Continental Oil Co.
Condea Vista

TOXICITY TEST SUMMARY

February 8, 1979

SAMPLE DESIGNATION: ALFOL 1618 Alcohol
S. A. Number 65469

SUBMITTED BY: Continental Oil Company
P. O. Box 1267
Ponca City, OK 74601

SUMMARY AND CONCLUSIONS:

1. Acute Oral Toxicity (LD_{50}) in Rats

Albino rats (Sprague-Dawley strain of both sexes) weighing 200-245 grams were fed measured single doses of a 20% aqueous suspension prepared in 0.5% gum tragacanth. The oral LD_{50} in rats was found to be greater than 10 grams per kilogram body weight.

2. Acute Oral Toxicity in Rabbits (M.L.D.)

New Zealand white rabbits were fed increasing single doses of a 20% aqueous suspension in a 0.5% aqueous gum tragacanth solution. The oral M.L.D. in rabbits was found to be greater than 10 grams per kilogram body weight.

3. Skin Irritation

The backs of three New Zealand albino rabbits were closely clipped. One area of the back was abraded and another left intact. The undiluted preparation was applied to the back of each rabbit. The treated areas were covered with plastic shields to keep the material in contact with the skin. Observations were made at various time intervals.

The scoring method of Draize, Woodward and Calvery (J. Pharmacol. Exp. Therap. 82y, 377(1944) was used in evaluating the skin-irritating properties of the compound at 24, 48, and 72 hours. The compound was found to produce no discernible irritation.

4. Eye Irritation

The method of Draize, et al (Ibid) was used to determine the degree of ocular irritation resulting from the addition of the preparation into the eyes of albino rabbits. In testing the material, 0.1 cc of a 20% suspension was instilled into the conjunctival sac of the right eye of each of three animals. Observation of the animals was continued for a period of several days following treatment. Fluorescein staining (2% aqueous solution) was used to determine the degree of ocular damage at 24, 48, and 72 hours. The compound was found to produce no discernible irritation.

5. Inhalation

Six albino rats (Sprague-Dawley strain of both sexes) were placed in a chamber and exposed to an atmosphere of concentrated vapors for six hours by passing a stream of warm air over the material (the temperature of the sample was kept at the melting point throughout the experimental period). The animals were observed for 14 days for fatalities at which time they were sacrificed and macroscopically examined for signs of systemic damage. All of the animals survived the six-hour exposure and the 14-day observation period. Autopsy of the sacrificed animals revealed no remarkable systemic damage with the exception of slight pulmonary congestion.

6. Toxicity by Skin Absorption

Measured doses of undiluted compound* were applied to the clipped backs of New Zealand white rabbits. The treated areas were covered with plastic shields to keep the preparation in close contact with the skin. The animals were observed for 14 days following the application of the compound. The M.L.D. by skin absorption was found to be greater than 10 grams per kilogram body weight.

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*The preparation was made fluid by increasing the temperature to the melting point.

7800 FOREST PARK BOULEVARD
ST. LOUIS 5, MISSOURI • JEFFERSON 1-5922 May 15, 1961

CERTIFICATE OF ANALYSIS

SUBJECT: Toxicity Tests on ALFOL 1618

SUBMITTED BY: Continental Oil Company
Ponca City, Oklahoma

SAMPLE DESIGNATION: Lot 8529A
S.A. 65469

EXPERIMENTAL PROCEDURE:

1. Acute Oral Toxicity(LD_{50}) in Rats

Albino rats(Sprague-Dawley strain of both sexes) weighing 240-245 grams were fed measured single doses of a 20% aqueous suspension(prepared in 0.5% gum tragacanth) by means of a rubber catheter attached to a hypodermic syringe. After the approximate Minimum Lethal Dose had been determined, groups of five rats were fed at levels designed to blanket the toxicity range in a manner sufficient to supply data for the calculation of the LD_{50}. The animals were observed for 14 days and the LD_{50} was calculated by the method of Gaddum(J. Pharmacol. Expt. Therap. 85:1(1945)). Animals were autopsied and macroscopic examination made. Results are shown in Table I.

2. Acute Oral Toxicity in Rabbits(M.L.D.)

New Zealand white rabbits were fed increasing single doses of a 20% aqueous suspension(in a 0.5% aqueous gum tragacanth solution) by means of a rubber catheter attached to a hypodermic syringe. The Minimum Lethal Dose Data are shown in Table II.

3. Skin Irritation

The backs of three New Zealand albino rabbits were closely clipped. One area of the back was abraded and another left intact. The undiluted preparation was applied to the back of each rabbit. The treated areas were covered with plastic shields to keep the material in contact with the skin. Observations were made at various time intervals.

The scoring method of Draize, Woodward and Calvery(J. Pharmacol. Exp. Therap. 82:377(1944)) was used in evaluating the skin irritating properties of the compound. Results are shown in Table III.

4. Eye Irritation

The method of Draize, et.al.(Ibid) was used to determine the degree of ocular irritation resulting from the addition of the preparation into the eyes of albino rabbits. In testing the material 0.1 cc. of a 20% suspension was instilled into the conjunctival sac of the right eye of each of three animals. Observation of the animals was continued for a period of several days following treatment. Fluorescein staining(2% aqueous solution) was used to determine the degree of ocular damage. Results are shown in Table IV.

5. Inhalation

Six albino rats(Sprague-Dawley strain of both sexes) were placed in a chamber and exposed to an atmosphere of concentrated vapors for six hours by passing a stream of warm air over the material(the temperature of the sample was kept at the melting point throughout the experimental period). The animals were observed for 14 days for fatalities at which time they were sacrificed and macroscopically examined for signs of systemic damage. Results are shown in Table V.

6. Toxicity by Skin Absorption.

Measured doses of undiluted compound* were applied to the clipped backs of New Zealand white rabbits. The treated areas were covered with plastic shields to keep the preparation in close contact with the skin. The animals were observed for 14 days following the application of the compound. Results are shown in Table VI.

* The preparation was made fluid by increasing the temperature to the melting point.

RESULTS AND DISCUSSION:

1. Acute Oral Toxicity in Rats(LD_{50})

TABLE I

Oral Mortality Data

Dose(gm/Kg)	Mort./No. Animals	% Mortality
2	0/5	0
4	0/5	0
5	0/5	0
10	0/5	0

The oral LD_{50} in rats was found to be greater than 10 grams per kilogram body weight.

2. Acute Oral Toxicity in Rabbits(MLD)

TABLE II

Oral Mortality Data

Animal No.	Dose(gm./Kg.)	Fate
1	1.0	Survived
2	2.5	Survived
3	5.0	Survived
4	10.0	Survived

The oral MLD in rabbits was found to be greater than 10 gram per kilogram body weight.

3. Skin Irritation

TABLE III

Skin Irritation Data

Animal No.	24 hours		48 hours		72 hours	
	I°	A°	I	A	I	A
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Average	0	0	0	0	0	0

Maximum Score Attainable: 8.0 I°=Intact A°=Abraded

The compound when applied undiluted to the intact and abraded skin of rabbits was found to produce no discernible irritation.

4. Eye Irritation

TABLE IV

Eye Irritation Data

Animal No.	Numerical Evaluation at the End of 1 hour	24 hours	48 hours	72 hours
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
Average	0	0	0	0

Maximum Score Attainable: 110.0

The compound when instilled into the conjunctival sac of rabbits produced no discernible ocular irritation.

5. Inhalation

TABLE V

Inhalation Data

Animal No.	Fate
1	Survived
2	Survived
3	Survived
4	Survived
5	Survived
6	Survived

All of the animals survived the six hour exposure and the 14 day observation period. Autopsy of the sacrificed animals revealed no remarkable systemic damage with the exception of slight pulmonary congestion.

6. Toxicity by Skin Absorption

TABLE VI

Skin M.L.D. Data

Animal No.	Dose gm./kg.	Fate
1	1.0	Survived
2	2.5	Survived
3	5.0	Survived
4	7.5	Survived
5	10.0	Survived

The M.L.D. by skin absorption was found to be greater than 10 grams per kilogram body weight.

SUMMARY:

1. The oral LD_{50} in rats was found to be greater than 10 grams/Kg. body weight.

2. The oral M.L.D. in rabbits was found to be greater than 10 grams/Kg. body weight.

3. The compound produced no skin irritation when applied to the intact and abraded skin of rabbits.

4. The compound when instilled into the conjunctival sac of rabbits produced no discernible ocular damage.

5. Rats exposed to a concentrated atmosphere of vapors survived the exposure and the 14 day observation period.

6. The M.L.D. by skin absorption was found to be greater than 10 gm/Kg.

SCIENTIFIC ASSOCIATES

BY: Peter Rauhauser

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6200 S. LINDBERGH, ST. LOUIS, MO. 63123 • TELEPHONE: 314-487-6776

December 31, 1968

SUBJECT: Inhalation Toxicity Test of ALFOL 1618-C in Rats

SAMPLE DESIGNATION: ALFOL 1618-C
Conoco number 8331E
S. A. Number 149726

SUBMITTED BY: Continental Oil Company
Research and Development Department
Ponca City, Oklahoma 74601

SUMMARY AND CONCLUSIONS:

1. Ten young adult albino rats (5 males and 5 females, Carworth CFN strain) weighing 215 to 298 grams were exposed to a series of ten daily inhalation exposures (each of 8 hours duration) at a delivery flow concentration of approximately 0.56 mg./liter (avg. delivery flow concentration of the ten exposures - range of 0.16 mg./liter to 0.89 mg./liter) of undiluted nebulized Alfol 1618-C.

2. All animals survived the ten exposures and the 14 day observation period which followed the final (10th) exposure.

3. No gross signs of ill effects were observed in any of the animals during or prior to any of the ten exposures to the test material.

4. Body weight loss, feed intake reduction, weakness and emaciation were recorded in the majority of the animals during the observation period (particularly during the third week). At termination (28 days) weakness (2 animals) and emaciation (3 animals) were still evident. These effects could not be definitely attributed to the inhalation exposure of the animals to the test material.

5. Gross necropsy of the test animals sacrificed at termination showed pulmonary lesions (8 animals) and pale tan coloration of the kidneys (6 animals).

SUMMARY AND CONCLUSIONS: (continued)

6. Microscopic examination of sections of lung tissue from the test animals showed three animals with some bronchopneumonic changes, however, most of the lung changes observed in the ten test animals were similar to the findings of the two control animals.

It appeared that the test material was not specifically associated with the broncho-pneumonic involvement observed.

EXPERIMENTAL PROCEDURE:

Ten healthy young adult albino rats (five males and five females, Carworth CFN strain) weighing 215 to 298 grams were used to determine the respiratory effects of ALFOL 1618-C in rats when introduced into a 133 liter capacity stainless steel chamber in the form of a mist.

Each of the animals selected for the test were acclimated to laboratory conditions for one week prior to testing, and only those animals without grossly observable defects were used.

The undiluted ALFOL 1618-C was introduced into the chamber by means of a DeVilbiss Nebulizer (submerged in a boiling water bath) at a delivery flow rate of 36 liters per minute for a period of 8 hours per day. The animals were subjected to a total of 10 exposures (five consecutive daily exposures for two weeks). The particle size of the test material was five microns or less. A fresh sample of ALFOL 1618-C was weighed from the stock container and used for each of the 10 exposures. The animals were observed at 30 minute intervals for gross effects during each exposure; upon removal from the chamber, the animals were placed in their respective individual wire-bottomed cages elevated above the droppings. Feed, consisting of Purina Laboratory Chow (pelletized), and tap water were freely available to the animals at all times (excluding the actual exposure period).

EXPERIMENTAL PROCEDURE: (continued)

The animals were observed daily for 14 days following the final (10th) exposure. Weekly body weight and feed consumption records were maintained for each animal during the 28 day test period.

Following the observation period, all surviving animals were weighed, sacrificed and necropsied. Specimens of lung tissue from each of the ten test rats and two control rats (one male and one female - no treatment) were removed and preserved in 10% v/v formalin. Slides were prepared of lung tissue (stained with hematoxalin and eosin) from each animal and submitted to a pathologist (Wm. R. Platt, M.D.) for micropathological evaluation.

RESULTS:

TABLE No. 1

Estimated Daily Delivery Flow Concentration of Alfol 1618-C During 8 hour Exposure

Exposure No.	Estimated Delivery Flow Concentration of Test Material (mg./liter)
1	0.38
2	0.63
3	0.64
4	0.51
5	0.31
6	0.16
7	0.50
8	0.89
9	0.70
10	0.84
Avg. Estimated Delivery Flow Concentration of Alfol 1618-C =	0.56 mg./liter

Although the procedure for the introduction of the test material into the chamber remained essentially constant for each day of exposure, Table No. 1 shows that the estimated delivery flow concentration of Alfol 1618-C varied from 0.16 mg./liter (Day 6) to 0.89 mg./liter (Day 8). No explanation can be offered for this variance. With the exception of Day 6, each of the remaining daily exposure delivery flow concentrations of test material and the average delivery flow concentration (ten exposures) was in excess to the minimum desired concentration (0.25 mg./liter of test material) which was discussed with the client prior to initiation of the test.

RESULTS: (continued)

TABLE No. 2

Mortality Data

The mortality results during the exposure period (10 exposures within 14 days) and the 14 day observation period which followed are presented below.

During Exposures Mort./No. Animal	% Mortality During Exposures	During 14 Day Observation Period Which Followed Mort./No. Animal.	% Mortality During 14 Day Observation Period
0/10	0	0/10	0

All of the animals survived the series of ten "eight hour" exposures and the 14 day observation period which followed the final (10th exposure). No gross signs of adverse effects were noted in any of the animals during or prior to each of the 10 exposures.

During the 14 day observation period, bodyweight loss (Table 3), anorexia (Table 4), generalized weakness and slight emaciation (Nos. 2, 3, 4, 5 & 7) were recorded, however, at termination the latter gross signs were no longer evident in Animals Nos. 3 and 5. The above gross reactions could not be definitely attributed to the inhalation exposure of the animals to the test material.

RESULTS: (continued)

TABLE No. 3

Individual Weekly Body Weights, grams

Animal Number	Sex	Initial Weight	Test Period - Weeks 1	2	3	4	Change (gm.)	% Change
1	M	298	316	328	319	317	+19	+ 6.38
2	M	282	294	289	237	222	-60	-21.28
3	M	298	312	340	302	286	-12	- 4.03
4	M	290	299	309	279	279	-11	- 3.79
5	M	279	280	274	255	306	+27	+ 9.68
6	F	222	222	237	231	236	+14	+ 6.31
7	F	215	216	207	179	193	-22	-10.23
8	F	215	235	224	229	230	+15	+ 6.98
9	F	220	232	234	240	244	+24	+10.91
10	F	216	226	226	232	236	+20	+ 9.26

During the first week of the study, normal bodyweight gains or a constant weight were recorded in all animals.

Four animals (Nos. 2, 5, 7 & 8) showed slight bodyweight loss during the second week of the study; a normal weight picture was evidenced for the remaining animals. Slight bodyweight loss (Nos. 1, 5 & 6) and marked bodyweight loss (Nos. 2, 3, 4 & 7) were recorded for the third week; animals nos. 8, 9 & 10 showed normal gains.

At termination (28 days) normal gains were recorded for six animals for the entire test period. Three animals showed slight to moderate loss (-3.79 to -10.23%) and one animal marked loss (-21.28%) for the study period.

The decrease of bodyweight observed at various intervals among the animals generally corresponded to a reduction of feed intake for that period (See Table No. 4)

RESULTS: (continued)

TABLE No. 4

Individual Weekly Feed Consumption, grams

Animal Number	Sex	Test Period - Weeks 1	2	3	4	Total (gm.)
1	M	144	143	131	133	551
2	M	132	119	73	81	405
3	M	124	160	108	110	502
4	M	138	143	108	112	501
5	M	113	116	76	166	471
6	F	100	112	113	122	447
7	F	103	102	60	89	354
8	F	114	104	104	118	440
9	F	116	123	138	142	519
10	F	101	105	121	127	454

With the exception of Animals Nos. 2 and 8 (slight feed intake reduction during 2nd week) all animals displayed normal appetite and feed consumption during the first two weeks of the study (exposure period).

During the third week of the study, a marked decrease in feed consumption was noted in five animals (Nos. 2, 3, 4, 5 & 7); the remainder consumed a normal quantity of feed. This finding was again evident during the final week (4th) with the exception that Animal No. 5 showed normal feed consumption during this period.

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RESULTS: (continued)

TABLE No. 5

Individual Gross Necropsy Results of

Animals Sacrificed at Termination (28 days)

Group	Animal No.	Sex	Findings
Control	1	M	No gross lesions.
(Animals received no exposure treatment-used for comparative pathology purposes only)			
Control	2	F	No gross lesions.
Test	1	M	Pale tan kidneys, rough granular darkened upper portion of left lung.
Test	2	M	Animal thin and weak. Hemorrhagic lungs consolidation of 50% of lungs.
Test	3	M	Pale tan kidneys, anterior lobes of lun rough, darkened and granular.
Test	4	M	Animal thin. Hemorrhages, consolidation thickening and whitening of lungs (approximately 50%).
Test	5	M	Same as Animal No. 1.
Test	6	F	Pale tan kidneys.
Test	7	F	Animal thin and weak. Pale tan kidneys lung thickened, hemorrhagic and consolidated (approximately 20%.)
Test	8	F	Pale tan kidneys.
Test	9	F	Pale tan kidneys, approximately 20% of lung surface hemorrhagic.
Test	10	F	Approximately 40% of lung surface hemorrhagic.

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RESULTS: (continued)

TABLE No. 6

Microscopic Description of Lung Tissues of Rats Sacrificed at Termination (28 days)

Group	Animal No.	Sex	Findings
Group I			
Control	1	M	Sections of lung tissue showed some interstitial congestion. The alveolar walls were intact and there was no infiltrate of any inflammatory cell patt
Control	2	F	The amount of congestion was minimal, wi slight congestion in one or two vessels. The rest of the lung tissue was not remarkable.
Group II			
Test	1	M	There were two sections cut from this lu One section showed no significant microscopic changes. Some interstitial vesse were congested. The other section showe a diffuse marked inflammatory exudate present in the bronchiolar lumens and within the alveolar spaces. This was characteristic of a bronchopneumonia process in response to an irritative or inflammatory agent.
Test	2	M	Sections of lung tissue showed a moderat amount of vascular congestion with some interstitial congestion, but this was similar in quantity and quality to that seen in the No. 1 Control animal.
Test	3	M	Sections of bronchi and bronchioles show interstitial inflammatory reaction which also involved the lumen. The rest of the lung tissue showed focal lymphocytic proliferation and hyperplasia and also a exudate in the alveolar lumens.

RESULTS: (continued)

TABLE No. 6

Microscopic Description of Lung Tissues of Rats Sacrificed at Termination (28 days)

Group	Animal No.	Sex	Findings
Group II			
Test	4	M	Sections showed a marked inflammatory reaction response involving bronchioles and their lumens and the surrounding tiss This was also characterized by focal hyperplasia of lymphoid elements. The adjacent alveolar areas were filled with exudate consisting of polys, lymphocytes and mononuclear cells.
Test	5	M	Sections showed some congestion of the smaller vessels and interstitial tissues, but this was not much more than was seen in the No. 1 Control animal. The only additional finding was the presence of lymphoid aggregates, which is common in normal animal lung tissue.
Test	6	F	Sections of lung tissue showed some moder amount of congestion of the vessels and interstitial tissues and lymphocytic aggregate around the branches of the bronchioles. This was not remarkable.
Test	7	F	These sections showed a minimal to moderat response. There were focal areas of lung tissue in which there was a poly exudate i and surrounding the lumen. The lung tissu had an interstitial inflammatory reaction. Predominately, there was a lymphocytic-lik reaction, but this is normal for rats.
Test	8	F	Sections showed moderate congestion of the larger vessels and some interstitial congestion. This was similar to that observed for Rat No. 1 (Control).

RESULTS: (continued)

TABLE No. 6

Microscopic Description of Lung Tissues of Rats Sacrificed at Termination (28 days)

Group	Animal No.	Sex	Findings
Group II			
Test	9	F	Sections showed moderate congestion of interstitial vessels and of some of the branches of the moderate-sized vessels. Otherwise this was not more remarkable than Rat No. 1 (Control).
Test	10	F	Sections showed minimal to moderate interstitial congestion; however, this w less than Rat No. 1 (Control).

CONCLUSION:

Although there were three lung sections from the ten test animals which showed some bronchopneumonic changes, most of the lung changes were similar to those observed in the Control sections. It appears that the test material was not specifically associated with the bronchopneumonic involvement observed.

SCIENTIFIC ASSOCIATES, INC.

By: John Eibert Jr

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S. A. Number 149726

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