ACUTE TOXICITY OF SELECTED CRUDE AND REFINED SHALE OIL DERIVED AND PETROLEUM-DERIVED SUBSTANCES

Lawton H. Smith, Wanda M. Haschek, and Hanspeter Witschi

Biology Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830

This research was sponsored by the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

DISCLAIMER

Introduction

To help evaluate and predict hazards of substances to which humans may be exposed in the workplace, several kinds of tests with laboratory animals can be used. These tests are usually designed to give information on overall toxicity of a given compound and to detect its potential to cause untoward effects.

It was the objective of the present study to obtain general information on the toxicity of selected samples of crude Paraho shale oil and some of its derivatives, some crude petroleums, and 3 refined petroleum products. Five tests were used to determine the acute toxicity of these substances: acute lethality in mice following oral or intraperitoneal administration of a single dose; acute dermal toxicity of a single dose in rats; delayed-type allergic contact hypersensitivity in guinea pigs; primary eye irritation and primary skin irritation of a single dose in rabbits. In addition, we have examined histopathologic changes induced in mice following intraperitoneal injection of a single large dose of crude shale oil and two of its hydrotreated derivatives. Studies also have been initiated to examine the tumor inducing potential of selected samples. The test system used was the mouse lung adenoma bioassay. The present report describes our findings and shows that all compounds tested have very low or no acute toxic effects in laboratory animals.

Materials and Methods

1. Substances evaluated

Bulk samples the materials were received from the ORNL repository. Viscous samples were thoroughly mixed by shaking after being warmed in a water-bath at 70-80°F. Where feasible, samples were applied undiluted. In the experiments where it was necessary to administer graded doses measured portions were diluted with corn oil (laboratory grade, Fisher Scientific) to provide the desired final concentrations; the density of all substances was assumed to BE 1.0.

The following substances were studied:

ORNL REPOSITORY NO.	NAME
a) Shale oil and its products	
4601	Crude shale oil
4602	Hydrotreated shale oil
4607	Hydrotreated shale oil residue
4608	JP-5 product (shale oil derived jet fuel)
4609	JP-8 product (shale oil derived jet fuel)
4610	Diesel fuel marine product (shale oil derived)
ORNL REPOSITORY NO.	
b) Petroleum and its products	
5107	Mixed petroleum crudes
5301	Wilmington crude oil
5305	Recluse crude oil
4614	JP-5 product (petroleum derived jet fuel)

4615	JP-8 product (petroleum derived jet fuel)
4616	Diesel fuel marine product (petroleum derived)

2. Acute LD_{50} in mice

The objective of this test was to determine acute toxicity following single oral or intraperitoneal administration of a substance. The animals used were Balb/c male or female mice that weighed 20 to 25 g.

Mice were randomly grouped 6 per cage and were fasted for 18 hours before administration of a substance. For oral administration a ball-tipped needle (20 gauge) fitted to a 1 ml syringe was used. The administration volume was 10 µl per g body weight except when a dose of 16 g/kg was delivered; in this case 20 µl/10 g body weight of a solution containing 0.8 ml/ml of the test agent was administered. The doses were 2, 4, 8, and 16 g per kg. For intraperitoneal injections, a 1 ml syringe fitted with a 22 gauge needle was used. The injection volume was 10 µl per g body weight, and doses were 1, 2, 4, and 8 g per kg. Additional doses were tested when necessary. Corn oil was the vehicle used for both routes of administration.

Animals were returned to their cages with free access to food and water and were observed for signs of toxicity. The number of survivors was recorded daily. Two weeks after administration of the substance, the surviving mice were killed. The LD_{50} was calculated

from the number of 14-day survivors according to the procedures of Weil (1952).[1]

3. Acute dermal toxicity in rats*

The objective was to determine the acute toxicity of a substance following a single application to the skin. The animals used for this test were albino male (300-400 g) and female (200-250 g) rats. Each substance was tested on five or six animals of each sex.

The animals were lightly anesthetized with ether, and the hair was clipped from the sides and back. The substance was applied to the center of the back and spread over an area of 25 to 50 cm^2. An attempt was made to cover the same area on each animal and to apply the substance uniformly. Two ml per kg body weight of undiluted sample was applied with a microliter pipetting device. The rats were then placed in individual cages where they remained for four hours. During this time they were observed for signs of systemic toxicity. At the end of the four hour period any residual substance was removed and signs of skin injury were noted. The animals were returned to maintenance cages and observed over a period of fourteen days. At the end of this period, body weights were obtained and necropsies were performed

---

*The test methods used are based on those described in Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington, D.C., 1977. Acute toxicity, Pages 10-17; dermal toxicity, pages 23-26; delayed-type sensitivity, pages 36-39; eye irritation, pages 123-124; skin irritation, pages 28-31.

4. Delayed-type allergic contact hypersensitivity*

The objective was to determine the delayed-type allergic response resulting from cutaneous contact with a substance. The animals used for this test were Hartley albino male and female guinea pigs about three months old. Each substance was tested on 4 different animals of each sex.

Hair was clipped from a 3 to 4 cm^2 area on the back between the shoulder blades. Clipping was done on the first day of injection and when necessary during the course of further injections. One-tenth ml of a 0.1% solution of the substance in 20% DMSO-80% saline or in propylene glycol was injected intradermally three times a week for three weeks. The injections were within a 3 to 4 cm^2 field at different sites within the field. Twelve days after the ninth injection, a challenge dose of 0.05 ml of the 0.1% solution of the substance was injected intradermally into a fresh site of the clipped area. Readings for skin reactions were made 24 and 48 hours after each of the nine injections and after the challenge dose. Grading and evaluation of the response were made according to the method of Draize et al (1944)[2] which scores sensitization in terms of redness, edema, blistering, and necrosis.

5. Primary Eye Irritation*

The objective was to determine surface irritation to ocular tissues by a substance following a single application. The animals used were New Zealand albino male rabbits that weighed about 2 kg. Each substance was tested on one eye of four animals; the other eye served as a control. The lower lid was gently pulled away from the

eye and 0.1 ml of the undiluted agent was instilled into the conjunctival sac by use of a pipetting device. The eyes were examined for signs of irritation one, two, and three days after instillation. Grading and evaluation of a response were made according to a modified Draize method (Draize et al., 1944)[2] which scores irritation in terms of redness of the lid and the conjunctiva, edema of the lid and conjunctiva, and opacity of the eye surface.

6. Primary Skin Irritation*

The objective was to determine skin irritation by a substance following a single application. The animals used for this test were New Zealand albino male rabbits that weighed about 2 kg. Each substance was tested on six different animals.

Hair was clipped from the sides of the rabbits and 0.5 ml of the undiluted substance was distributed evenly over a 6 to 7 cm^2 area of flank skin by use of a pipetting device. The area was covered with a gauze pad secured with strips of adhesive tape, and the flank was wrapped with an elastic bandage. Four hours later, the bandage and pad were removed and any residual substance was wiped off with dry gauze. At this time the first reading for a skin response was made followed by further readings at 24 and 72 hours after application. If a definite response was observed at 72 hours, another reading was made seven days after application. Grading and evaluation of a response were made according to the method of Draize et al (1944)[2] which scores irritation in terms of redness and edema of the skin.

7. Histopathology mice

Male Balb/c mice 6 to 8 weeks old, 10 per group, were given a

single intraperitoneal injection of the test agent. The mice were killed 2 or 4 days later and gross necropsy performed. Tissues were removed and fixed in 10% buffered formalin. They were embedded in paraffin, sectioned at 4-6 μm, and stained with hematoxylin and eosin. The following organs were examined histologically: brain, heart, liver, kidney, esophagus, stomach, intestine, colon, pancreas, spleen, gall bladder, adrenal, lung, trachea, lymph node, and thymus.

8. Lung Adenoma Assay in strain A mice

The objective was to determine if multiple intraperitoneal injections of a substance can induce lung adenomas in strain A/Jax male mice, 6 to 8 weeks old. In preliminary toxicity studies, the maximum tolerated dose (MTD) was determined. The MTD is defined as the highest dose producing no mortality when given in a total of six injections over a two week period. After 6 weeks, survivors were killed and a gross necropsy performed. In the adenoma test proper, each substance was injected into 30 mice three times a week (Monday, Wednesday, Friday) for eight weeks. The following doses were used: the MTD, one-half the MTD, and one-fifth the MTD. Control groups were 1) 30 mice injected with the same vehicle (corn oil) used to dissolve the test substance, 2) 50 mice given a single injection of urethane at 1 g per kg body weight (positive control), and 3) 50 mice which received nothing.

Body weights were recorded every 2 weeks, and the mice were killed 20 weeks after the first injection. The lungs were fixed in Tellyesniczky's solution and the number of tumors on the lung surface counted. Selected tissues were fixed in 10% buffered formalin and Bouin's fixatives for histopathology.

Results

1. Acute toxicity in mice and rats

Data for acute toxicity of shale oil and its derivatives are given in Table I. Crude shale oil was the only one that produced acute deaths in both male (at does of 8 and 10 g/kg) and female (at doses of 4, 8 and 16 g/kg) mice when given orally. Following intraperitoneal injection, deaths also occurred at doses of 2 g/kg. Because the mortality data were similar for both sexes, substances 4608-4610 were tested in female mice only. The LD_{50} was between 6 and 8 g/kg for substances 4608 and 4609 and greater than 16 g/kg for substances 4610. (T-I)

Results for petroleum and its derivatives are shown in Table II. Acute toxicity data were for all practical purposes similar to the ones observed with shale oil and its derivatives. (T-II)

In both male and female rats, there were no visible toxic responses arising from the dermal application of 2 g/kg of compounds 4601, 4602 and 4607. Neither visible skin lesions nor manifestations of central nervous system pertubations were observed. Deaths did not occur during the 2 weeks following application of the substances, and all rats showed a progressive gain in body weight (Table III). (T-III) Similar observations were made when animals were treated with 2 g/kg of mixed petroleum-derived JP-5, JP-8 or Diesel fuel marine product: no animals diet and weight gains over a 2 week period were normal.

2. Delayed-type contact hypersensitivity

Guinea pigs were sensitized with crude shale oil, hydrotreated shale oil, hydrotreated shale oil residue or shale-oil derived and

petroleum-derived jet and Diesel fuels. The data are summarized in Table IV. During the sensitization procedure, only slight redness and focal scab formation was noted in some of the animals for all nine substances; edema and necrosis was not seen. After the challenge injection, all animals sensitized with shale oil, hydrotreated shale oil and with residue developed a degree of redness and edema greater than that observed for the sensitizing injections. The challenge injection of these 3 substances also produced a slight necrosis in most guinea pigs, and for 2 of the substances, a marked lesion with a necrotic center developed. No difference in reaction between the sensitizing and challenge injection was observed in animals exposed to the shale-oil or petroleum-derived jet and Diesel fuels.

(T-IV)

3. Eye and skin irritation in rabbits

The data in Table V show that only the shale oil and to a lesser extent its hydrotreated derivative produced a visible redness of the rabbit eye. However, irritation was slight and transcient and is considered insignificant in evaluating substance toxicity. None of the other substances produced visible irritation at the 3 observation intervals. There were no indications that the shale oil, its 5 derivatives, or the petroleum products were irritating to rabbit skin to the extent of producing visible redness or edema (Table VI).

(T-V)

(T-VI)

4. Histopathology

Mice killed 2 or 4 days following intraperitoneal injection of 4601 (4 g/kg), 4602, or 4607 (16 g/kg) showed superficial pyogranulomatous serositis of the peritoneal cavity resulting from

local reaction to the substances. Lymphoid depletion of the thymic cortex was present in all animals at 2 and 4 days, but was most marked in mice injected with 4602. Serositis and thymic cortical depletion were the only histopathologic alterations observed for these substances. No lesions were present in animals injected with corn oil alone.

5. Lung adenoma assay

This assay was done essentially as described by Shimkin and Stoner (1975).[3] In preliminary toxicity studies, the MTD for 4601 was determined to be 2.5 g/kg per injection and for 4602, 16 g/kg. However, during the course of the adenoma assay it was found that the MTD, as determined, was too high. Only in one group was the survival rate higher than 50%. Cumulative mortality rates are shown in Figure 1 a and b and average weight ~~Gain~~ gain of survivors in figure 2. It is interesting to note that with both crude shale oil and hydrotreated shale oil animals continued to die even after cessation of exposure to the test agents. The reasons for this delayed toxicity remains to be established. Treated animals also generally failed to gain weight at rates comparable to controls.

As shown in data of Table VII, tumor incidence was from 30 to 60% in treated animals and not significantly different from animals injected with vehicle (corn oil) alone. On the other hand, tumor multiplicity was significantly higher in animals injected with 1.25 g/kg of crude shale oil. In all other groups, the difference from the ~~con-rol~~ control value were statistically not significant. However, few animals survived when injected with 2.5 g/kg of crude shale oil or

T-VII

with 16, 8 or 3.2 g/kg of hydrotreated shale oil (Table VIII). In view of this general toxicity, the adenoma assay in these groups is probably not representative and needs to be repeated at lower dose levels. Detailed results on histopathology will be reported later.

T-VIII

Discussion

Based on our data, crude shale oil may be considered slightly toxic when given orally and all other samples are practically non-toxic; no sex difference was found where both male and female mice were tested. Following intraperitoneal injection the substances were slightly toxic. Acute skin toxicity was invariably greater than 2 g/kg and no evidence was obtained to document appreciable eye and skin irritation. Histopathologic findings following acute administration of large amounts of test material were not remarkable. Lymphoid reduction in the thymic cortex was interpreted as a stress reaction rather than a substance-specific effect.

Compared to petroleum and some of its derivatives, shale oil and its derivatives appear to have practically the same overall toxicity. Fuels from both sources have about the same LD_{50}'s and were essentially nonirritating to the skin and eye. We would not expect, therefore, that the commercial use of these jet and diesel fuels produced from shale oil to be any more hazardous upon acute exposure than their petroleum-derived equivalents currently in use.

From the present data, we would conclude that a single exposure of humans to any one of the six Paraho/SOHIO substances tested would result in little if any systemic toxicity or in skin or eye injury.

One possible exception arises from the findings of the skin-sensitizations bioassay. Our data suggest that repeated topical exposures to the crude shale oil or the hydrotreated products 4602 or 4607 could possibly elicit an immunologically mediated reaction in humans. This type of reaction which could take weeks or months to develop may become manifest as itching, reddening, eruption, and edema of the skin. These reactions may disappear if there is no further contact with the sensitizing substance, but the state of sensitization may be permanent.

The data obtained in the lung adenoma bioassay suggest that crude shale oil has tumorigenic potential. This would appear to agree with preliminary data obtained in skin painting experiments (Holland, J. M., 1980). Before any more definitive conclusions can be drawn, particularly as to the carcinogenic potency of shale oil, additional studies with the lung adenoma bioassay will have to be completed.

It is interesting to note that the oral LD_{50} of shale oil or petroleum and their derivatives are consistently higher than the oral LD_{50} found with materials derived from various coal conversion processes. Table VIII list some data for comparative purposes.

In conclusion, acute toxicity of Paraho shale oil and of five derivatives does not appear to be a problem of immediate concern. The substances produced as little acute effects as do the petroleum-derived materials. Our data essentially agrees with those in a report by Weaver and Gibson (1979) except that these authors failed to obtain a positive response in the assay for skin

sensitization. This discrepancy might need to be resolved in further studies.

Acknowledgement

The authors thank Marsha Harden, Carol Paton and Clarissa Shelton for their excellent assistance.

References

1. Weil, C. S. (1952) Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8: 249-263.
2. Draize, J. H., G. Woodard, and H. O. Calvery (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82: 377-390.
3. Shimkin, M. B., G. D. Stoner (1975) Lung tumors in mice: Application to carcinogenesis bioassay. Adv. Cancer Res. 21, 1-58.
4. Weaver, N. K., R. L. Gibson (1979) The US oil shale industry: a health perspective. Am. Ind. Hyg. Assoc. J. 40: 460-467.
5. Briefing on Oak Ridge National Laboratory Life Sciences Synthetic Fuels Program. Eds. K. E. Cowser et al., September 27, 1979, pp. 86-94.
6. Holland, J. M. (1980) These proceedings.

Table I. Acute Lethality Test in Mice: Shale oil and Derivatives

	LD_{50}(g/kg)			
	Oral		Intraperitoneal	
Substance	Male	Female	Male	Female
4601	11.3	11.3	6.1 (4.5-8.2)*	4.3 (2.0-6.1)
4602	>16.0	>16.0	>16.0	>16.0
4607	>16.0	>16.0	>16.0	>16.0
4608	ND**	>16.0	ND	8.0 (5.4-12.8)
4609	ND	>16.0	ND	6.4 (5.0-8.0)
4610	ND	>16.0	ND	>16.0

* 95% confidence limits
** ND = not done

Table II. Acute Lethality Test in Mice: Petroleum and Derivatives

	LD_{50} (g/kg)			
	Oral		Intraperitoneal	
Substance*	Male	Female	Male	Female
5107	>10.0	ND**	ND	ND
5301	>16.0	ND	ND	ND
5305	>16.0	ND	ND	ND
4614	ND	>16.0	ND	>11.2 (8.13-15.5)***
4615	ND	>16.0	ND	8.0 (5.5-12.8)
4616	ND	>16.0	ND	>16.0

*ND = not done

**95% confidence limits

*** - mixed petroleum crudes; 5301 - Wilmington crude oil; 5305 - residue crude oil

Table III.

a) Weight gain in male and female rats over 2 weeks following dermal application of 2 g/kg of shale oil, hydrotreated shale oil and residue

	Body weight (g)*					
	Male			Female		
Substance No.	No. of animals	Day 0	Day 14	No. of animals	Day 0	Day 14
4601	6	358 ± 8	415 ± 7	6	209 ± 5	241 ± 2
4602	6	352 ± 9	422 ± 9	5	209 ± 3	238 ± 4
4607	6	370 ± 4	435 ± 7	6	225 ± 5	256 ± 6

* Data are means ± SE.

Table IV. Delayed Type Allergic Contact Hypersensitivity Test in Guinea Pigs

Substance	Average response* 24 and 48 hours after each of 9 sensitizing injections			Average response* 24 and 48 hours after the challenge injection		
	Redness	Edema	Necrosis	Redness	Edema	Necrosis
Shale oil and Derivatives						
4601	±	0	0	1-2	2	±
4602	0	0	0	2	1	±
4607	0	0	0	2	1	±
4608	0-1	0	0	0	0	0
4609	0-1	0	0	0	0	0
4610	0-1	0	0	0	0	0
Petroleum Derivatives						
4614	0-1	0	0	0	0	0
4615	0-1	0	0	0	0	0
4616	0-1	0	0	0	0	0
Propylene Glycol**	0-1	0	0	0	0	0
Dinitrochlorobenzene***	1-2	0-1	1-2	1-2	1-2	2-3

* Scale: 0, ±, 1-4, ** Vehicle, *** Positive Control

Table V. Primary Eye Irritation Test in Rabbits

Substance	Response at indicated times after instillation*		
	24 hours	48 hours	72 hours
Shale oil and its Derivatives			
4601	6 of 6 showed grade 1 redness**	0	0
4602	2 of 6 showed grade 1 redness**	0	0
4607	0	0	0
4608	0	0	0
4609	0	0	0
4610	0	0	0
Petroleum Derivatives			
4614	0	0	0
4515	0	0	0
4616	0	0	0

* 4 or 6 eyes per substance

** On the basis of a 0 to 4 response of lid, conjunctiva, and cornea.

Table VI. Primary skin Irritation Test in Rabbits

Substance	Number of rabbits responding at indicated times after application*					
	4 hours		24 hours		72 hours	
	Redness	Edema	Redness	Edema	Redness	Edema
Shale oil and its derivatives						
4601	0	0	0	0	0	0
4602	0	0	0	0	0	0
4607	0	0	0	0	0	0
4608	0	0	0	0	0	0
4609	0	0	0	0	0	0
4610	0	0	0	0	0	0
Petroleum derivatives						
4614	0	0	0	0	0	0
4615	0	0	0	0	0	0
4616	0	0	0	0	0	0

*Six animals per substance

Table VII. Lung Adenoma Assay

Substance	Dose per injection[1] (g/kg)	No. of mice initially	No. of survivors	No. of mice with tumors	No. of tumors per mouse[2]
4601	2.5	30	5	3	0.6 ± 0.3
	1.25	30	12	6	1.3 ± 0.5*
	0.5	30	16	7	0.6 ± 0.2
4602	16	30	0	—	—
	8	30	1	0	0
	3	30	6	3	1.0 ± 0.6
CORN OIL	10	20	19	6	0.4 ± 0.1
UNTREATED	—	20	20	3	0.2 ± 0.1
URETHAN	1.0[3]	20	20	20	23.2 ± 1.7

[1]Given 3 times a week for 8 weeks.

[2]Total number of tumors divided by total number of surviving animals; mean ± SE.

[3]One single injection ip.

*P <0.05 compared to corn oil controls.

Table VIII. Acute Oral Lethality Test of Selected Coal Conversion Products

Substance	ORNL Repository Number	LD_{50}* (g/kg)
H-Coal		
H-coal distillate (raw)	1601	3.6 (2.8-4.5)**
HTD distillate (low severity)	1602	4.0 (3.4-4.7)
HTD distillate (high severity)	1604	5.5 (3.8-7.2)
HTD fuel oil (low severity)	1617	6.2 (3.7-8.7)
HTD fuel oil (high severity)	1619	6.2 (5.1-7.3)
Atmospheric bottoms (ASB)	1313	2.3 (1.9-2.6)
Vacuum overhead (VSOH)	1314	2.6 (2.2-3.2)
Atmospheric bottoms (ASB)	1309	3.6 (2.4-5.2)
Vacuum overhead (VSOH)	1310	2.5 (1.7-3.1)
Coal SRC I		
HDT light organic liquid (low severity	1606	3.6 (3.1-4.1)
HDT light organic liquid (high severity)	1608	~10.0
HDT recycle solvent (low severity)	1614	2.6 (1.9-3.2)
HDT recycle solvent (high severity)	1616	5.3 (4.4-6.3)
Coal SRC II		
Fuel oil blend	1701	3.1 (2.7-3.5)
Zinc Halide Hydrocracking Process		
Product distillate	1801	3.4 (0.5-2.3)

* Balb/c male mice, ** 95% confidence limits

Fig. 1A

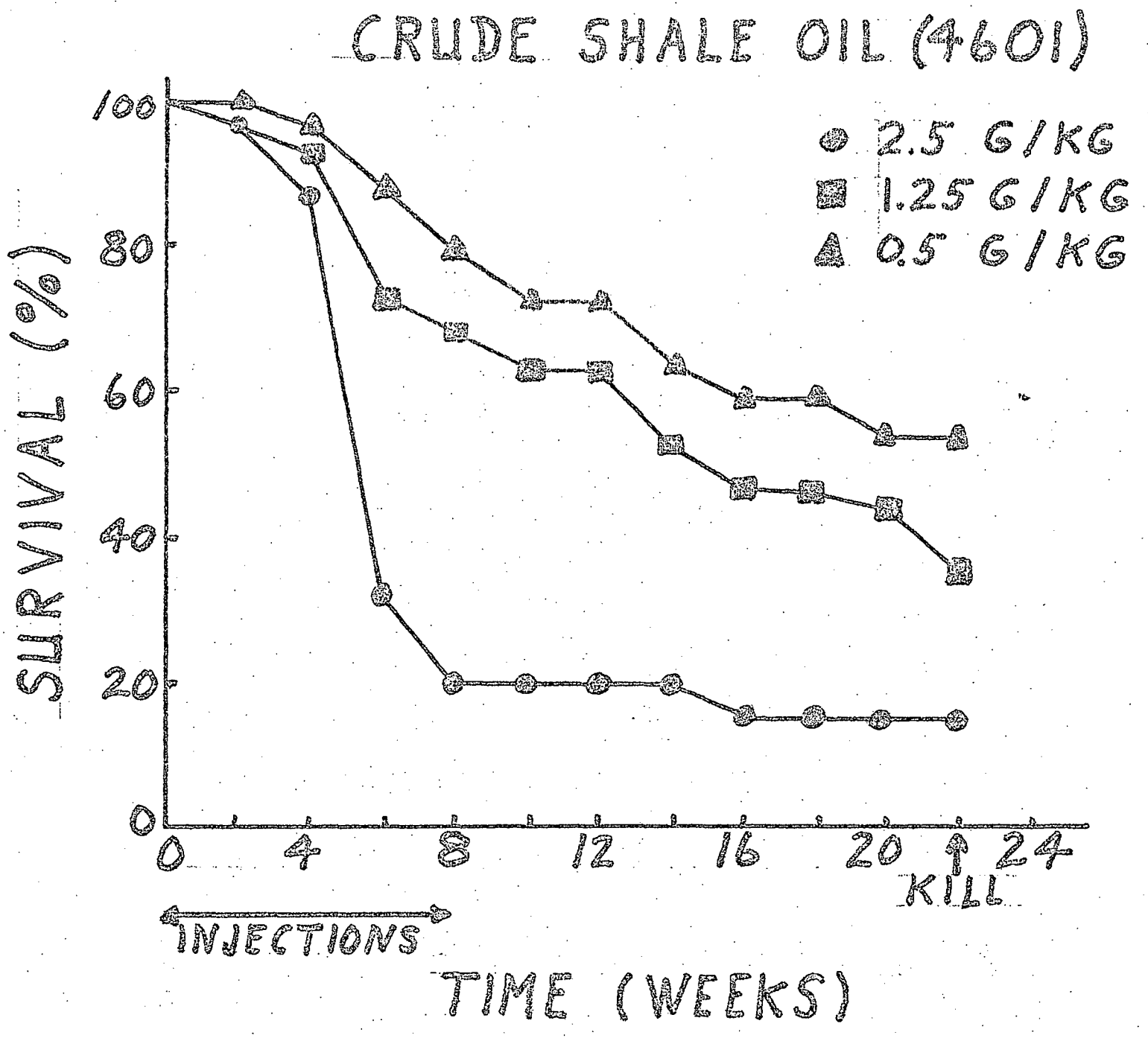
CRUDE SHALE OIL (4601)
2.5 G/KG
1.25 G/KG
0.5 G/KG
SURVIVAL (%)
100
80
60
40
20
0
0
4
8
12
16
20
24
KILL
INJECTIONS
TIME (WEEKS)

Fig. 13

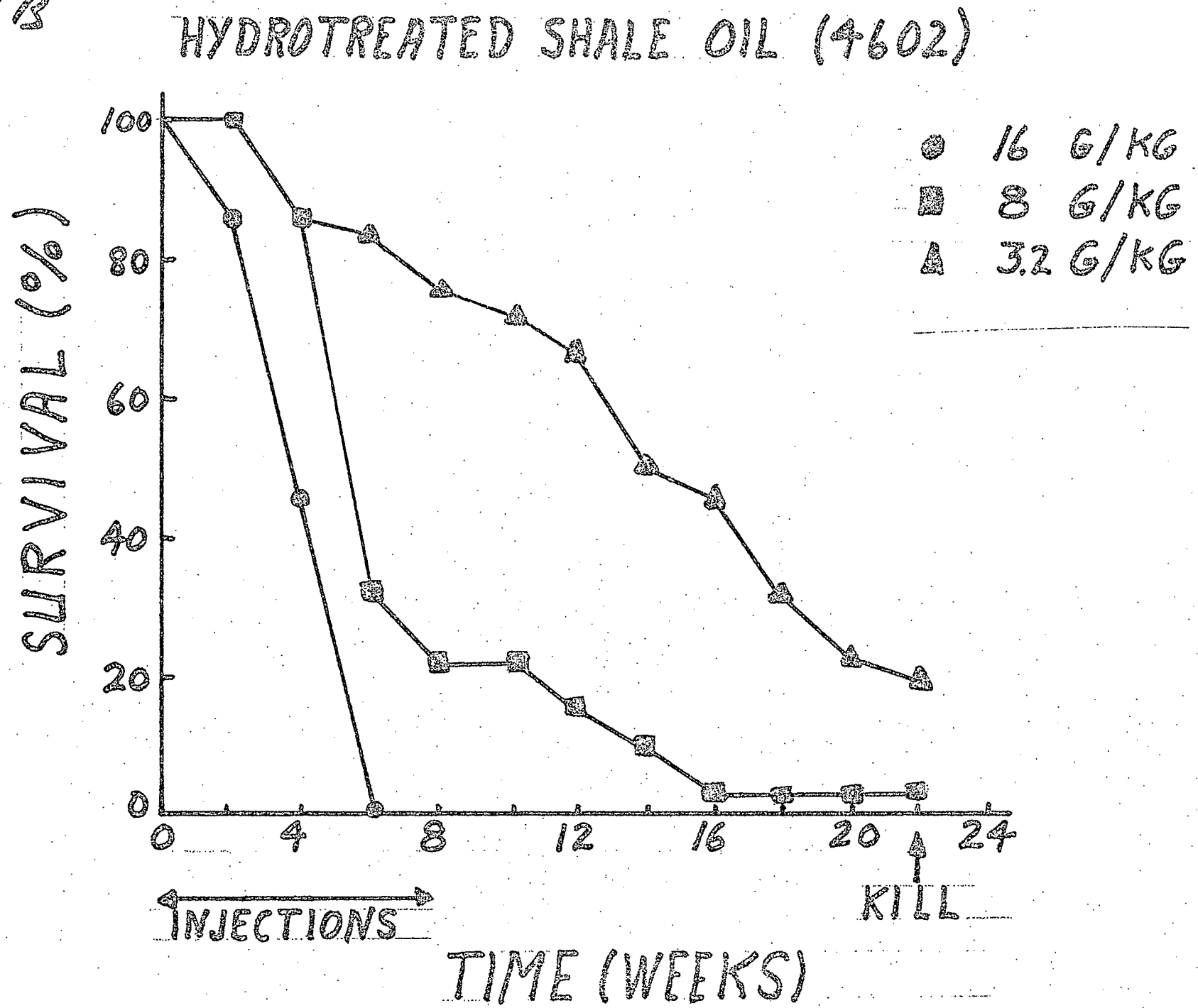
HYDROTREATED SHALE OIL (4602)
16 G/KG
8 G/KG
3.2 G/KG
SURVIVAL (%)
100
80
60
40
20
0
0
4
8
12
16
20
24
INJECTIONS
KILL
TIME (WEEKS)

Fig. 2 Monthly weight gain