m^3	3/6	0,0,4	53-68 gm.	coordination loss
4 hr.	175 mg/m^3	0/6	-	51-68 gm.	coordination loss

LD_{50} = 325 (157-671) mg/m^3

Gross Pathology: In victims, livers mottled; lungs red with dark foci; stomachs and intestines gas-filled; nothing remarkable in survivors.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadequate number of doses.

i. Inhalation, Single by male Rats

Dust exposure at room temperature

Time	Measured Concentration	Dead/ Dose	Days of Death	Weight Change	Toxic Signs
4 hr.	325 mg/m^3	6/6	in exposure	-	coordination loss
4 hr.	173 mg/m^3	1/6	in exposure	100-106 gm.	coordination loss

Gross Pathology: In victims, livers loss mottled; lungs red with dark foci; stomachs and intestines gas filled. Nothing remarkable in survivors.

LD_{50} = 223 (183-270) mg/m^3

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadequate number of doses.

j. Inhalation, Single, by Rats

Aerosol exposure at 22°C; delivered as 13% w/w in distilled H_2O.

LC_{50} = 1040 (398-2700) mg/m^3

Time	Measured Concentration	Dead/ Dosed	Days of Death	Weight Change	Toxic Signs
3 hr. 31 min.	3360 mg/m^3	6/6	in exposure	-	tremors
6 hr.	700 mg/m^3	2/6	0,1	56-76 gm.	tremors

Gross Pathology: In victims, lungs dark red with many small dark foci; stomachs and intestines gas-filled. In survivors, nothing remarkable.

Toxicity Category II: Warning

Classification: Supplementary Data

(a) Inadequate number of doses.

k. Skin Irritation, Rabbit, uncovered

Applied in distilled water (DW)

Results: No irritation on 5 rabbits from a 40% suspension in DW.

Classification: Supplementary Data

(a) Detailed results not provided.

1. Eye Irritation, Rabbit

   Instilled as a solid or in DW

   Results: No corneal injury on 5 eyes from 40 mg per eye of the powder or from 0.5 ml per eye of a 40% suspension in DW.

   Classification: Supplementary Data

   (a) Detailed results not provided.

8. Document 8. Acute Dermal Administration in Rabbits with Larvin Insecticide Technical (UC-51762) (Hazelton Project No. 400-614, December 17, 1979)

   Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

   Results: No deaths; $LD_{50} > 6.31$ gm/kg

   Toxicity Category III: Caution

   Classification: Core-Minimum Data

9. Document 9. Acute Dermal Toxicity in Rats with UC-51762 technical; (CDC Research, Inc., Study No. CDC-UC-012-79; November 9, 1979)

   One group of 4 male Sprague-Dawley rats received a dosage of 640 mg/kg on the intact skin of the fur clipped trunk under an impervious cuff for 4 hours. Observations for 7 days.

   Results: No deaths, $LD_{50} > 640$ mg/kg

   Toxic Signs: None observed

   Skin Reactions: No adverse reactions

   Necropsy: No gross lesions

   Classification: Supplementary Data

   (a) Inadequate number of doses.
   (b) Abraded skin not tested.
   (c) Exposure period only 4 hours.

10. Document 10. Acute Dermal Administration in Rabbits with Larvin-500 Insecticide Formulated (UC-51762) (Hazelton Project No. 400-614, December 17, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Results: LD_{50} > 2.04 gm/kg

Toxicity Category III: Caution

Classification: Core-Minimum Data

10a. Document 10a. Acute Dermal LD_{50} Study in Rabbits with Larvin-75 WP (Hazelton Report No. 400-620; April 7, 1980)

One group of 5M + 5F NZW rabbits received a dosage of 2000 mg/kg on the abraded skin of the fur clipped trunk under an impervious cuff for 24 hours. Observation for 14 days.

Results: No deaths, LD_{50} > 2000 mg/kg

Toxic Signs: None observed

Skin Irritation: Erythema in 9 rabbits on day 1 which lasted to day 3. Slight edema in 2 rabbits on day 1 which last to Day 3.

Necropsy: No gross lesions.

Histopathology: Slight dermal irritation in 4/10 rabbits, characterized by minimal or slight diffuse acanthosis, hyperkeratosis, and diffuse subepidermal pleocellular infiltrate. Necrotic cell debris was present on the epidermal surface of two of the rabbits. An additional rabbit had focal subepidermal pleocellular infiltrate and minimal focal acanthosis possibly resulting from the abrading process. Skin sections from five rabbits were histologically normal.

Toxicity Category III: Caution

Classification: Core-Minimum Data

11. Document 11. Acute Inhalation Toxicity Study in Rats with Larvin Technical Dust (UC-51762) (Hazelton Project No. 400-615; November 30, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Results: No deaths; LC_{50} > 5.31 mg/L (nominal)
LC_{50} > 0.32 mg/L (gravimetric)

Toxicity Category II: Warning

Classification: Core-Minimum Data

12. Document 12. Acute and 9-Day Dust Inhalation Study on Rats with UC-51762 Technical (Carnegie-Mellon; Project Report#42-63; July 5, 1979)

Reviewed in memo of 10/6/80 from William Dykstra to Jay Ellenberger; 264-GUG.

Results: LC_{50} = .126 mg/L; males
LC_{50} = .115 mg/L; females

Toxicity Category I: Danger

Classification: Core-Minimum Data

Nine-Day Dust Inhalation: No NOEL; LEL = 4.8 mg/m^3, pinpoint pupils and tremors.

Classification: Core-Minimum Data

13. Document 13. Acute Inhalation Toxicity Study in Rats with Larvin 500 (UC-51762 flowable formulation) (Hazelton Project No. 400-618; February 8, 1980)

The test material, Larvin 500 (UC-51762 flowable formulation, was evaluated for acute inhalation toxicity in rats. Two exposures, designated Exposure A and Exposure B were conducted, the second indicated by a low gravimeteric concentration obtained in the first exposure.

Exposure A:

One group of 5 male and 5 female rats was exposed to a nominal active ingredient aerosol concentration of 5.0 mg/L of air for four hours under dynamic conditions in a 100-liter glass and stainless steel chamber operated at 16.7L of air/minute. The mean gravimetric concentration of airborne solids was 0.022 mg/L of air. A second group was exposed to air alone.

Results: No deaths, LC_{50} > 5.0 mg/L (nominal)
LC_{50} > 0.022 mg/L (gravimetric)

Toxic Signs: Activity, preening, sneezing, on Day 1 through 4 of the post-exposure observation period, two rats (1 male and 1 female) were observed to have urogenital fur stains.

Body Weight: Initial weight loss immediately following exposure (more pronounced in male rats). The weight was subsequently regained by the end of 14 day period.

Necorpsy: No gross lesions.

Toxicity Category I: Danger (gravimetric)

Classification: Supplementary Data

(a) Inadequate number of doses tested.
(b) LD_{50} not adequately calculated.

Exposure B:

One group of 5 male and 5 female rats was exposed to a nominal active ingredient aerosol concentration of 7.0 mg/L of air for 4 hours under dynamic conditions in a 100-liter glass and stainless steel chamber operated at 16.7 L of air/minute. The mean gravimetric concentration of airborne solids was 0.0839 mg/L of air. A second group was exposed to air alone.

Results: No deaths; LC_{50} (nominal) > 7.0 mg/L
LC_{50} (gravimetric) > 0.0839 mg/L

Toxic Signs: Sniffing, excessive preening, excessive swallowing, restless activity, chewing. One male rat exhibited a reddish brown crust around both eyes on Days 1 through 8 following exposure.

Body Weight: Significantly lower mean body weights for male rats.

Necropsy: No gross lesions.

Toxicity Category I: Danger (gravimetric)

Classification: Supplementary Data

(a) LD_{50} not adequately calculated.
(b) Inadequate number of doses.

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14. Document 14. 4-Hour LC_{50} Determination in Rats with Larvin Wettable Powder (75% active) (Hazelton Project No. 400-623; March 26, 1980)

Groups of 5 male and 5 female rats were exposed for four hours to nominal concentrations of 2.21, 4.50, 5.65 and 6.88 mg/L (gravimetric concentrations of 0.441, 0.734, 0.776 and 1.14 mg/L). Observation for 14 days.

Results: LC_{50} = 6.23 (5.59-6.94) mg/L (nominal)
LC_{50} = 0.776-1.14 mg/L (gravimetric)

Toxic Signs: Sniffing, preening, labored breathing, tremors.

Necropsy: Lung discoloration

Toxicity Category II: Warning

Classification: Core-Guideline

15. Document 15. UC-51762-Formulation (75 WP) 9-Day Dust Inhalation Study on Rats (Carnegie-Mellon Institute of Research; Project No. 42-62; June 28, 1979)

Groups of 10 male and 10 female Hilltop Sprague-Dawley rats were subjected for 6-hours per day, for 9 days, to repeated inhalation of UC-51762 formulation (75 WP) particulates at mean measured atmospheric concentrations of 57.8, 18.8 and 4.7 mg/m^3 for males and 61.6, 14.7 and 4.5 mg/m^3 for females. Control groups inhaled chamber air that did not contain UC-51762 formulation particular. Body weight per se and as a change form pre-exposure values, liver, lung, kidney and brain weight per se and as a percentage of body weight, food consumption and plasma or serum, erthrocyte and brain cholinesterase per se and as a percentage of the control mean were compared to evaluate toxic response.

Results:

For both males and females, inhalation of a target concentration of 60 mg/m^3 resulted in tremors, pinpoint pupils and mastication, in addition to abnormal righting reflex, toe pinch, gait and locomotion. At this same level, significant depression were found in body weight, body weight change, food consumption and erythrocyte and brain cholinesterase for the test groups. These clinical signs (indicative of toxic effect) were seen to a lesser degree in animals that inhaled dust target concentrations of 20 mg/m^3, and they were not seen, except for a few cases of pinpoint pupils, lacrimation and mastication, in animals

that inhaled dust target concentrations of 5 mg/m^3. Significant depressions in body weight gains were also seen at 20 mg/m^3 (both sexes) and 5 mg/m^3 (males only).

Conclusion:

No NOEL was observed for inhalation of UC-51762 formulation (75 WP) in this study. Even at the lowest levels tested (target concentration of 5 mg/m^3) some clinical signs typically associated with anticholinergic effects (pinpoint pupils and lacrimation) and a significant reduction in body weight gain (in males) were observed.

Classification: Core-Minimum Data

16. Document 16. Acute Eye Irritation Study in Rabbits with Larvin Insecticide Technical (UC-51762) (Hazelton Project No. 400-616; November 23, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Conclusion: Toxicity Category II: Warning

Classification: Core-Guidelines

17. Document 17. Acute Eye Irritation Study in Rabbits with Larvin-500 Insecticide Formulated (UC-51762) (Hazelton Project No. 400-616; November 23, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Conclusion: Toxicity Category III: Caution

Classification: Core-Minimum Data

18. Document 18. Primary Eye Irritation Study in Rabbits with Larvin 75 WP (Hazelton Report No. 400-621; April 1, 1980)

Twenty-four hours prior to instillation of the test material, the left eye of each rabbit was examined following staining with 2% fluorescein sodium solution to confirm the absence of corneal defects. 58 mg of the test material was placed into the conjunctival sac of the left eye of each of nine NZW rabbits with the right eye serving as a control. The treated eyes of 3 rabbits were rinsed for one minute with lukewarm water 20-30 seconds post-instillation.

The treated eyes of the remaining 6 rabbits were left unwashed. Observation was at 1, 2, 4 and 7 days.

Results:

Conjunctival redness was noted in two unwashed eyes at 24 hours, while some compound still remained in the conjunctival sac of six unwashed eyes at 24 hours. No corneal opacity iritis, or conjunctival chemosis or discharge were noted in any of the test animals. Washed eyes did not show any irritation. Ocular lesions were observed to clear in all animals by 48 hours.

Toxicity Category III: Caution

Classification: Core-Minimum Data

19. Document 19. Primary Skin Irritation Study in Rabbits with Larvin Insecticide Technical (UC-51762) (Hazelton Project No. 400-617, November 23, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Conclusion: Toxicity Category IV: Caution

Classification: Core-Guidelines

20. Document 20. Primary Skin Irritation Study in Rabbits with Larvin-500 Insecticide Formulated (UC-51762) (Hazelton Project No. 400-617; November 23, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Conclusion: Toxicity Category IV: Caution

Classification: Core-Minimum Data

21. Document 21. Primary Skin Irritation Study in Rabbits with Larvin 75 WP (Hazelton Project No. 400-622; February 27, 1980)

0.5 gm of test material was applied to two intact and two abraded skin sites on the fur-clipped trunk of 6 NZW rabbits under an impervious cuff for 24 hours. Observation and scoring at 24 and 72 hours according to Draize.

Results:

Nine abraded and eight intact exposure sites exhibited very slight erythema and one abraded and one intact site exhibited well-defined erythema at 24 hr., while no abraded or intact sites showed any erythema at 72 hr. Only one abraded site had very slight edema at 24 hr., while no sites showed edema at 72 hr. No other dermal effects were observed.

P.I. = 0.45

Toxicity Category IV: Caution

Classification: Core-Guideline

22. Document 22. Skin Sensitization in Guinea Pigs (CDC Study No. CDC-UC-003-79; July 12, 1979)

Test Material: UC-51762 technical

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

There were no skin changes seen in the skin during the induction and challenge periods. Necropsy was unremarkable.

Classification: Core-Minimum Data

23. Document 23. Dermal Sensitization in the Guinea Pig with UC-51762 technical purified grade (Carnegie-Mellon Institute of Research, Report No. 42-61; June 22, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

In this study UC-51762 (technical grade) produced weak sensitization reactions in guinea pigs as compared to reactions produced by UC-51762 - 75 WP and UC-51762 - 4F (CMI Project Report No. 42-26).

Classification: Core-Minimum Data

24. Document 24. Skin Sensitization in Guinea Pigs (CDC Study No. CDC-UC-004-79, July 12, 1979)

Test Material: UC-51762 - 4F

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

There were no changes seen in the ten guinea pigs during the challenge period. There were no detectable gross lesions found at necropsy.

Classification: Core-Minimum Data

25. Document 25. Dermal Sensitization Potential in the Guinea Pigs (Carnegie-Mellon Institute of Research, Special Report#42-26); March 28, 1976)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

UC-51762 - 75WP produced moderate sensitization, whereas UC-51762-47 was a mild to moderate sensitizer.

Classification: Core-Minimum Data

25a. Document 25a. Skin Sensitization in Guinea Pigs (CDC Project No. CDC-UC-002-79, June 25, 1979)

Test Material: UC-51762 - 75WP

The test material was studied for Guinea Pig Skin Sensitization according to the Code of Federal Regulations. The method employed was modified from the method of Buehler and the method of Draize. Ten young adult male White Hartley guinea pigs were clipped free of hair over the back, prior to the study and at least once a week during the induction phase. A 0.5 ml aliquot of the test material was then applied by gentle inuction to an area of the back, left of midline, and allowed to air dry for 5 to 15 minutes. The back was then covered with a gauze pad and held in place with hypoallergenic adhesive tape. The patch was removed after 24 hours. The applications were made three times weekly for three weeks or a total of 9 applications at the same site. Following a 17 day rest period in which no applications were made, the animals were reclipped and twice challenged at the original induction site and a virgin site, with the same volume and concentration of material for a 24 hour period.

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The challenges were made on Days 37 and 39 and the readings were taken on Days 38, 40, and 43, according to the method of Draize. A gross necropsy was performed on all animals at the end of the study.

Results:

There were no skin changes seen in the skin during the induction and challenge periods. Necropsy was unremarkable.

Classification: Core-Minimum Data

26. Document 26. Clinical Safety Evaluation of Larvin UC-51762 Powder and Larvin 500 Flowable (FDRL OE No. 2177; November 14, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

Larvin UC-5172 powder and Larvin 500 flowable both may be capable of inducing mild sensitization in human and subjects.

Classification: Core-Minimum Data

27. Document 27. Approximate Acute oral Toxicity (LD_{50}) in Hens and Estimation of Efficacy of Atropine Prophylaxis vs. a greater than LD_{50} dose (FDRL No. 6064, 12/22/78)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

LD_{50} = 582 mg/kg (hens)

The efficacy of atropine sulfate (15 mg/kg) prophylaxis 15 min. prior to administration of test material was seen to be sufficient to warrant recommendation of a reduction in the LD_{75} (830 mg/kg) size of test group in subsequent delayed neurotoxicity study from 40 to 22 hens.

Classification: Core-Minimum Data

27a. Document 27a. UC-51762; Antidote Study using Atropine Sulfate (Carnegie-Mellon Institute of Research, Project Report 41-59; March 30, 1978)

Groups of 5 or 10 male Hilltop Wister albino rats 190 to 120 gm, were given doses by peroral intubation, equivalent to 2 or 2.5 LD_{50}'s of UC-51762. The LD_{50} of the UC-51762 used in this study was 149 mg/kg.

At various periods, after the single peroral dose of the toxicant, atropine sulfate was administered initially intravenously at a dosage of 10.0 mg/kg (1 ml = 20 mg atropine sulfate in 0.9% saline). In most cases, when given 5 minutes after the toxicant, atropine sulfate saved one-half or more of the dosed rats. However, to optimize survival, it is necessary to protect the rats from over-atropinization and dehydration. Repeated doses of atropine sulfate by intraperitoneal, intravenous or subcutaneous injection were given in combination with fluid therapy (lactated Ringer's solution) by intraperitoneal or subcutaneous injections. These trials prolonged the life of the rats but did not accomplish 100% survival. The administration of atropine sulfate eliminated the signs of lacrimation and salivation, delayed and lessened the severity of tremors and prolonged the life of the animals.

Classification: Core-Minimum Data

28. Document 28. Evaluation of UC-51762 #40-488 as a Potential Delayed Neurotoxic Agent following Oral Administration to hens protected by Atropine Sulfate (FDRL No. 6065; June 14, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE, PP#9G2152.

Results:

UC-51762 #40-488 displayed a potential for delayed neurotoxicity which was manifested as marginal symptoms up to 21 days in 8 birds. These clinical signs were not apparent in the redosed test animals after 42 days.

Classification: Core-Minimum Data

28a. Document 28a. Acute Delayed Neurotoxicity Study in Hens (IRDC Report No. 369-047; September 2, 1980)

Reviewed in memo of 10/6/80 from William Dykstra to Jay Ellenberger; 264-GUG.

Results:

Larvin thiodicarb was not a delayed neurotoxic agent in this study.

Classification: Core-Minimum Data

29. Document 29. Acute Intraperitoneal LD_{50} of C-51762 in the Mouse (Pharmakon Study No. PH-405-UC-001-79)

Five groups of 5M + 5F mice were administered UC-51762 intraperitoneally at 10, 15, 20, 50 and 60 mg/kg. Observation was for fourteen days.

Results: LD_{50} = 34 (21.9-52.7) mg/kg

Toxic Signs: Slight tremors, salivation, convulsions.

Body Weight: Survivors gained weight.

Necropsy: Unremarkable

Classification: Core-Minimum Data

30. Document 30. Acute Intraperitoneal LD_{50} of UC-51762 in the Rat (Pharmakon Study No. PH-404-UC-001-79; May 30, 1979)

Five groups of 5 male and 5 female rats were administered UC-51762 intraperitoneally at 10, 20, 30, 50 and 60 mg/kg. Observation for 14 days.

Results: LD_{50} = 23 (15.9-33.1) mg/kg

Toxic Signs: Tremors, twitches, convulsions, salivation, piloerection, crusty nose, exophthalmus, and chromodacryorrhea.

Body Weight: Survivors gained weight.

Necropsy: Lungs bright red, black spots, red sac on stomach, intestines fluid-filled.

Classification: Core-Minimum Data

31. Document 31. UC-51762; Inclusion in the Diet of Rats for Thirteen Weeks (Carnegie-Mellon Institute of Research Project Report 41-63; April 6, 1978)

Reviewed in memo 5/23/79 from William Dykstra to Frank Sanders; 1016-EUP-LE; PP#9G2152.

Conclusion: Systemic NOEL = 3.0 mg/kg/day

Cholinergic NOEL = 10 mg/kg/day.

Classification: Core-Guidelines

32. Document 32. UC-51762; Inclusion in the Diet of Dogs for Thirteen Weeks (Carnegie-Mellon Institute of Research Project Report 44-98; May 31, 1978)

Reviewed in memo of 5/29/79 from William Dykstra to Frank Sanders; 1016-EUP-LE, PP#9G2152.

Conclusion: Systemic + cholinergic NOEL = 15 mg/kg/day.

Classification: Core-Minimum Data

33a. Document 33a. Evaluation of Larvin (UC-51762) in 21-Day Dermal Toxicity Study in Rabbits (Snell Project#02413-090; 3/14/80)

The sample was diluted with physiological saline to obtain a paste consisting of approximately 60 percent Larvin (UC-51762). The dose levels were as follows:

Group	Dose/Level	T/C Sample
I	1 ml/kg	physiological saline (vehicle)
II	1 gm/kg	UC-51762
III	2 gm/kg	UC-51762
IV	4 gm/kg	UC-51762

The trunk of each animal was clipped free of hair prior to the initiation of the study and as necessary thereafter to keep it relatively free of hair. From each test group of twenty, 10 rabbits (5 males and 5 females) were further prepared by abrading the test site. This was repeated on a weekly basis. The skin of the other 10 rabbits (5 males and 5 females) was not abraded. The vehicle control (physiological saline) or test material was applied by gentle inunction over the clipped area of the abraded and unabraded skin. The animals were exposed to the test material for a six hour period, five days a week for three weeks. The treated skin was covered with surgical gauze secured in place with hypoallergenic adhesive tape. The trunks of the rabbits were then covered with a nonreactive heavy gauge plastic. At the end of each daily exposure, the wrappings were removed and the treated areas were rinsed with water to remove any remaining material. The skin of each rabbit was evaluated daily for signs of erythema and edema prior to dosing. Food consumption was recorded at two to four day intervals throughout the study. Body weights were recorded on the first day of the study (Day 0) and at 3 to 4 day intervals thereafter. Animals were observed daily for signs of toxicity. The following clinical tests were performed prior to study initiation and at the end of the treatment period:

Hematology:

Hematocrit	total and differential WBC
Hemoglobin	platelet count
Erythrocyte count	

Blood Chemistry:

Calcium	BUN	Total protein
potassium	Total cholesterol	Direct Bilirubin
LDH	SAP	Total Bilirubin
SGPT	Albumin	Glucose
SGOT	Globulin	

The animals were sacrificed by injecting pentobarbital sodium (5 grains/ml) into the heart. The external surface, all orifices, cranial cavity, carcass, external and cut surfaces of the pelvic cavities and their visceral and cervical tissues and organs were examined. The weights of liver, kidneys, heart, gonads, thyroid (with parathyroid), adrenals and pituitary were recorded. The following organs were taken from all animals and preserved in 10 percent neutral buffered formalin:

brain	optic nerve	stomach	gonads
eye	liver	pancreas	bone marrow
thyroid	spleen	small intestine	urinary bladder
lung	kidneys	large intestine	sciatic nerve
heart	adrenals	spinal cord	unusual lesions
skin (treated/ untreated)			

The tissues were processed routinely, embedded in paraffin and stained with hematoxylin and eosin prior to microscopic evaluation.

Results:

Eleven mortalities occurred during the course of this test[a]:

Group	Test Material	Dose	Sex	Date of Death	No. Days on Study
I	Saline	1 ml/kg	M	10/09/79	22
I	Saline	1 ml/kg	F[b]	10/08/79	21
II	Larvin	1 gm/kg	F	10/08/79	21
II	Larvin	1 gm/kg	F[b]	10/07/79	21
II	Larvin	1 gm/kg	F	10/08/79	20
III	Larvin	2 gm/kg	F[b]	10/08/79	21
III	Larvin	2 gm/kg	F[b]	10/08/79	21
III	Larvin	2 gm/kg	F[b]	10/08/79	21
IV	Larvin	4 gm/kg	M	10/10/79	23
IV	Larvin	4 gm/kg	M	10/02/79	15
IV	Larvin	4 gm/kg	F[b]	10/08/79	21

[a] terminal sacrifice of all animals was conducted from 10/9/79 to 10/11/79.

[b] —Died during final bleeding.

The deaths were not dose-related. Skin desquamation eschar formation and pimples were noted in all groups and apparently resulted from the procedures of application and removal of test substances from the skin. However, desquamation appeared in a greater number of animals as the dose increased.

No treatment-related observations of general toxicity or behavior were noted in this experiment. There were no significant treatment-related differences in body weight during the test. There were no significant treatment-related differences in food consumption values during the test. No erythema or edema were observed on day 1. As the test progressed, scores for both erythema and edema gradually increased in number and severity across all groups. Marked increases were observed over the last four to five days of the test period. Scores of 4 (erythema) and 2 (edema) were recorded for all groups in the final days of the test, however, a greater number of males were affected in the compound treated groups than in the control group. The incidence of affected females did not appear to differ between treatment groups. Most differences from the test results of the final bleeding were either within the normal and expected range of values or they were not dose-related. However, red blood cell count, hemoglobin, hematocrit, and platelet count suggested a significant treatment-related macrocytic anemia in both males and females.

This conclusion is supported by the increase in mean corpuscular volume (MCV) in males and females and mean corpuscular hemoglobin (MCH) in males.

No relative organ weight differences were found among female treatment groups. In males, relative organ weight differences in the liver (increase in group 4) and adrenals (decrease in group 2) were found. The changes in organ weights were not associated with microscopic structural change. No microscopic evidence of systemic, compound-induced or dose-related effects were observed except for the skin. Skin alterations such as generalized hyperkeratosis, abscesses and ulceration were observed and did appear to be dose-related.

Conclusion:

Application of Larvin (UC-51762) to the skin of rabbits daily (5 days/week for three weeks) at doses of 1, 2 and 4 gm/kg/day produced a dose-related macrocytic anemia. Induction of a compound related dermatologic effect manifested as erythema and edema was observed. No histopathologic signs of anemia were observed.

Classification: Core-Guideline

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33b. Document 33b. Raw Data of Document 33a.

33c. Document 33c. Raw Data of Document 33a.

33d. Document 33d. Raw Data of Document 33a.

34. Document 34. UC-51762; 16-Dose Rabbit Dermal Study (Carnegie-Mellon Institute of Research Project Report No. 42-52; June 12, 1979)

Five male NZW rabbits weighing between 2.3 and 3.0 kg (14-15 weeks old) were randomly assigned to each of three dosage groups. One group of five animals received 4.0 gm/kg/day and a second group received 1.0 gm/kg/day of UC-51762 both of which were administered as a paste wetted with physiological saline. The third group of rabbits received dermal applications of saline and were treated exactly as the animals which received UC-51762. The exposure took place on abraded skin under an impervious cuff for 6 hours daily on each of 5 days per week for 3 consecutive weeks. Exposure continued on the Monday following the third week, and the sacrifice took place on the following day. All clinical signs of toxicity and skin irritation were recorded daily. Body weight and food consumption were measured twice weekly, Monday and Thursday, and individual doses of UC-51762 were adjusted for changes in body weight.

The following hematological determinations were made on each animal and at the beginning (4 days before dosing) and at the end of the study (following 15 doses): hematocrit, hemoglobin, RBC count, reticulocyte count and total and differential WBC count. Plasma and RBC cholinesterase activities were determined once prior to dosing (4 days before), at the end of the second week, and near the end of the study (following 15 doses).

The rabbits were sacrificed by CO_2 narcosis at which time they were 18 to 19 weeks of age. Complete gross necropsy examinations were performed and the following tissues from all sacrificed rabbits were taken for possible future examination and were fixed in 10% neutral buffered formalin:

thyroids
parathyroids
adrenals
heart
great vessels
thymus
spleen
cervical lymph nodes
sublumber lymph nodes
inguinal lymph nodes
esophagus
stomach
duodenum
jejunum
ileum
cecum
colon
liver
gall bladder
pancreas

(continue from last page)

trachea	brain
lungs	skin (treated and untreated)
testes	mammary gland
epididymics	lumbar muscle
prostate	femoral marrow smear
kidneys	lesions
urinary bladder	

Organ weights were recorded for the liver, kidneys, adrenals and testes. In addition, a sample of brain approximately 2 x 4 x 6 mm in size was obtained for cholinesterase assay. This piece of brain was from the left cerebral hemisphere at the level of interthalamic adhesion (intermediate mass) and included the hypothalamus. After adeqaute fixation in 10% neutral buffered formalin, the following tissues from all treated and control rabbits were processed by standard techniques, stained with hematoxylin and eosin and examined microscopically.

livers	treated skin
kidneys	untreated skin
lesions	

Statistical analyses of the data were performed.

Results:

The only sign of toxicity noted during the study was the appearance of soft feces and/or diarrhea in both treated and control groups. At the 4 gm/kg dose level, 4 out of 5 rabbits exhibited this condition after 7 to 10 doses. At the 1 gm/kg level, 5 out of 5 had soft feces or diarrhea after 7 to 14 doses. One rabbit in the control group also exhibited this condition. No signs of skin irritation were noted on the UC-51762 dosed rabbits. One rabbit in the 4 gm/kg group died after 12 doses. The death was attributed to cardiovascular failure secondary to fluid imbalance brought on by an acute enterotyphilitis. There was an apparent reduction in weight gain in the 4.0 gm/kg UC-51762 treated animals, but this reduction was not statistically significant at any time during the study. The high dose (4 gm/kg) group actually showed a net loss of weight during the study. The absence of statistical significance may be attributed to the small sample size and to the variability among animals within groups. For example, one animal in the high dose group lost 815 grams during the study, almost one-third of its initial weight . Although the total food consumption in the high dose (4 gm/kg) rabbits was approximately 20% lower than the control value, the difference between the groups was not statistically significant at any

time during the study. There were no statistically significant effects in any of the organs examined. Apparent dose-related decreases in absolute kidney and testicular weights were observed, but these differences disappeared when the organ weights were expressed as percentages of body weight. The only statistically significant difference in hematology between treated and control groups at sacrifice was a reduction in red blood cell (RBC) count in the high dose (4 gm/kg) group. Other related parameters in the high dose animals such as hemoglobin concentration and reticulocyte count, did not differ significantly from the saline control values. Because of the significant differences in the pretreatment values of some RBC parameters, all the RBC parameters were analyzed also as changes from the pretreatment values. This analysis confirmed the reduction in RBC count, packed cell volume and in hemoglobin concentration. These results suggest a trend towards anemia in the rabbits which received 4 gm/kg of UC-51762. There were no statistically significant differences in plasma, RBC and brain cholinesterase levels between the UC-51762 treated rabbits and their saline controls. Plasma cholinesterase activity in the rabbits assigned to UC-51762 treatment were higher than those in the saline control group prior to treatment, and the values in both groups appeared to have decreased by the time of sacrifice. The change from the pretreatment values in the UC-51762 treated groups was not significantly different from that in the saline controls.

UC-51762 treatment did not result in a significant change in cholinesterase activity of brain, plasma, or erythrocytes.

There were no gross or histopathologic lesions related to treatment. Histologic lesions involving the bile ducts and gall bladder of control and experimental animals were attributed to infestation with a coccidian protozoan parasite.

Conclusion:

Effects at 4 gm/kg of UC-51762 consisted of diarrhea, reduction in erythrocytes and hemoglobin, and a reduction in body weight. A dose of 1.0 gm/kg resulted in diarrhea and/or soft feces.

Classification: Core-Minimum Data

35. Document 35. Pilot Teratology Study in Rats with UC-51762 (IRDC Report No. 369-028; September 10, 1979)

    Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

    Conclusion:

    Due to high material toxicity, a dosage level of 40 mg/kg/day is considered excessive for a teratology study.

    Classification: Supplementary Data

36. Document 36. Teratology Study in Rats with UC-51762, (IRDC Report No. 369-029; December 28, 1979)

    Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

    Conclusion:

    The test material produced signs of maternal and fetotoxicity as evidenced by dose-related decreases in mean maternal body weight gain and mean fetal body weight and dose-related increases in reduced fetal ossification. The test material was not teratogenic when administered to pregnant rats at dosages up to 30 mg/kg/day. However, the NOEL for fetotoxicity was not established in the study.

    Classification: Core-Minimum Data

37. Document 37. Rat Teratology Study with UC-51762 (Carnegie-Mellon Institute for Research Project Report 42-48; June 1, 1979)

    Reviewed in memo of 5/2/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

    Conclusion:

    UC-51762 was not teratogenic at dosages up to 100 mg/kg/day given during gestation days 6-15 or 0-20. The fetotoxic NOEL is 3 mg/kg/day.

    Classification: Core-Minimum Data

38. Document 38. Pilot Teratology Study in Mice (IRDC #369-030; August 29, 1979)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP9G2152.

Conclusion:

A dosage level greater than 200 mg/kg/day for a teratology study would produce excessive maternal toxicity.

Classification: Supplementary Data

39. Document 39. Teratology Study in Mice with UC-51762 (IRDC Report #369-031; February 26, 1981)

Reviewed in memo of 2/21/80 from William Dykstra to Charles Mitchell; 1016-EUP-LE; PP#9G2152.

Conclusion:

The NOEL for maternal toxicity is 100 mg/kg/day. UC-51762 is not teratogenic or fetotoxic at 200 mg/kg/day or less. Fetotoxic effects would likely be present at maternal toxic levels.

Classification: Core-Minimum Data

40. Document 40. UC-51762 technical; Inclusion in the Diet of rats for Three-Generations and Dominant Lethal Mutagenesis Studies (Carnegie-Mellow Institute of Research Project Report 42-65; July 2, 1979)

A. 3-Generation Reproduction Study

Fischer 344 albino rats were used as the parent generation (F^0). Their median birthdate was August 15, 1977. They were observed clinically and weighed weekly until dosing started on September 27, 1977.

Fifteen male and 26 female rats were randomly assigned to each dosage level, and 10 and 20 of these were randomly selected for mating at approximately 100 days of age. Target dosage levels were 10.0, 3.0, 1.0 and 0.5 mg/kg/day. There were two control groups which received untreated Purina Chow only. The UC-51762 rats and those in the two control groups were randomized from the same lot.

Each generation was bred once. The first generation, designated F_0, served as parents for the F_{1a} offspring, which subsequently became parents of the next generation. The offspring of the next mating trial, F_{2a}, became parents for the next generation. The study terminated when the F_{3a} Pups were weaned.

The F_0 Parents received their respective UC-51762 treated diets from the day of the first dose, 9/27/77, until they were 98 days of age when they were first bred, 11/21/77. Individual body weight and group food consumption data were recorded weekly. Twenty females and 10 males at equivalent dosage levels were mated, one male and 2 females to a cage. Cohabitation was allowed to continue for 15 days. The date of parturition (day 0 of lactation) and the number of live and dead newborn were recorded for each litter. Dams and pups were observed daily for changes in appearance and behavior. The litter size was reduced to 10, if necessary, on day 4 postpartum. The offspring so retained were selected at random. Offspring were weighed as litters at 4 and 14 days and individually at 21 days postpartum. The sex of each animal was determined at 21 days postpartum and any abnormal conditions recorded. The offspring not selected to be parents of the next generation and the F_0 Parents were then killed. Stillborn and progeny that died during the study were necropsied.

The parentage of each weanling was recorded to avoid brother-sister mating. F_{1a} rats were randomly selected within each dosage group for the next mating trial. Each litter was represented in this selection insofar as was practicable; litters born late in the mating trial were not used due to time limitations imposed by the study schedule. The selected F_{1a} rats were caged so that sexes were separated and continued on their diets until 3/27/78, when they were 98 days of age and when they were first mated. Following the previously described procedures, the randomly selected F_{2a} offspring were first mated at 105 days of age, on 8/7/78. The median birthdates of the F_0, F_1 and F_2 rats were 8/15/77, 12/19/77 abd 4/24/78 respectively.

Necropsies were performed on 5 males and 5 females, randomly selected from each dosage level of the F_2 parents as well as of F_{3a} weanlings. Microscopic examination was performed on fixed and stained portions of accessory sex glands, testicles, and epididymis or uterus and ovaries, oviducts, urinary bladder and mammary tissue for the F_2 parents. For the F_{3a} weanlings, all tissues were fixed in situ.

Records were kept of individual rat reproductive behavior. The following calculations were made and the results subjected to the appropriate statistical analyses:

A. Indexes

Fertility Index - The proportion of females that were pregnant of the number that were mated or the proportion of males shown to be fertile of the number that were mated.

Gestation Index - The proportion of pregnancies that resulted in litters with live pups.

Gestation Survival Index - The proportion of newborn pups that were alive at birth.

Viability Index - The proportion of liveborn that survived 4 days.

14-Day Survival Index - The proportion of pups retained on day 4 that survived 14 days.

Lactation Index - The proportion of pups retained on day 4 that survived 21-days.

B. Weight Data

1. Mean body weight, mean body weight gain, average daily food consumption, average daily compound and the efficiency of food utilization.

2. Mean litter weight at 4 and 14 days; mean pups at 21 days.

C. Progeny Data

Group values for newborn litter size.

D. Gestation

Individual values of gestation duration; days from first mating to the birth of a litter.

Results

One female rat (2226, 0.08, F_{1a}) was killed at 150 days when signs of clinical illness were noted. There were no gross lesions. No other parental rats died or were killed during the study. No dosage-related mortality in neonatal deaths was observed. For each index and mean in all three generations the data of each dosage group were compared statistically to those of the two control groups. None of the criteria examined indicated a deleterious effect in the F_{1a} F_{2a} or F_{2a} F_{3a} generations. Body weight changes of the F_0 male rats for all dosage groups were depressed significantly during the first week of dosing. This change was not dose-related and did not recur during subsequent periods. It is not indicative of a deleterious effect on reproduction. Furthermore, all indices of reproduction were within normal limits for all three generations.

F_{3a} pups were approximately 21 days of age when killed while the F_{2a} males and females were approximately 183 and 177 days of age at sacrifice, respectively. No treatment-related lesions associated with the ingestion of UC-51762 were noted in those rats examined.

Conclusion:

Although non dose-related transitory body weight depression was noted in the male rats during the early portion of the first mating trial, none of the criteria of reproductive performance in the three-generation study was affected. In order to determine the systemic NOEL for the study pathology records of the 3.0, 1.0, and 0.5 mg/kg/day pups of the F_{3a} generation and pathology records of the 3.0, 1.0, and 0.5 mg/kg/day adult male and female rats of the F_{2a} generation are required to be submitted.

Classification: Supplementary Data

(a) Complete pathology reports not submitted.

B. Dominant Lethal Mutagenic Study

The male F_2 rats from the three-generation reproduction study were removed from their respective UC-51762 dosing regimens on 9/26/78 at 155 days of age. Concurrently, on 9/26/78, 74 F_2 male rats derived from the two control groups of the three-generation reproduction study were each given a single intraperitoneal dose of 0.25 mg/kg triethylenemelamine in distilled water. These males served as the

positive controls. In a test for dominant lethal mutagenesis, starting on 9/27/78 and continuing for 3 weeks, groups of naive, virgin female Fischer 344 rats were first mated at 107 days of age. The rats were mated 1 male:1 female in each weekly mating period. Females were introduced into male cages. Cohabitation was allowed to continue until a vaginal plug was seen; if no plug was seen, the mating pairs remained together for the 7-day period. Successfully mated males were not re-mated within the weekly period. Mating was started on Wednesday of each week. The males were 170 days of age at the beginning of the third week of mating. Each group of females was killed midway in gestation, 12 days after a vaginal plug (VP) was seen (or when no vaginal plug was seen, on the thirteenth day after first mating). The results for each of the 315 females so mated were recorded individually. The ovaries, uterus and urinary bladder were dissected from each female and fixed in 10% buffered formalin phosphate solution. Corpora lutea were counted only for the high level and controls.

Statistical analyses of the data were performed.

Results:

All parameters of the UC-51762 treated rats were similar statistically to those of both negative control groups. In contrast, the following changes were noted for the triethylenemelamine treated rats:

(1) Statistically significant reductions for the median number of total implants and median number of viable implants (week 3);

(2) Statistically significant increase for median percentage of fetal deaths per dam (week 2) and numerical increases for this parameter (weeks 1 and 3);

(3) Statistically significant increase of the ratio of the total number of females with early fetal deaths to the number of pregnant females.

Conclusion:

In all criteria of effect in the dominant lethal mutagenesis study, the UC-51762 rats were not significantly different from the control rats. Responses noted in the positive control, TEM, group show that the rats were susceptible to a known mutagen.

Classification: Core-Minimum Data

41. Document 41. Mutagenicity Evaluation of UC-51762 in the Ames Salmonella/Microsome Plate Test (LBI Project No. 20838; April, 1978)

The technical UC-51762 was tested at doses of 1.0, 10, 100, 500 and 1000 ug per plate with and without metabolic activation in Salmonella typhrmurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 and Saccharomyces cerevisiae D-4. Positive controls were tested.

Results:

The test compound, UC-51762, did not demonstrate genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Classification: Core-Minimum Data

42. Document 42. Micronucleus Test (Pharmakon Study No. PH-309-UC001-79; May 22, 1979)

In a preliminary dose range study, technical UC-51762 was administered i.p. once daily for two days to a group of ten mice at a concentration of 15 mg/kg resulting in the death of one mouse. Based upon these findings 10 mg/kg was chosen as the high dose. UC-51762 was administered i.p. once daily for two days to 4 male and 4 female CF-1 mice at the high dose of 10 mg/kg and to a similar group of mice at a low dose of 5 mg/kg. Concurrently, triethylenemalamine at 0.5 mg/kg as a positive control, and water at 20 ml/kg as a negative control, were administered to two groups of eight mice. All the mice were sacrificed by the inhalation of CO_2, six hours after the second dose and their femurs removed. The bone marrow is removed from the femurs smeared on a slide, stained and one thousand (1000) polychromatic erythrocytes are counted for the presence of micronuclei. Roughly 8% of the cells are erythrocytes and half are polychromatic. The results are statistically evaluated.

Results:

The results for UC-51762 were negative in the micronucleus test, based upon the inability of the chemical to produce a statistically significant increase in the number of micronuclei per 1000 polychromatic erythrocytes in the treated versus the control mice.

Classification: Core-Minimum Data

43. Document 43. UC-51762 technical; Reverse Mutation in Saccharomyces cerevisiae (Pharmakon Study No. PH-303-UC-001-79; June 1, 1979)

UC-51762 technical was evaluated for its ability to induce eukaryotic reverse mutation in the homoallelic ilv I-92/ilv I-92 diploid strain D_7 of Saccharomyces cerevisiae at concentrations of 0.25, 0.0625, 0.025, 0.006625 and 0.0025 mg/ml.

Results:

The results for UC-51762 technical were negative in the Saccharomyces cerevisiae strain D_7 eukaryocyte reverse mutation assay.

Classification: Core-Minimum Data

44. Document 44. UC-51762 technical; Mitotic crossing over in Saccharomyces cerevisiae (Pharmakon Study No. PH-302-UC-001-79; June 1, 1979)

UC-51762 was evaluated to test the ability to induce mitotic crossing over in the heteroallelic ade 2-40/ade 2-119 diploid strain D_7 of Saccharomyces cerevisiae. The concentrations of UC-51762 in the mitotic crossing over assay were 0.25, 0.0625, 0.025, 0.00625 and 0.0025 mg/ml. 4-nitroquinoline-N-oxide was used as a positive control.

Results:

The results of UC-51762 were negative in the Saccharomyces cerevisiae strain D_7 mitotic crossing over assay.

Classification: Core-Minimum Data

45. Document 45. UC-51762 technical; Mitotic Gene Conversion in Saccharomyces cerevisiae (Pharmakon Study No. PH-304-UC-001-79; June 1, 1979)

UC-51762 was evaluated to test the ability to induce mitotic gene conversion in the heteroallelic diploid trp 5-12/trp 5-27 strain D_7 of Saccharomyces cerevisiae. The concentrations of UC-51762 in the mitotic gene conversion assay were 0.25, 0.0625, 0.025, 0.00625 and 0.0025 mg/ml. 4-nitroquinoline-N-oxide was used as positive control.

Results:

UC-51762 technical produced a significant increase in mitotic gene conversion in the heteroallelic trp 5-12/trp 5-27 diploid strain D_7 of Saccharomyces cerevisiae at the

0.25, 0.0625 and 0.025 mg/ml levels compared to the negative control as determined by the student's "t" test.

Classification: Core-Minimum Data

46. Document 46. UC-51762 technical; Primary DNA Damage (Pharmakon Study No. PH-305-AM-002-79; May 14, 1979)

UC-51762 was tested to determine if the chemical is able to modify the DNA of the repair deficient strain p.3478 differentially than the DNA repair competent strain W3110. Escherichia coli strains W3110 and p.3478 were grown to exponential phase in HAT/broth. For the direct acting assay (without metabolic activation) the test compound was added at concentrations of 10, 1, 0.1, 0.01 and 0.001 mg/ml of sterile 7 mm discs at a volume of 20 ul. All dilutions were made in DMSO. For the metabolic activation assay, 50 ul of S-9 mix were added to the test wells cut in the center of the petri plates, and the compound was added at concentrations of 10, 1, 0.1, 0.01 and 0.001 mg/ml at a volume of 50 ul. Positive controls were tested.

Results

UC-51762 did not produce a substantial zone of inhibition in either strain. The lack of a zone of inhibition can be a result of the inability of the test chemical to diffuse through the media or the chemical's inability to produce genetic effects. These two alternatives can be further elucidated through the suspension assay.

Classification: Core-Minimum Data

49. Document 49. Miscellaneous Toxicity Studies (Carnegie-Mellon Institute of Research Project Report 42-27; March 21, 1979)

UC-45650

S-methyl-O-(N-methylcarbamoyl)acetothia hydroximate
Sample 41-100
Peroral, single dose to rats
LD_{50} = 20 (9.56-41.9) mg/kg; 1 ml = 100 mg in corn oil

UC-54172

1-methylsulfinyl-N-(methylcarbamoyloxy)-acetimidate
Sample 41-102
Peroral, single dose to rats
LD_{50} = 476 (311-727) mg/kg; 1 ml = 100 mg in corn oil

UC-527[illegible]

S-methylacetothiolhydroximate
Sample 41-101
Peroral, single dose to rats
LD_{50} = 1000 (636-1572) mg/kg; 1 ml = 100 mg in corn oil

UC-58614

1-methylthioacetaldehyde-O-(hydroxymethylcarbamoyl) oxime
Sample 100-403
Peroral, single dose to rats
LD_{50} = 238 (156-363) mg/kg; 1 ml = 100 mg in corn oil

Toxic Signs: tremors, sluggish, prostate

Gross Pathology: In victims, lungs with petechiae; medullae of kidneys pink; adrenals pink or red; stomachs transparent, distended, fluid-filled; intestines opaque or transparent in sections, gas filled. In survivors, nothing remarkable.

Classification: Supplementary Data

(a) Individual animal data not presented.

50. Document 50. Miscellaneous Toxicity Studies (carnegie-Mellon Institute of Research Special Report 39-40; March 1, 1976)

UC-45650

S-methyl-O-(N-methylcarbamoyl) acetothiohydroximate
Sample 38-459
Peroral, single dose to rats
LD_{50} = 47.6 (31.1-72.7) mg/kg; 1 ml = 10 mg in PEG 400

Toxic Signs: tremors, salivation, lacrimation

Gross Pathology: In victims, livers mottled; kidneys speckled and slightly congested; intestines liquid-filled and injected. Nothing remarkable in survivors.

Classification: Supplementary Data

(a) Individual animal data not presented.

51. Document 51. UC-45650; Results of Feeding in the Diet of Rats for 7 days (Carnegie-Mellon Institute of Research Project 41-102; June 19, 1976)

UC-45650 was incorporated in the diet of 5 male and 5 female Wistar albino rats for 7 days at dosage levels of 40.6, 17.0, 5.0, 0.0 or 0.0 mg/kg/day for males and 38.6, 15.0, 6.3, 0.0 or 0.0 mg/kg/day for females. Criteria of effect included mortality, diet consumption, growth, and weights of the livers and kidneys.

Results:

No deaths occurred in this study. At the high and intermediate levels in both sexes body weights were reduced throughout the duration of the study. Diet consumption was reduced at the highest level fed in both sexes and in the females at the intermediate level. Evaluation of effects on organ weights revealed significant reduction of absolute liver and kidney weights in both sexes at the highest level and in the females at the intermediate level. Relative kidney weight was increased for the low level females with respect to the control, but this parameter was not affected in the intermediate and high dosage levels. This change is considered to be a statistical anomaly, not indicative a compound-related effect since it didn't occur in a dose-related manner.

Conclusion:

Based on the limited parameters evaluated, the NOEL is 5.0 mg/kg/day for males and 6.3 mg/kg/day for females.

Classification: Supplementary Data

(a) No hematologics or clinical chemistries were evaluated.

52. Document 52. UC-45650; Methomyl; S-methyl-O-(N-methylcarbamoyl) acetothiohydroximate; Inclusion in the Diet of Rats for 13-Weeks (Carnegie-Mellon Institute of Research Project Report 41-64; April 6, 1978)

Methomyl (UC-45650) was administered in the diet to 10 Males + 10 females young adult Fischer 344 rats for thirteen weeks at mean dosage rates of 30.2, 10.2, 3.0, and 1.0 mg/kg/day to males and 29.8, 9.9, 3.0, and 1.0 mg/kg/day to females. Two control groups of 10 rats/sex were used. Criteria of effect included clinical signs, mortality, diet consumption, body weight, clinical chemistries, hematologies, plasma, RBC and brain cholinesterase, urinalyses, organ weights, gross pathology and histopathology.

Results:

These dosages produced no mortality or clinical signs attributable to toxicity throughout the study, in particular there were no clinical signs attributable to cholinesterase inhibition. Diet consumption was unaffected by treatment. There was an equivocal increase in water consumption by males at 10.2 mg/kg/day, and a decrease at all levels in females. Body weight gain of males was unaffected, but was decreased all levels in females from day 28 on. Mean kidney weights, as percent of body dosage levels were increased,

but without histologic changes, and may be attributable to the body weight decrease.

Statistically significant alterations of spleen weights in both sexes were not felt to be of toxicologic significance. There were no statistically significant changes in serum chemistry in either sex, although a few individual serum calcium values were outside the generally accepted normal range for rats. Slight but statistically significant reductions in red blood cell parameters of males at 30.2 and 10.2 mg/kg/day were not considered biologically significant. Plasma and brain cholinesterases of both sexes were within acceptable ranges, but erythrocyte cholinesterase values of male and female rats at the highest dosage level were statistically elevated, possibly representing a compensatory process. There were no biologically significant gross lesions or treatment-related histopathologic effects when compared to controls.

Conclusion

The significant alterations noted in this study were decreased body weight gains in females at all levels. No NOEL methomyl was established in this study.

Classification: Core-Minimum Data

53. Document 53. UC-52702; S-methylacetothiolhydroximate; Range Finding Toxicity Studies (Carnegie-Mellon Institute of Research Special Report 39-25; February 16, 1976)

1. Stomach Intubation, Rat - LD_{50} = 794 mg/kg; 1 ml = 100 mg in corn oil.

2. Skin Penetration, 4-hr., Rat - LD_{50} > 1000 mg/kg; 1 ml = 100 mg in corn oil.

3. Inhalation, Rat - aerosol, 3% (w/w) in distilled water; 0.820 mg/L for 4 hours killed 0 of 6.

4. Uncovered Skin Irritation, Rabbit - none from 25% in acetone or from a one-gram + 2 ml dimethyl phthalate (DMP) suspension.

5. Eye Injury, Rabbit - severe from the solid or from the above suspension in DMP.

Classification: Supplementary Data

(a) Individual animal data not presented.
(b) Inadequate number of doses.

53a. Document 53a. UC-52702; Results of Feeding in the Diet of Rats for 7 days. (Carnegie-Mellon Institute of Research Project Report 41-104; June 21, 1978)

UC-52702 was incorporated in the diet of 5M + 5F Wistar albino rats for 7 days at dosage levels of 199.4, 141.5, 79.7, 0.0 or 0.0 mg/kg/day for males and 243.2, 120.8, 78.4, 0.0 or 0.0 mg/kg/day for females. Criteria of effect included mortality diet consumption, growth and weights of the livers and kidneys.

Results:

Reduced body weights, diet consumption and organ weight changes were observed in both sexes at all dosage levels fed. Of the parameters examined, only the relative liver and kidney weights for the males at 79.7 mg/kg/day and the females at 78.4 mg/kg/day were equivalent to the controls.

An acrid, pungent odor produced by the test material at all dosage levels, rather than any latent toxicity associated with the ingestion of UC-52702 is possibly the variable that resulted in the abnormally low diet consumption and consequent body weight reduction and organ weight changes. It is questionable, therefore, whether determination of a meaningful NOEL is practicable by inclusion of UC-52702 in the diet.

Conclusion:

No NOEL was established in this study.

Classification: Suppelementary Data

(a) Clinical chemistries and hematologies not evaluated.

54. Document 54. UC-54172; Miscellaneous Toxicity Studies (Carnegie-Mellow Institute of Research Project Report 41-25; February 15, 1978)

UC-54172

1-methylsulfinyl-N-(methylcarbamoyloxy)-acetimidate
Peroral, single dose to rats
LD_{50} = 453 (296-696) mg/kg; 1 ml = 10 mg in corn oil

Classification: Supplementary Data

(a) Inadequate number of doses.
(b) Individual animal data not presented.

55. Document 55. UC-54172; Results of Feeding in the Diet of Rats for 7 days (Carnegie-Mellon Institute of Research Project Report 41-106; June 22, 1978)

UC-54172 was incorporated in the diet of 5M + 5F Wistar albino rats for 7 days at dosage levels of 193.2, 138.7, 54.1, 0.0 or 0.0 mg/kg/day for males and 247.0, 143.2, 62.0, 0.0 or 0.0 mg/kg/day for females. Criteria of effect included mortality, diet consumption, growth, and weights of the livers and kidneys.

Results:

Diet consumption, body weight gain and absolute liver and kidney weights were depressed in male rats at 193.2 and 138.7 mg/kg/day. For female rats at 247.0 and 143.2 mg/kg/day, relative kidney weight was the only parameter equivalent to the controls. No adverse effects were noted for the amles or females at the lowest dosages fed, 54.1 and 62.0 mg/kg/day, respectively.

Conclusion: The NOEL for the study is 54.1 mg/kg/day.

Classification: Supplementary Data

(a) Blood chemistries and hematologies not performed.

56. Document 56. UC-58614; Miscellaneous Toxicity Studies (Carnegie-Mellon Institute of Research Project Report 41-133; Sept. 25, 1978)

UC-58614

1-methylthioacetaldehyde-O-(hydroxymethylcarbamoyl) oxine
Sample 40-449
Peroral, single dose to rats
LD_{50} = 200 (123-326) mg/kg; 1 ml = 100 mg in corn oil

Classification: Supplementary Data

(a) Individual animal data not presented.
(b) Inadequate number of doses.

57. Document 57. UC-58614; Results of Feeding in the Diet of Rats for 7 days (Carnegie-Mellon Institute of Research Project Report 41-107; June 23, 1978)

UC-58614 was incorporated in the diet of 5M + 5F Wistar albino rats for 7 days at dosage levels of 145.6, 48.8, 18.0, 0.0, or 0.0 mg/kg/day for mlaes and 141.6, 48.3, 183.0, 0.0, or 0.0 mg/kg/day for females. Criteria of effect included mortality, diet consumption, growth, and weights of the livers and kidneys.

Results:

Body weight gain was depressed for the duration of the study in male rats at 145.6 mg/kg/day, females rats at 141.6 mg/kg/day and at day 7 in male rats at 48.8 mg/kg/day. Absolute liver weights were reduced in both sexes at the high level and in males at the intermediate level. Absolute kidney weights were reduced in males at the high level, in both sexes at the intermediate level and in females at the low level.

Diet consumption was reduced moderately in males at the high level and in females at the high and intermediate levels.

Conclusion:

NOEL for males is 18.0 mg/kg/day and LEL for females in 18.3 mg/kg/day.

Classification: Supplementary Data

(a) Clinical chemistries and hematologies not performed.

58. Document 58. Range Finding Toxicity Test of Acetonitrile (Carnegie-Mellon Institute of Industrial Research Report 10-23; February 14, 1947)

Oral Doses

Standard albino rats
LD_{50} = 3.8 gm/kg

Death was usually prompt; survivors gained weight and gross pathology of victims was confined to digestive tract irritation.

The LD_{50} value found shows acetonitrile to have an oral toxicity quantitatively similar to that of acetic and propionic acids, and to be 1/25 to 1/40 as toxic as propionitrile and acrylonitrile.

Dermal Exposure

Rabbit LD_{50} close to 5 ml/kg

Inhalation Exposure

Inhalation vapors substantially saturated at room temperature for four hours resulted in the death of all of 6 rats. The animals were anesthetized in 30 minutes and the first death occurred in 2 hours. There were no delayed deaths. All victims had hemorrhagic lungs.

At a concentration of 8,000 ppm (0.8% by volume, about half of saturation), one of six rats died as a result of four hours of exposure with severe lung injuries. The five survivors gained weight well during the next two weeks. These data suggest that one must inhale 16 times as high a concentration of acetonitrile as of acrylonitrile for a given degree of injury.

Irritation

In the rabbit belly vesicant test the undiluted sample produced no irritation at all.

In the rabbit eye the sample is placed in grade 5 for potential injury, making it no more hazardous than acetone or triethanolamine.

A volume of 0.02 ml is necessary to produce severe injury.

Classification: Supplementary Data

(a) Raw data not provided.

59. Document 59. The Toxicity of Acetonitrile (Carnegie-Mellon Institute of Industrial Research Report 18-34; February 7, 1955)

The acute oral toxicity acetonitrile is 2.34 (2.03-2.70) gm/kg in female Wistar rats and 2.46 (1.60-3.78) gm/kg in male Wistar rats which is approximately 1/25th that of acrylonitrile and lies between methyl ethyl ketone and methyl isobutyl ketone. The last three compounds have respectively LD_{50}'s of 0.093, 4.0 and 2.1 gm/kg. The results of single known ppm exposures indicate acetonitrile is less toxic for rats then methyl ethyl ketone, methyl isobutyl ketone, or acrylonitrile. A concentration of 64,000 ppm acetonitrile for 4 hours was necessary to kill all of 6 rats, whereas the lethal concentrations for the other 3 chemicals were 8,000, 4,000, and 1000 ppm respectively. The hazard of acetonitrile as indicated by the results of the concentrated vapor test was also less than that for the two ketones and acrylonitrile.

The toxicity of acetonitrile appears to be of the same order of magnitude as methyl ethyl ketone in repeated exposures.

The foregoing conclusions would be valid if several danger signals were not present. First, the wide range of species susceptibility to acetonitrile makes the estimation of safe levels of acetonitrile for humans more difficult than were there a smaller difference between species susceptibility.

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If the metabolic behavior of man is similar to that of the dog in respect to acetonitrile, the threshold limits for humans could be set higher than were man similar to the rabbit. Second, if the metabolic behavior of man is similar to that of the rat, the very wide range of susceptibility of various individuals of this species completely invalidates the direct comparison made between acetonitrile and the two ketones previously mentioned. Third, the appearance of thiocyanate in the urine of animals exposed to acetonitrile vapors would not cause the formation of dangerous amounts of cyanide in vivo.

Classification: Supplementary Data

(a) Detailed results not provided.

60. Document 60. The Toxicity of Acetonitrile, Part II. (Carnegie-Mellon Institute of Industrial Research Report 21-100; November 13, 1958)

Acetonitrile had a wide range of lethal activity for rats in single oral doses as LD_{50}'s ranging from 1.7 to 8.5 ml/kg signified. The compound seemed to be somewhat less toxic undiluted than when fed in corn oil, water or 1% aqueous tergitol 7. The difference in response between males and females was reduced when the animals were fasted overnight before dosing.

Intraperitoneal injection of undiluted acetonitrile caused slightly greater toxicity in rats than did intraveous injection.

It is hypothesized that absorption from the peritoneal cavity by the blood vessels of the portal circulation (which lead directly to the liver), and the liver caused the immobilization or destruction of some liver enzymes.

The rabbit skin assay resulted in moderate to high toxicity by the percutaneous route with an LD_{50} of 0.5 ml/kg when applied as a 75% of 1.25 ml/kg when applied undiluted.

The response of rats that inhaled 655 ppm acetonitrile vapors 7 hours per day for 90 days was similar to that observed in rats inhaling 644 ppm vapors for a like period in the 1955 tests (Special Report 18-43).

Significant micropathological lesions involving the kidneys, lungs, and livers occurred. There appeared to be no significant alteration of the hematocrit value (packed erythrocyte volume), and hemoglobin values of females at this level. The significance of the body weight and organ weight response of the males at the 1320 ppm level was equivocal because the animals lost weight between the 86th and 90th exposure days. Weight gains and organ weights of the females did not differ significantly from the controls.

One rhesus monkey died after two 7-hour exposures to 2510 ppm and two other monkeys succumbed after 23 and 50 exposures to 660 ppm acetonitrile vapors. Three rhesus monkeys survived ninety-one 7-hour exposures to approximately 350 ppm acetonitrile vapors. A body weight loss of 7% was observed in one animal. One monkey showed an erythrocyte count which was persistently higher than the pre-exposure values, but two monkeys showed only a transitory elevation of the erythrocyte count during the exposure period. A maximum of 5.4 micrograms of cyanide/100 ml was found in the blood of the monkeys in the 12th exposure week after 5 consecutive exposures. A maximum of 11.7 micrograms of cyanide/100 ml, determined as SCN^-, was formed in the monkey's urine during the 9th exposure week. The most notable gross pathology was slight hemorrhage of the superior and inferior sagittal sinuses in the brains of all three monkeys.

The mean weight, as percentage of original weight, of 3 dogs exposed concurrently with the monkeys, fell significantly below that of 2 control dogs several times during the exposure period, but there were no significant weight changes during the last 19 exposure days. The hematocrit and hemoglobin values of all three exposure dogs were significantly depressed during the 5th exposure week with a general return to pre-exposure values toward the end of the exposure period. Blood cyanide and urinary thiocyanate concentrations were similar to those observed in the monkeys. No significant gross pathology was observed at autopsy. The blood cyanide of 3 dogs that inhaled 16,000 ppm actonitrile for 4 hours reached a maximum concentration of 435 micrograms/100 ml after the third hour and fell to a minimum of 170 micrograms at about the 5th hour.

Classification: Supplementary Data

(a) Detailed animal data not provided.

61. Document 61. Intraportal Injection of Acetonitrile (Carnegie-Mellon Institute of Industrial Rsearch Report 22-2; January 23, 1959)

Report 21-100 gave the following results for acetonitrile: rat tail vein injection, LD_{50} 1.68 (0.80-3.21) mg/kg; rat intraperitoneal injection, undiluted, LD_{50} 0, 95 (0.58-1.54) ml/kg.

That report discussed hypotheses to explain the unexpectedly higher toxicity by intraperitoneal than by intravenous injection. It appeared possible that (1) dilution of acetonitrile injected peripherally reduced it to a concentration less inhibitory to liver enzyme systems than when it reaches the liver from the peritoneal capillary bed, that (2) loss of acetonitrile to alveolar air between tail vein injection and the liver is appreciable, or, not mentioned in the report, (3) a major part of the detoxication of acetonitrile occurs peripherally, not in the liver.

Partly to explore this situation, and partly to learn a new technique, surgical means were used to inject undiluted acetonitrile directly into the portal vein of the rat liver. By this technique the LD_{50} was 0.71 (0.57-0.89) ml/kg. This value is closer to the intraperitoneal toxicity where acetonitrile reaches the liver and the peritoneal capillary bed and portal vein, than that when it goes through the peripheral circulation and the lung first. The result does not distinguish between the three hypotheses outlined above, but does confirm the high intraperitoneal toxicity.

Classification: Supplementary Data

(a) Detailed results not provided.

62. Document 62. Pharmacological Screening of Acetonitrile (Carnegie-Mellon Institute of Industrial Research Report 19-115; Sept. 17, 1956)

Acetonitrile is difficult to classify under the formal pharmacological classifications. Initially and briefly, it manifests the properties which are characteristic of the parasympatholomimetic-like compounds. This stageis followed by a prolonged period when it resembles a sympathomimetic-like compound. Then, terminally, if there has been a fatal absorption, it again reverts to the terminal symptoms of the parasympathomimetic. Therefore, there is an initial hypotension followed by a prolonged hypetensive state and then a terminal and gradual hypotension. Respiratory rate is increased during the period of intoxication until just before death when it decreases gradually.

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Respiratory volume is initially decreased, then increased for a prolonged period and finally it also gradually decreases. There is a central nervous system stimulation which lasts until just before death and then it also shows evidence of considerable depression.

Vagal stimulation is noted frequently. Acetonitrile is felt to be cumulative after repeated administrations of the material in anaesthetized dogs. It causes an evident increase in the clotting of even arterial blood which is easily controlled with heparin, an anti-coagulant. Recommended antidotes include the sympathomimetics such as epinephrine, ephedrine sulfate and neosynephrine. Intravenous fluids and artifical respiration combined with even a central stimulant such as metrazol can be used advantageously in the terminal stages of intoxication. Adrenergic blocking agents, antihistamines and atropine sulfate are contra-indicated due to their ability to enhance the depressant effects of the acetonitrile. Fortunately, there appears to be sufficient time between generalized depression and ultimate death to allow for the administration of all antidotal measures. Death is initially due to respiratory cessation followed by cardiovascular collapse.

Classification: Supplementary Data

(a) Detailed results not provided.

63. Document 63. Range Finding Test; Journal of Industrial Hygiene and Toxicology, Vol. 30, No. 1, 1948, pp 64-68.

Little information on acetonitrile presented, e.g., single dose oral LD_{50}, sub-acute oral toxicity, skin absorption, vapor exposure, skin irritation, eye injury and skin sensitization.

Classification: Supplementary Data

(a) Detailed results not provided.

64. Document 64. Range Finding Toxicity Data: List VI; American Industrial Hygiene Association Journal, Vol. 23, pp 95-107, 1962.

A table presents acute toxicity and irritation data on more than 300 compounds, accumulated in a continuing program for screening potential commercial products. The standardized experimental methods are described. No data on acetonitrile was present.

Classification: Supplementary Data

(a) Detailed information on results not provided.

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65. Document 65. An Exploration of Joint Toxic Action: 27 Industrial Chemicals Intubated in Rats in all possible pairs; Toxicology and Applied Parmacology 14, 340-347 (1969).

Prediction of the safety or hazard of various exposures to mixtures of chemicals must often be made in the absence of knowledge of the mode of their joint toxic action. The rat peroral LD_{50} was determined for 50% by volume mixtures of all possible pairs among 27 commercial organic chemicals. The results indicate that the harmonic mean formula for additive joint toxicity satisfactorily predicted the toxicity of a large proportion of the pairs. The soundest hypothesis for the joint action of untested pairs is that of additive toxic action. Pairs deviating most markedly from this mode of joint action are listed.

Classification: Supplementary Data

(a) Detailed information on results not provided.

66. Document 66. An Exploration of Joint Toxic Action. II. Equitoxic Versus Equivolume Mixtures. Toxicology and Applied Pharmacology 17, 498-503 (1970).

With 53 pairs of industrial organic chemicals in which the ratio of the rat peroral LD_{50} values of the 2 components ranged from 1 to an extreme of 140, the harmonic mean formula for additive joint toxicity predicted the toxicity of equitoxic mixtures as satisfactorily as it previously was shown to do for equivolume mictures. Pairs deviating most markedly from this mode of joint toxic action are listed.

Classification: Supplementary Data

(a) Detailed information on results not provided.

67. Document 67. Acetonitrile Poisoning in Connection with a Lethal Case. European Journal of Toxicology, 7:91-97 (1974) (Translation-French)

The study of this fatal human intoxication with acetonitrile recalls the clinical aspects of acetonitrile intoxication.

Classification: Supplementary Data

(a) Raw data not provided.

68. Document 68. Experimental intoxication by acetonitrile.1. Acute intoxication, administered intraperitoneally European Journal of Toxicology 8:94-101 (1975) (Translation-French).

The experiment has demonstrated that the lethal dose of acetonitrile for rats is relatively high and that this compound manifested itself with rather little toxicity. This low toxicity is connected with rapid transformation of the acetonitrile into hydrocyanic acid. In fact the lower this velocity is the greater the possibility for the organism to react with detoxication. But with very high doses it is possible that the amount of the liberated hydrocyanic acid is too great and is responsible for ensuing death which occurs in all cases.

It was also ascertained in the last series of experiments, that acetonitrile is eliminated in relatively small amounts in the urine compared to the injected dose, and elimination by pulmonary respiratory can be considerable.

However, the role of the intact molecule of acetonitrile, in its toxic action, is not known.

Classification: Supplementary Data

(a) Raw data not provided.

69. Document 69. Experimental intoxication with acetonitrile.2. Acute intoxication via inhalation European Journal of Toxicology 8:102-106 (1975) (Translation-French)

The acute intoxication via inhalation permit certain conclusions to be made:

(1) The death of the rats occurs always when the concentrations of the free cyanides are close to those found after intraperitoneal intoxication. Thus, there must be a relation between the death of the rats and the amount of cyanides.

(2) The amounts of hydrocyanic acid are not directly related to the amounts of acetonitrile found in the organs.

(3) The symptoms of these intoxications vary according to sensitivity of the animal and according to the inhaled concentrations, but anuria occurs constantly after these acute intoxications independent of the method of administration and persists generally for a long time.

Classification: Supplementary Data

(a) Raw data not presented.

70. Document 70. Experimental intoxications with acetonitrile.3. Medium term intoxication, administered with repeated intraperitoneal injections. European Journal of Toxicology 8:107-112 (1975) (Translation-French)

This series of intoxications made it possible to ascertain that the acetonitrile degraded into hydrocyanic acid very slowly so that the rats could bear repeated doses of 50 mg/kg.

The amounts of the free and combined hydrocyanic acid found in the urine indicates a percentage in the order of magnitude of 5% degrade acetonitrile related to the injected dose. The rest is eliminated by the kidney and mainly through respiration. The amounts of free and combined cyanides found in the organs are of the same order of magnitude as those found after acute intoxications, which proves that the hydrocyanic acid is not the only compound responsible for the observed deaths. The changes encountered at the level of the cerebellum deserve a deeper study to determine whether they are provoked by the free hydrocyanic acid or by the intact molecule of acetonitrile.

Classification: Supplementary Data

(a) Raw data not presented.

71. Document 71. Experimental intoxication with acetonitrile.4. Influence of hydroxocobalamine on the medium term intoxication. European found of Toxicology 8:113-124 (1975)

The point of impact of hydroxocobalamine seems to be located at the structures which are sensitive to toxic effects, i.e., the central nervous system. In fact, neither the distribution in the tissues nor the global urinary elimination are noticeably different with or without treatment. Moreover, under the influence of the hydroxocobalamine one can see a slight increase of the combined cyanides in the organs of excretion: The kidneys, the intestines and in the urine.

This study has demonstrated that acetonitrile could have in medium and certainly in long term, toxic effects which are comparable to those of small doses of alkaline cyanides. These toxic effects should not be neglected from the point of view of occupational intoxications since they consist in the appearance of lesions in the nervous cells; these lesions are caused by the free cyanides by the acetonitrile but not by the intact nitrile molecule since they do not form if a cyanide detoxifying treatment is applied.

Classification: Supplementary Data

(a) Raw data not presented.

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47. Document 47: UC-51762; Chronic Oncogenicity Feeding Study in Mice (Carnegie-Mellon Institute of Research Project Report 43-10; January 25, 1980)

Charles River, CH:COBS CD-L (ICR)BR outbred albino mice were used in this study. They were 29 days of age when received by the laboratory on 10/19/76 and were, therefore, 42 days of age when dosing was started on 11/1/76. These mice were all shipped at the same time and were examined at the same time and were examined on receipt for health and general appearance, and a random sample examined for evaluation of health status. Mice were housed in suspended stainless steel cages, 2 to a cage for the first year of doses and singly thereafter. Water was provided by an automatic dispensing system with demand-controlled values in each cage. Ground diet was supplied ad libitum in 4 oz stainless steel feeders with stainless stell wire mesh followers. Each animal was assigned an identification number and was identified permanently by a toe-clipping procedure.

80 mice of each sex were assigned randomly to each of five groups, representing three treatment levels of UC-51762 and two untreated control groups. The experimental compound was administered for 24 months at concentrations in the daily diet to achieve dosage level goals of 10.0, 3.0 or 1.0 mg/kg/day. Parameters of effect examined included mortality, diet consumption, body weight change, tumor incidence and other gross and histologic changes.

During the study all mice were observed daily for physical condition and signs of clinical or behavioral effect. Any alterations were tabulated in body weight record books. Food consumption and body weight were recorded biweekly for the first year of the study. During the second year, body weights were recorded monthly.

Mice which died or were sacrificed were examined for gross anatomic and histologic alterations. From May 8, 1978 to May 26, 1978, twenty mice, randomly selected, of each sex per dosage group were sacrificed after receiving a median of 80 weeks of doses. From November 1, 1978 to November 9, 1978 the surviving mice were sacrificed for examination after receiving a median of 105 weeks of doses. All sacrificed mice were anesthetized with methoxyflurane and killed by dislocation of the cervical spinal cord. The following procedures were utilized for the dissection and examination of each mouse:

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(1) Thymus and associated mediastinal lymph nodes and other mediastinal tissues were removed in one piece, placed in a cassette and fixed in 10% neutral buffered formalin (NBF).

(2) The lungs were gently inflated with 10% NBF.

(3) The head was removed with the pituitary in situ and fixed in 10% NBF.

(4) The skin was removed from one rear leg and the leg was fixed in toto in 10% NBF. To ensure proper fixation of bone marrow, the femur was cracked the length of the bone before placing in fixative.

(5) A section of the spinal cord extending from the last rib to the pelvic bones was fixed in 10% NBF.

(6) All abdominal and thoracic viscera were removed, carefully examined and representative samples were fixed in 10% NBF.

(7) The four feet were removed and placed in the jar of tissues to ensure proper animal identification.

The following tissues were fixed in 10% NBF except where autolysis was so severe that only the organs necessary for diagnosis were saved.

Pituitary
Thyroid
Parathyroid
Adrenals
Heart
Great Vessels
Thymus
Spleen
Cervical Lymph Node
Mesenteric Lymph Node
Nares
Nasal Cavity
Larynx
Trachea
Lungs
Ovaries
Oviduct
Uterus
Cervix
Testes
Epididymides
Prostate
Seminal Vesicles
Coagulating Gland
Kidneys
Urinary Bladder
Submandibular Gland
Parotid Salivary Gland
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Liver
Gall Bladder
Pancreas
Brain
Spinal Cord
Sciatic Nerve
Eyes
Harderian Gland
Head (including inner & middle ears, trigeminal ganglia, etc.)
Skin
Mammary Gland
External Ear
Peritoneum
Adipose Tissue
Anterior Thigh Muscle
Femur
Femorotibial Joint
Sternum
Vertebrae

(continue from last page)

Sublingual Salivary Gland — Any Gross Lesions
Tongue
Teeth

To ensure that all tissues were taken, a careful examination was made against the necropsy check list prior to discarding the carcass. The check list was initialed and dated by the prosector performing the necropsy. The necropsy findings were recorded on individual Pathology Record Sheets or dicated by the prosector for later transcription onto the Pathology Records Sheets. The Pathology Record Sheets were approved and signed by one of the pathologists assigned to the study.

Tissues and handwritten obsevations made at necropsy were shipped to Biomed Research Laboratories, Seattle, Washington.

Tissues were trimmed by the histotechnicians, embedded, sectioned and stained with H&E for examination by one of the two pathologists assigned to the study. These tissues were given Biomed accession numbers which appear on all slides processed there (e.g. BM-842).

The following tissues were evaluated microscopically on each animal from which they were saved.

Cassette #

1. Sterum (Decal)
2a. Pituitary
2b. Adrenals (right and left) Multiple sections on slide
2c. Trachea with Thyroids
Esophagus Multiple sections on slide
3. Brain (3 sections)
4. Heart (Atrial and Ventricular)
Skeletal Muscle (Anterior Thigh)
5a. Spleen (Cross-section)
Liver (3 pieces) Gall Bladder with piece of Liver
5b. Lungs
6. Salivary Gland
7. Kidney - right and left
Urinary Bladder
8. Male - Testicles (one cross-section and one longitudinal)
Epididymis
Prostate
Female - Ovary with Attached Oviduct
Uterus - Longitudinal section
Uterine Horns - Cross-section

9. Duodenum with Pancreas
   Jejunum
   Ileum
   Colon
   Stomach (Glandular and Nonglandular)
   Mesenteric Lymph Node
10. Skin with Mammary Gland
11. Eyes
    Harderian Gland

In addition to the tissues in the above list, all gross lesions were examined microscopically.

Statistical evaluation of the data was performed.

Results:

The mortality of male mice which received UC-51762 was not significantly different from that of the control groups except for the 1.0 mg/kg/day group after 16 months of exposure. At that time, the mortality of this group was significantly different from that of control group A but not from control group B. In spite of the sharp increase in the mortality of the high-dose males from the sixteenth to the eighteenth month of the study, the mortality of this group never differed significantly from the controls at any time during the study according to the life table analysis.

Among the female mice, the mortality of the high-dose group appeared to increase over the last six months of the study, and differed significantly from that of control group B over the last 2 months.

When the two control groups were pooled, the difference was still statistically significant. The increase in the mortality of the high dose female mice is considered to be a biologically important effect related to ingestion of 10 mg/kg/day of UC-51762.

Body weight reductions noted for the high level males versus control group B at 29,309 and 449 doses are considered random changes and not deleterious. Similar body weight reductions were noted for the high level females versus control group B at 169, 281 and 309 doses. Furthermore, body weight reduction was noted for the intermediate and low level females from 169 to 337 doses and again from 449 to 505 doses. Since these changes were not dose-related they are not considered indicative of deleterious effect.

Mean dosage levels attained for each 90-day period (approximate) of the study were quite close to the dosage goals. From 340-438 doses, diet consumption was depressed marginally for the high and intermediate male mice versus control group A only. In the same period, diet consumption was increased for the high level females and decreased for the intermediate and low level females. In both cases, the values were quite similar to those of one or both control groups. These changes are, therefore, considered to be artifacts attributable to chance.

The incomplete presentation of primary neoplasms data precluded an evaluation of the effects of UC-51762 on the oncogenic potential on mice. Accession# is 099588.

Classification: Supplementary Data

(a) The registrant is required to submit by sex comprehensive table of primary neoplasms in male and female mice from the study, which represents the total of each of the primary neoplasms from the 18-month sacrifice, final sacrifice and mice that died or were euthanized when moribund.

48. Document 48. UC-51762; Chronic Toxicity and Oncogenicity Feeding Study in Fischer 344 Rats (carnegie-Mellon Bushy Run Research Center Project Report 43-18; March 24, 1980)

Fisher 344 Mai Br rats, 780 of each sex, were purchased from Microbiological Associates, Walkersville, Maryland. They were 28 days of age when received on January 3, 1977 and were, therefore, 50 days of age when dosing began on January 25, 1977. The rats were all shipped at the same time, and were examined on receipt for health and general appearance. A random sample of these rats was subjected to the quality control procedures described below.

The animals were housed in suspended stainless steel cages, three per cage for the males and five per cage for the females. Water was provided by an automatic dispensing system with demand-controlled values in each cage. Ground diet was supplied ad libitum in 12 ounce opal glass jars. Each animal was assigned a unique identification number, and was identified permanently by a toe-clipping procedure. The following quality control procedures were performed on a random samole (5 per/sex) of the rats on arrival, and at bi-monthly intervals throughout the study. Blood samples, taken from the abdominal vena cava, were sent to Microbiological Associates, Bethesda, Maryland for murine virus antibody determination. Nasopharyngeal and pulmonary swabs were tested for aerobic bacterial flora, and intestinal parasites were identified by Zinc Sulfate flotation of fecal

samples. The following tissues were examined microscopically: salivary glands, trachea, lungs, liver, kidneys and testes.

The cages were examined twice a day each weekday and daily on weekends to remove dead and moribund rats. Dead rats were carefully necropsied with particular attention given to a search for tumors. Approximate portions of representative organs were fixed for embedding, sectioning, staining and microscopic examination.

120 rats of each sex were assigned randomly to each of six groups representing four treatment levels of UC-51762 and two untreated control groups. The test substance was administered for 24 months at concentrations in the daily diet selected to achieve dosage level goals of 10.0, 3.0, 1.0 or 0.5 mg/kg/day.

Interim sacrifices were performed a 6, 12 and 19 months. Ten rats per sex per treatment group were sacrificed at 6 and 12 months, and 20 per sex per group at 19 months. Prior to each sacrifice clinical status was determined by examination of hematology, urinanalysis and clinical chemistry parameters as shown below. Criteria of effect also included mortality, clinical signs, diet consumption, body weight change, organ weights, and the incidence of tumors and other pathologic findings.

During the study all rats were observed daily for physical condition and signs of clinical or behavioral effects. All animals were weighed every two weeks for the first year and monthly thereafter. Food consumption was determined every other week on 50 rats from each dosage group until the body weights stabilized (561 doses). The following clinical chemistry parameters were measured at approximately 6-months intervals on the animals that were to be sacrificed: creatinine, calcium, albumin, glucose, BUN, SAP, SGOT, SGPT, and plasma and erythrocyte cholinesterase.

The following hematologic parameters were measured at approximately 6-month intervals on the animals selected for sacrifice: mean corpuscular hemoglobin (MCH), red blood cell count, mean cell volume, hematocrit hemoglobin, mean corpuscular hemoglobin concentration, nucleated red blood cell count, total and differential leucocyte count. Blood samples for hematology and clinical chemistry were collected by orbital puncture under methoxyflurane anesthesia after 48 hours on control diet.

Urinalysis, performed on the same group of animals as above, included measurements of urine volume, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood and nitrite. Urine samples were also examined for color, turbidity, and presence of phosphate, calcium oxalate, blood cells, urinary epithelia, spermatozoa, bacteria and yeasts.

Rats that died or were sacrificed when moribund were examined for gross and histologic alterations as noted below. The rats selected for sacrifice were placed on control diet 24 hours prior to sacrifices, anesthetized with methoxyflurane and killed by severing the brachial vessels to permit exsanguination. Opthalmologic examinations, utilizing the wet microscopic slide technique, were performed only on those rats with suspected abnormalities. Brains were removed immediately, weighed, divided sagitally, and one-half submitted for cholinesterase assays. A complete necropsy was performed on all rats. The liver, kidney, spleen, heart, adrenals, and testes of each animal were weighed.

The number of rats per dosage level that was sacrificed at the terminal sacrifice is as follows:

Dosage	Males	Females
10.0 mg/kg/day	60	65
3.0 mg/kg/day	61	63
1.0 mg/kg/day	59	58
0.5 mg/kg/day	52	63
0.0 (A) mg/kg/day	57	55
0.0 (B) mg/kg/day	62	64

The rats were approximately 780 days of age at the time of sacrifice.

Commercially prepared 10% neutral buffered formalin (NBF) was the fixative of choice for all tissue fixation. The pH of the fixation was 6.0 to 7.0.

As noted above, the rats were anesthetized with methoxyflurane and killed by severing the brachial vessels to permit exsanguination.

As noted above, ophthalmologic examination utilizing the wet microscopic slide technique were performed only on those rats with suspected abnormalities. The table below is a listing of the number of animals with these abnormalities by sex and dosage level.

Opthalmologic Abnormalities

	mg/kg/day - Males						mg/kg/day - Females				
	10	3	1	0.5	0.0(A)	0.0(B)	10	3	1	0.5	0.0(A)
Lenticular Opacity	1	4	1	2	3	1	5	0	3	4	3
Corneal Opacity	0	1	1	1	1	2	0	2	1	3	0
Atrophy	0	1	1	0	0	0	0	1	0	1	0
Abnormal in Size	1	1	2	1	1	2	0	1	1	0	2
Conjunctival Swelling	0	0	0	0	0	0	1	1	1	0	0
Periocular Hemorrhage	0	0	0	0	0	0	0	0	1	0	1
Periocular Encrustment	1	0	0	1	0	0	1	0	4	0	0

Consistent procedures were prescribed for the dissection and examination of each rat. The following specific procedures were carried out:

1. The eyes were removed, placed in a cassette and fixed in 10% NBF. Immediately following fixation (24 hours) eyes were processed to the paraffin block stage.

2. One sciatic nerve was exposed, but not dissected free, from each rat.

3. One femur was either opened or removed from each rat and a fresh bone marrow impression smear was prepared and stained with Wright-Giemsa stain.

4. The lungs were gently inflated with 10% NBF.

5. The brain was divided sagittally and the right half weighed and submitted for cholinesterase assays for 53 male and 60 female rats. The left half was in 10% NBF for histologic preparation.

6. The stomach, mesenteric lymph nodes and sections of the small and large intestines were removed and fixed in 10% NBF.

7. Urinary bladders not distended with urine were gently inflated and fixed in 10% NBF.

8. Organ weights were recorded on individual Pathology Record Sheets. Organ weighted included liver, kidneys, spleen, heart, brain, adrenals and testes.

9. All abdominal and thoracic viscera were removed and representative samples were fixed in 10% NBF.

10. The four feet were removed and placed in the jar of tissues to insure proper animal identification.

The following tissues from all sacrificed rats were fixed in 10% NBF:

Pituitary
Thyroids
Parathyroids
Adrenals
Heart
Great vessels
Thymus
Spleen
Cervical lymph node
Mesenteric lymph node
Nasal cavity
Larynx
Trachea
Lungs
Ovaries
Oviduct
Uterus
Testes
Epididymides
Prostate
Seminal vesicles
Coagulating gland
Kidneys
Urinary bladder
Submandibular salivary gland
Parotid salivary gland
Sublingual salivary gland
Tongue
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Liver
Pancreas
Brain
Spinal cord
Sciatic nerve
Eyes
Harderian gland
Head (including inner & middle ears, trigeminal ganglia, etc.)
Skin
Mammary gland
Peritoneum
Adipose tissue
Femoral marrow smear
Anterior thigh muscle
Femur
Costochondral junction
Femorotibial joint
Sterum
Vertebrae
Any gross lesions

To insure that all tissues were taken, a careful examination was made against the necropsy check list prior to discarding the carcass. The necropsy check list was initialed and dated by the prosector performing the necropsy.

The necropsy findings were dictated onto cassettes by the prosector and later transcribed onto individual Pathology Record Sheets. The Pathology Record Sheets were subsequently approved and sighed by the pathologist assigned to the study.

The following tissues were evaluated microscopically from rats at all levels of treatment as well as controls:

Pituitary	Urinary bladder
Thyroids	Salivary gland
Parathyroids	Esophagus
Adrenals	Stomach
Heart	Duodenum
Thymus	Jejunum
Spleen	Ileum
Mesenteric lymph node	Colon
Trachea	Liver
Lungs	Pancreas
Ovaries	Brain
Oviduct	Eyes
Uterus	Harderian gland
Testes	Skin
Epididymides	Mammary gland
Prostate	Anterior thigh muscle
Kidneys	Sternum

In addition, histologic preparations from tissue with gross lesions were prepared from animals in all groups.

Statistical evaluation of the data was performed.

Results:

The data for male rats show that the cumulative mortality in the high dose males was consistently higher than that of both control groups from the fourteenth to the twenty-second month of the study. This difference was not statistically significant versus control group A, but was significant versus control group B during the fifteenth, twentieth and twenty-first months of the study. By the termination of the study the cumulative mortality in both control groups exceeded that in the highest dose level males. Among female rats, there was no increase in mortality associated with ingestion of UC-51762. The dosages attained closely approximately the dosage goals. A fixed concentration of UC-51762 was fed from 8/11/78 (561 doses), after which date diet consumption was not measured.

Diet consumption in male rats was unaffected by ingestion of UC-51762. In female rats during the first 6 months of the study, diet consumption in all UC-51762 treated groups were significantly lower than than in control group A. However, the value for control group B was also significantly lower than that for control group A and was even lower than the values for the UC-51762 treated groups. It is not possible, therefore, to infer that diet consumption was affected by ingestion of UC-51762 during this period. During the second half of the first year, diet

consumption was unaffected by UC-51762 treatment. In the thirteenth to the eighteenth months of the study, a pattern similar to that of the first six months was seen. Diet consumption values for all the UC-51762 treated groups fell between those of the controls, which differed from each other once again; a simple interpretation is not possible.

Ingestion of UC-51762 had a more profound effect on the weight gain of female rats, than on the males at 10.0 mg/kg/day. In the high-dose males and females the difference from both controls was statistically significant from the third through the twenty-first months of the study. Males at 3.0 mg/kg/day of UC-51762 had significantly decreased body weight gain at doses 84, 307 and 391. However, the female rats which received 3.0 mg/kg/day of UC-51762 or less had no reduction in weight gain.

All male rats, UC-51762 treated and controls, experienced a significant weight loss between 503 and 531 doses, after which time the rats never regained their previous weights. The weight loss is attributed to an outbreak of sialodacryoadenitis (SDA) virus, the signs of which were first observed on July 12, 1978.

In addition to the weight loss, the rat showed cervical swelling and eye lesions and were generally debilitated for a period of approximately two weeks. As a result of the infection, the scheduled 18-month sacrifice was postponed for approximately one month. Female rats were also affected, through less severely than the males, by the SDA infection. They showed only a small weight loss, and resumed their normal weight gain within one month of the SDA outbreak. The incidence of cervical swelling was lower, but eye lesions were more frequently observed in females than in males.

No statistical comparisons were made between organ weights of the UC-51762 treated and control groups sacrificed at 24 months since many of the organs had tumors or other age-related lesions. The report states that any chronic toxic effects of UC-51762 on individual organs would have been observed in the animals sacrificed at 6, 12 or 19 months when age-related changes were less pronounced. The reviewer does not agree with this rationale.

No statistically significant effects on absolute or relative organ weights were observed in the rats sacrificed at 6 months. In the rats which were sacrificed after 12 months of treatment, no treatment-related effects were observed in the males, but there appeared to be a dose-related reduction in absolute and relative liver weights in females. Although no UC-51762 treated group differed significantly from the controls, the data suggest that there might have been a subtle effect related to ingestion of UC-51762.

The overall toxicological importance of this finding is diminished by the absence of this effect in the animals sacrificed at 6 or at 19 months.

Relative spleen weights of females fed 10 or 1.0 mg/kg/day of UC-51762 for 365 days were significantly higher than control B only. However, the values for UC-51762 fed rats were similar to the mean of control A, which was also significantly higher than that of control B. This effect was probably a random statistical occurrence unrelated to UC-51762 exposure.

The absolute heart weight of female rats which received 3 mg/kg/day of UC-51762 for 12 months was significantly lower than the means of both control groups. This effect is probably not biologically important since it was not dose-related and it disappeared when the heart weights were expressed as percentage of body weight.

The only statistically significant difference observed in rats which received UC-51762 for 19 months was a decrease in absolute and relative spleen weight in the 3 mg/kg/day group of females when compared to control B only. Since the means of the UC-51762 treated group were identical to those of control A, which also differed significantly from control B, no biologically important effect is inferred.

In general, cholinesterase activity was unaffected by treatment except for a significant increase in the erythrocytes of male rats which received 10 mg/kg/day of UC-51762 for 24 months. Although the mean of the high dose group was statistically different from that of only one control group (A), the magnitude of the difference from both controls was such that a real effect can be inferred. This increase in the cholinesterase activity of rats which were chronically exposed to a cholinesterase inhibitor may represent a compensatory process.

After 6 months of doses, the blood glucose levels of male rats which received 3.0 or 0.5 mg/kg/day of UC-51762 were significantly lower than that of control A only. Since these differences were significant versus only one of two control groups and were not dose-related, they were probably random occurrences without biological significance.

Two statistically significant differences were observed in male rats which were fed UC-51762 for 12 months, though neither appears to have biological importance. The BUN values of male rats which received 3.0 or 0.5 mg/kg/day of UC-51762 were significantly higher than that of control A but not control B. Secondly, the SGOT value of the high dose group was significantly higher than that of control B but not control A. In both cases, the values of the UC-51762 treated groups, especially those at the highest dose

level, were either identical with or very similar to those of one of the control groups.

There were no statistically significant findings in any parameters in the rats sacrificed after 19 or 24 months of exposure to UC-51762.

Among the rats sacrificed after 6 months of exposure, the females at the two highest doses had urinary pH values which were significantly higher than those of control A but not control B. Since the pH values of the two control groups were also significantly different from each other, it is not possible to infer, that the increase in pH was associated with ingestion of UC-51762. At the 12-month sacrifice, the urine volume of female rats which received the two highest doses of UC-51762 was significantly lower than that of both control groups. Although this reduction in urine output appears to be a real effect at this point in the study it should be noted that no such effect was was observed at the 19- and 24-month sacrifices. At the 19-month sacrifice, the high dose male rats had significantly lower urine volume than control A but not control B. The urinary specific gravity of the high dose rats was also higher (though not statistically so) than that of either control group. These changes may be indicative of a subtle renal effect of UC-51762. However, since the urinary parameters of the high dose males were normal after 24 months of treatment, the toxicological importance of the marginal effect at 19 months is doubtful.

The only statistically significant finding in hematology at the 6-month sacrifice was an increase in lymphocyte count in males which received the two highest doses versus control B only. Female rats which received the same doses of UC-51762 also had higher lymphocyte counts than the controls, but the differences were not statistically significant. Furthermore, no significant increase in the percentage of lymphocytes was observed after 12, 19 or 24 months of the study. At the 12-month sacrifice, the male rats which received the three highest doses of UC-51762 had red blood cell, hematocrit, and hemoglobin values that were significantly lower than those of one or both controls. The effect appeared to be dose-related with the high-dose animals more severely affected than those that received the lower doses. These reductions in red blood cell parameters were not observed at the 19 and 24 month sacrifices.

After 24 months of treatment, female rats which received 3 mg/kg/day of UC-51762 exhibited higher red blood cell counts and hematocrits than control B only. Since this difference was not dose-related, it is probably not indicative of a deleterious effect.

6-Month Interim Sacrifice:

There were no histopathological changes in the tissues examined which could be attributed to treatment with UC-51762. The frequency of spleenic hemosiderosis in females was statistically increased in the high level rats versus control group B (9/10 vs 2/10, $p < 0.01$). However, since the frequency of hemosiderosis was 6/10 in control group A, and since there were no associated hematologic abnormalities or spleen weight changes, the observed a effect is probably not biologically significant.

12-Month Interim Sacrifice:

None of the lesions observed in the rats sacrificed after 12-months of treatment appeared to be associated with ingestion of UC-51762.

19-Month Interim Sacrifice:

No biologically important treatment-related lesions were found in rats which received UC-51762 for 19-months. On first examination, there appeared to be a significant increase in the incidence of hepatocellular hyperplasia in the high-dose females. However, a subsequent re-evaluation of the livers did not confirm this increase.

Dead and Moribund Animals:

A total of 241 rats were euthanized when moribund or died during the study. A variety of neoplastic lesions were observed in rats from all groups, and there was no association with ingestion of UC-51762. A tabular presentation of non-neoplastic lesions was not included for the dead and moribund animals and, therefore, no conclusions regarding the histological findings were made.

24-Month Sacrifice:

A variety of neoplastic and non-neoplastic lesions were observed in the rats sacrificed at 24 months. When the incidence of a given lesion appeared to be different in the UC-51762 fed group than in the control groups, a statistical comparison was made. The following lesions deserve comment because their frequency or severity appears to be related to treatment with UC-51762. The incidence of pituitary cysts in high-dose males was higher than that of either control group and statistically higher than that of one group (14/60 vs 4/56 and 7/61). The pathogenesis and biological significance of these cysts in unclear, but they do not appear to be correlated with the appearance of hyperplastic or neoplastic lesions in the anterior pituitary.

The incidence of thymic epithelial hyperplasia in the high-dose males was higher than in the controls though the difference barely failed to achieve statistical significance. In addition, two high-dose males had thymomas, which are relatively rare in Fischer 344 rats. No other thymomas were observed in the present study, and only two cases were reported out of 1794 aging male Fischer 344 rats (Goodman, et al., Toxicol., Appl. Pharmacol. 48:237-248, 1979). These historical data are less than ideal since the rats were obtained from different sources. Intralaboratory historical data for rats of this strain are not presently available. Therefore, with the evidence at hand, it is impossible to definitively interpret the occurence of the thymomas with respect to its toxicological importance.

The frequency of hemosiderosis of the mediastinal lymph nodes was significantly higher in the high dose females than in control group B and in the pooled controls. In addition, the frequency of interstitial prostatitis was significantly higher in males at the three highest doses than in control group B. Both lesions appear to be associated with ingestion of UC-51762, although prostatitis may be of uncertain toxicological significance.

The frequency of hepatocellular hyperplasia appeared significantly higher in male rats at all dosages of UC-51762 than in control group B. The frequencies were also numerically higher than that in control group A, though statistical significance was attained only at 1.0 mg/kg/day. An association between this lesion and ingestion of UC-51762 was also suggested by the distribution of the more severe lesions (those graded 2+ or higher). The frequency of these severe lesions appeared substantially higher in the control groups, though no dose-response was apparent. These data suggested an association between the presence of hepatocellular hyperplasia and ingestion of UC-51762.

The frequency of neoplastic nodules in the livers of UC-51762 fed rats was numerically higher at all dosages than that in either control group, though the differences were not statistically significant. This pattern was not observed in the case of hepatocellular carcinomas. Thus, while there was no definitive evidence that ingestion of UC-51762 was associated with the development of hepatic neoplasia in male rats, hepatic hyperplasia, discussed above, appeared to be associated with treatment.

Therefore, it was decided to resolve the question by evaluating additional sections of liver from each of the male rats. The 10% neutral buffered formalin-fixed tissues were pulled and three additional pieces of liver from each of the 351 male rats were embedded in one block, sectioned and stained with H and E for evaluation. One pathologist examined all of the liver sections and graded the altered (hyperplastic) foci on a scale of 0 to 4+ based on the following scheme:

0 = no altered cellular foci
1+ = 1-5 altered cellular foci
2+ = 6-15 altered cellular foci
3+ = 16-30 altered cellular foci
4+ = above 30 altered cellular foci

Altered cellular foci were foci of basophilic or clear cells occupying a portion of or an entire lobule. The scoring was not entirely based on numbers of foci, however, since the size of the foci and general appearance of the cells entered inot consideration. For instance, a liver possessing 13 to 14 foci, some of which were quite large was scored as a 3+ or a liver having 5 foci that were large, some of which occupied nearly an entire lobule, was rated as a 2+. On the other hand, livers with 6-7 very small not easily definable foci was graded a 1+. All three sections were considered before arriving at a grade. Only 1 of the 351 slides had only 2 sections instead of 3.

It can be readily seen from Table 1 that there was no compound-related effect observed when the livers were evaluated in this manner. It was evident throughout this liver evaluation that different pieces liver from the same rat had markedly different number of altered cellular foci. Not infrequently, one or two liver sections would possess a moderate to marked (2-3+) number of foci while the third piece was negative, or two pieces could be negative and the third pieces have 6-10 foci making the overall grading 2+ for the liver.

Because of the marked variation between liver sections, it was decided to compare the previous grading of the liver section used to generate the initial pathology report with the present grading. While many discrepancies existed, as would be expected based on the variation between sections, the most important finding was that in only 11 of the 351 (3.1%) rats did all 4 pieces of liver grade 0 (have no altered cellular foci) and in 8 of these 11 there was generalized leukemic infiltrate in the liver which possibly compromise the ability of the host's liver to respond with hyperplastic foci.

Neoplastic nodules were found in 11 rat livers during this evaluation compared with 24 livers in the initial study. In only 2 rats were nodules found in the liver sections during both evaluations. It was obvious that most of these neoplastic nodules were not detected grossly. This further underscores the necessity of examining more than one piece of liver per rat in order to obtain a more adequate evaluation of the pathologic changes in this organ.

TABLE 1

Hepatocellular Hyperplasia and Neoplastic Nodules in the Livers of Male Rats Receiving UC-51762

Group	Hepatocellular Hyperplastia 0	1+	2+	3+	4+	Neoplastic Nodules
10.0	9/60	19/60	27/60	5/60	0/60	2/60
3.0	2/61	24/61	31/61	3/61	1/61	3/61
1.0	2/59	21/59	29/59	7/59	0/59	2/59
0.5	8/52	29/52^{b-}	10/52	5/52	0/52	1/52
0.0(A)	5/57	18/57	28/57	6/57	0/57	1/57
0.0(B)	6/62	29/62	24/62	2/62	1/62	2/62

Footnote: First superscript denotes degree of significance versus Control A; second superscript denotes degree of significance verse Control B.

- = no significance
b = 0.01 > P > 0.001

Conclusion:

No conclusions regarding the NOEL for cholinergic and systemic toxicity and oncogenic potential of the study can be made until the following data are submitted:

a) Tabular presentation of the microscopic findings of the 241 animals found dead or enthanized while moribund.

b) Comprehensive tabular presentation of histologic findings of all rats examined in the study.

c) Comprehensive table of primary neoplasms of all rats examined in the study.

d) A 90-day rat feeding study at dosages of 10, 3, 1 and 0.5 or less mg/kg/day (plus controls) of UC-51762 to determine the NOEL for plasma, RBC and brain cholinesterase. The samples taken for determination of cholinesterase levels shall be from rats which are neither fasted or placed on control diets for any period of time.

e) The addendum to the pathology report (Vol. 12, pp. 17275-17295) states that neoplastic nodules were found in 11 rat livers during the examination of 4 additional pieces of the 351 male rats remaining at final sacrifice compared with 24 livers with neoplastic nodules in the initial study (Vol. 15, table 5, pp. 14930). Oral communication with Dr. Edward H. Fowler, pathologist for the study, indicated that the 9 of the 11 neoplastic nodules were found in addition to the initial 24 neoplastic nodules. This aspect of the addendum pathology report is not clear from the report and is required to be submitted in writing together with a new statistical evaluation of all neoplastic nodules.

Classification: Supplementary Data

(a) Reasons stated in conclusions.

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