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NO. 467

TOXICOLOGY OF DMMPA

PART II

THE EFFECTS OF CHRONIC APPLICATION OF DMMPA TO TEST ANIMALS (U)

by

I.W. Coleman

PROJECT NO. 13E11

August 1977

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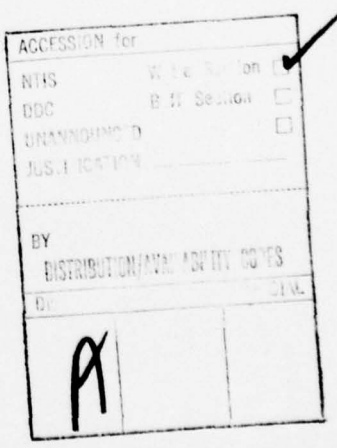

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FOREWORD

In conducting the experiments described in this report, the author and his staff have adhered to the code of ethics and the methods promoted by the Canadian Council on Animal Care. These are contained in their bulletin "Principles of Care of Experimental Animals: a Guide for Canadian Users".

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TOXICOLOGY OF DMMPA
PART II
THE EFFECTS OF CHRONIC APPLICATION OF DMMPA TO TEST ANIMALS (U)

by

I.W. Coleman

ABSTRACT

The effects of chronic daily dosing of *dimethyl morpholinophosphoramidate* (DMMPA) have been assessed in rabbits for dermal application and in rats for parenteral application.

One hundred day application of DMMPA dermally to rabbits was without effect.

One hundred day application to rats, both male and female, was without effect on breeding both in the incidence of pregnancies, number of pups per litter and the number of still-born.

Three separate experiments are described in which male and female rats were dosed with DMMPA in periods from 100 to 240 days. Some thirty separate pathological screening tests were examined including nutritional, physiological, haematological, blood chemistry, tissue weight and water content procedures as well as macroscopic and microscopic pathology. Of these, only water intake, heart rate and lung damage were found to be affected. The highest dose of DMMPA having no effect on these latter parameters was 0.5 gm/kg/day.

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TOXICOLOGY OF DMMPA
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THE EFFECTS OF CHRONIC APPLICATION OF DMMPA TO TEST ANIMALS (U)

by

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INTRODUCTION

The previous report on the toxicology of DMMPA covered only the results of acute, single dosing of the material (1). In the following report the results of chronic dosing on a daily exposure are summarized for periods of exposure varying from 100 to 240 days. All methods used in the chronic exposure study are identical to those described in Annex A of the previous paper. Equally, the chronic studies were performed in the same experimental facilities and with the same staff as were the acute investigations. One change was introduced in the reporting of the experiments, however. The long term experiments with repeated dosing resulted inevitably in accidents resulting in the deaths of animals. All such deaths were autopsied and, when found due to accident, such animals were removed from the study completely so that none of the data of these animals contributed to the results as shown.

SECTION A:

THE EFFECTS OF CHRONIC APPLICATION OF DMMPA TO THE SKIN OF RABBITS

Male New Zealand white rabbits were dosed daily, 5 days per week but not on weekends. Six animals were dosed with 50 µl (62 mg) and six at 100 µl

(123 mg) on an area of clipped skin shaved with fine clippers so that the skin was not broken or scraped. Doses were continued for a total of 100 days at both levels, at which time the animals were sacrificed, autopsied and the area of skin excised, fixed and stained with haematoxylin and eosin.

The results of the experiment were completely negative in all aspects. The animals showed no signs of skin damage by the DMMPA application whatsoever. Sections of skin taken proved histologically normal. The autopsy did not show any evidence of dose related lesions. From these results it was concluded that doses of DMMPA as high as 123 mg/animal/day are innocuous. Since the animals weighed between 2-4 kg, doses as high as 60 mg/kg applied dermally are without adverse effects after dosing for 100 days.

SECTION B:

THE EFFECTS OF CHRONIC APPLICATION OF DMMPA ON BREEDING CHARACTERISTICS OF RATS

Male and female rats were orally dosed with DMMPA at 200 µl (244 mg) per animal per day, 7 days per week for 100 days. The animals were then transferred to breeding cages with a ratio of 5 females to one male and retained for 3 weeks after which the females were segregated into individual cages for littering. The pattern of breeding included:

(1) DMMPA treated females with untreated males,
(2) DMMPA treated males with untreated females,
(3) DMMPA treated males and females.

Offspring from Group 3 were allowed to mature and breed in the same pattern as before to test if the first generation whose parents were exposed to DMMPA were in any way adversely affected.

The results obtained are shown in Table I. These results do not indicate any significant difference in (a) the number of pregnancies, (b) the average number of pups per litter or (c) the number of stillborn, between any of the DMMPA treated group in comparison with the untreated controls. All pups from each group were kept segregated until weaning and, although no data were kept on growth rate or percent survival, there was no evidence of any difference between the offspring of the treated versus the untreated parents.

TABLE I

BREEDING RESULTS IN RATS CHRONICALLY DOSED WITH 228 M[illegible] PER DAY FOR 100 DAYS

Breeding Group		Percentage of Pregnancies	Total Number of Live Pups	Still Born	Abnormal Pups	Average Number of Pups per Litter
Females	Males					
Dosed DMMPA - 125	Untreated - 25	81	802	2	0	9.9
Untreated - 125	Dosed DMMPA - 25	85	765	4	0	9.0
Dosed DMMPA - 125	Dosed DMMPA - 25	79	893	2	0	11.3
Untreated - 125	Unteated - 25	79	814	4	0	10.3
First Generation Untreated - 125	First Generation Untreated - 125	81	745	2	0	9.2

All offspring except those of Group 3 were sacrificed at weaning, but those of Group 3 were maintained to maturity and offspring of their breeding did not vary from the results of the untreated control breeding. Although this experiment did not assess resorbed foeti, since the mean number of live pups per litter was not different in the DMMPA treated animals from those of the controls, there would appear to be little likelihood that DMMPA affected this parameter or was altering foetal development to any significant degree.

SECTION C:

EFFECTS OF CHRONIC APPLICATION OF DMMPA TO RATS

Cumulative LD_{50} (100 days) (2)

Five attempts to obtain experimental data to quantify this parameter were unsuccessful. Doses of DMMPA above 0.9 of an acute LD_{50} value (5.9 gm/kg males) were found to accumulate to lethal effects in 2-3 days, while values below this level either did not produce death at all even with 100 repetitions of the daily dose or produced deaths so erratically that there was always doubt that the deaths were due to DMMPA accumulative effects. The only value obtained from all chronic dosing daily for 100 days or longer is that of 2.5 gm/kg (male rats) which is the lowest dose repetitively given at which cumulative lethality was observed. This value must approximate LD_{50} (100 days) but has no statistical validity.

I. EFFECTS OF LOW DOSE - 100 DAY APPLICATION - FEMALE RATS

One hundred and fifty female rats were selected and grouped into 10 groups of 15 animals each, such that no significant difference in mean weight existed from one group to another. They were allowed time to adjust to the metabolism cages for a period of two weeks before beginning the oral application of DMMPA by stomach tube and were subsequently dosed daily, 7 days per week for a total of 100 days. Animals were dosed at the same time each day at 1300 hours, following the dose schedule given in Table II. The procedures used in handling, sacrifice and analysis of the animals are those described in Annex A of Reference (1). Animals were sacrificed on the 10th,

TABLE II

DOSE SCHEDULE FOR CHRONIC LOW LEVEL EXPOSURE OF RATS TO DMMPA

Groups	Dose per animal* (mg)	Dose per Kilogram (mg)
A	123	570
B	62	280
C	31	150
D	15	80
F	7.5	38
H	3.8	19
J	1.9	9
K	0.9	5
G	Control 1.0 ml water orally	
L	Control handled only	

* All doses given orally at a volume of 1.0 ml per animal with DMMPA made up in fresh aqueous solutions daily.

15th, 30th, 45th, 60th, 65th, 70th, 80th, 95th and finally on the 100th day, taking one animal from each group, both test and control, at each time of sacrifice. This procedure was followed in order to determine the time required at a given dosage for the lesions produced to develop. The procedure had the effect, however, of reducing the number of animals exposed to the 100 day dosing to 5, with a comparable number of controls. It was intended, however, to repeat the chronic exposure trials later such that this difficulty was overcome by postponing sacrifice times for a period of 100 days or longer so that all animals would have this period of dosing (see part III of this Section).

NUTRITIONAL PARAMETERS

Data on food intake, water intake and weight gain were measured on each animal daily to a total of 150 observations per day. To deal more conveniently with this quantity of information, averages were taken for 5 day intervals in which the mean of this period was averaged for each parameter. These data were then incorporated into data cards and processed by the computer such that the trends for each parameter could be plotted with time and compared with the equivalent control group values. As well, the significance of any difference between the means of the test dose and control groups was determined by the "t-test".

In Figure 1, the comparison between the two control groups labelled L and G are plotted for food intake, water intake and weight gain. As can be seen, although the only difference between these control groups lies in the fact tnat one, Group G, received 1.0 ml water daily by stomach tube, there are frequent times during the 100 days when highly significant and significant differences are observed in food intake and water intake. No apparent explanation for such differences has come to light in the careful examination of the procedures of operation. They can not be assigned to differences in temperature, air supply, light or to staff since the latter were rotated so that the same people were not always assigned to the same test groups. Seemingly, such difference occurs by virtue of some uncontrolled factor in the experimental design. This evidence has forced the conclu-

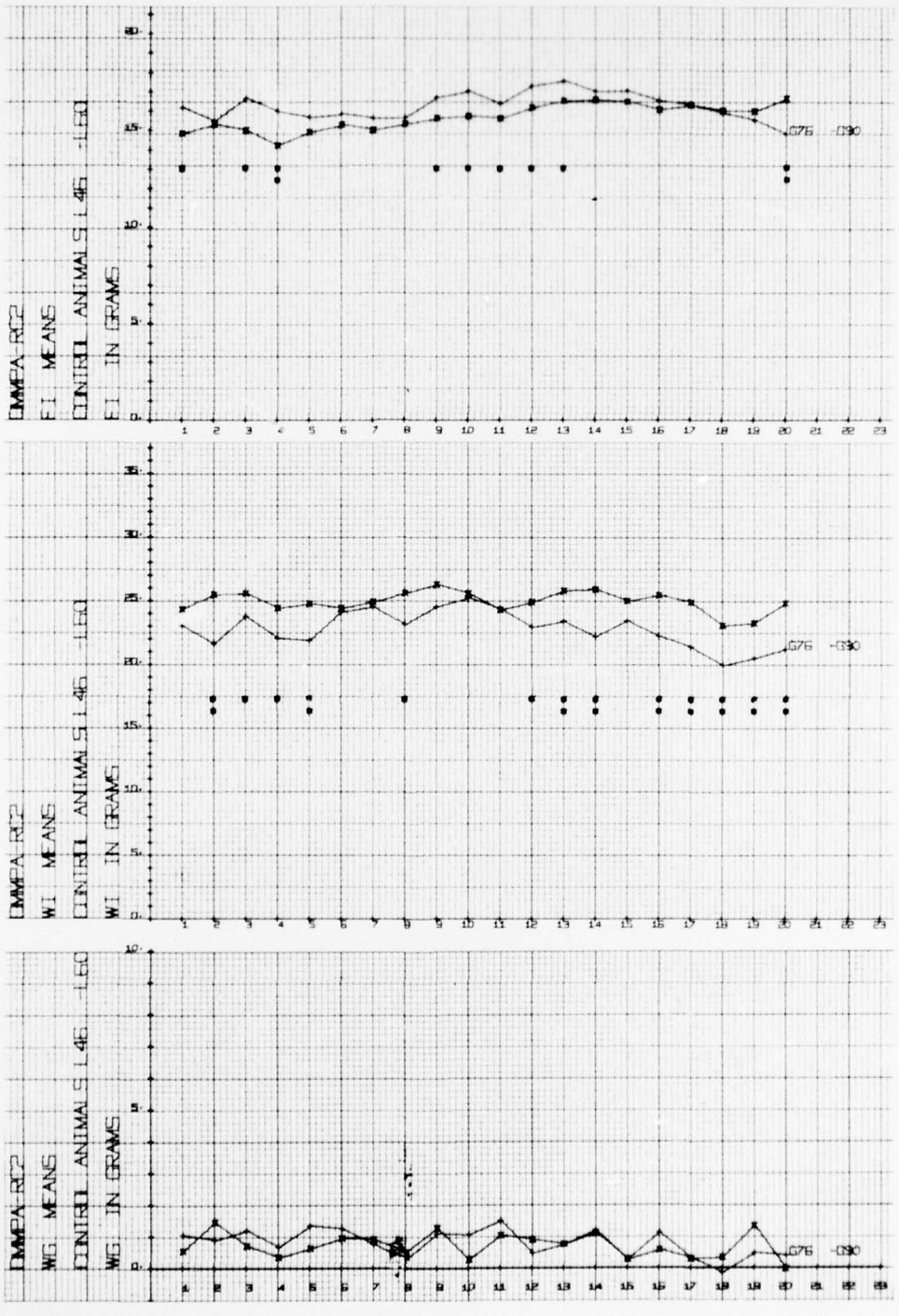

FIGURE 1- FOOD INTAKE (FI), WATER INTAKE (WI) AND WEIGHT GAIN (WG) IN FEMALE CONTROL RATS GROUPS L AND G. THE MEAN OF EACH GROUP OF 15 ANIMALS FOR A FIVE DAY PERIOD (INTERVAL WEEK) IS PLOTTED AGAINST THE NUMBER OF INTERVALS FOR A TOTAL OF 100 DAYS. SIGNIFICANT DIFFERENCE BY 'T-TEST' IS SHOWN AS A SINGLE BLACK SQUARE, HIGHLY SIGNIFICANT DIFFERENCE BY TWO BLACK SQUARES.

sion that in the dosed groups, differences in food intake and water intake observed will be considered of importance only if those differences follow a pattern proportional to the dose of DMMPA applied.

The Effect of DMMPA on Food Intake

The variations in food intake with differing doses of DMMPA over the period of the experiment are shown in Figure 2 A, B and C for the 8 doses of DMMPA applied. As can be observed, there are frequent occasions where the food intake in DMMPA-dosed animals is significantly and highly significantly different from the control group. However, there is no evidence that the difference between the dosed and control groups follows any pattern related to the level of DMMPA. The conclusion was drawn that DMMPA with daily dosing at levels of 123 mg/animal/day for a period of up to 100 days has no effect on the food intake of female rats.

The Effect of DMMPA on Water Intake

The variations in water intake with differing doses of DMMPA over the period of 100 days are shown in Figure 3 A, B and C for the 8 doses of DMMPA applied daily. Again frequent significant and highly significant differences in the water intake of animals dosed with DMMPA are observed, but there is no observable regularity in such differences such that the relationship to the dose of DMMPA applied can be seen. The conclusion was drawn that DMMPA in doses as high as 123 mg/animal/day for a period of up to 100 days has no effect on the water intake of female rats.

The Effect of DMMPA on Weight Gain

The variations in weight gain in rats dosed with differing doses of DMMPA over a period of 100 days are shown in Figure 4 A, B and C for the 8 doses levels of DMMPA applied daily. There is no evidence of significant differences in weight gain for any dose of DMMPA at or below 123 mg/animal/day from the control group receiving no DMMPA. The conclusion was drawn that DMMPA dosing at these levels is without effect on the rate of weight change.

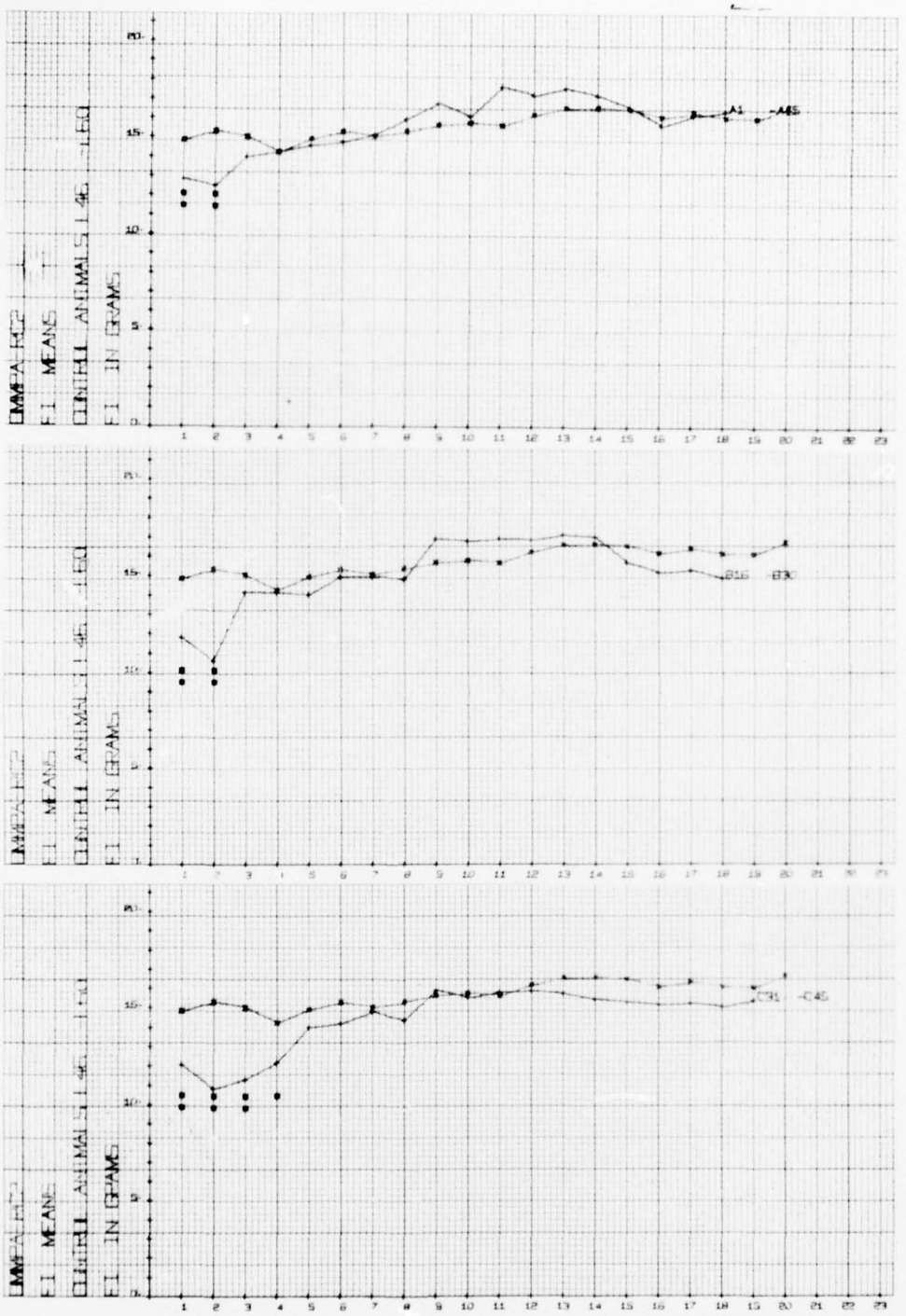

FIGURE 2A- FOOD INTAKE IN GRAMS PER ANIMAL PER DAY OF GROUP A (123 MG DMMPA/ANIMAL/DAY), GROUP B (62 MG DMMPA/ANIMAL/DAY) AND GROUP C (31 MG DMMPA/ANIMAL/DAY). THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY WEEK PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING. TWO BLACK SQUARES INDICATES DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE SIGNIFICANT.

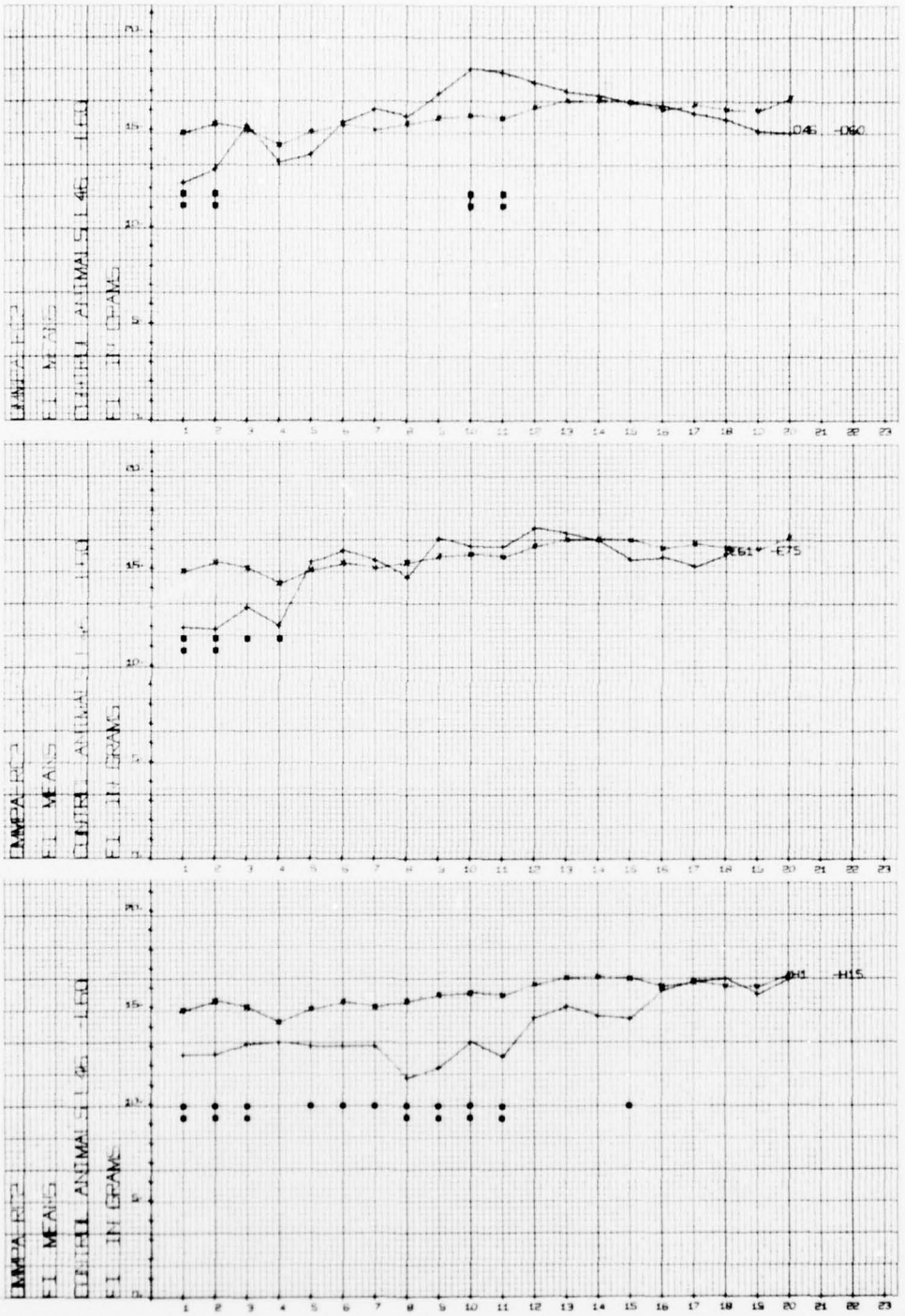

FIGURE 28- FOOD INTAKE IN GRAMS PER ANIMAL PER DAY OF GROUP D (15 MG DMMPA/ANIMAL/DAY), GROUP E (7.5 MG DMMPA/ANIMAL/DAY) AND GROUP H (3.8 MG DMMPA/ANIMAL/DAY). THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY WEEK INTERVAL IS PLOTTED AGAINST THE CONTROL GROUP FOR 100 DAYS OF DMMPA DOSING. TWO BLACK SQUARES INDICATES DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE SIGNIFICANT.

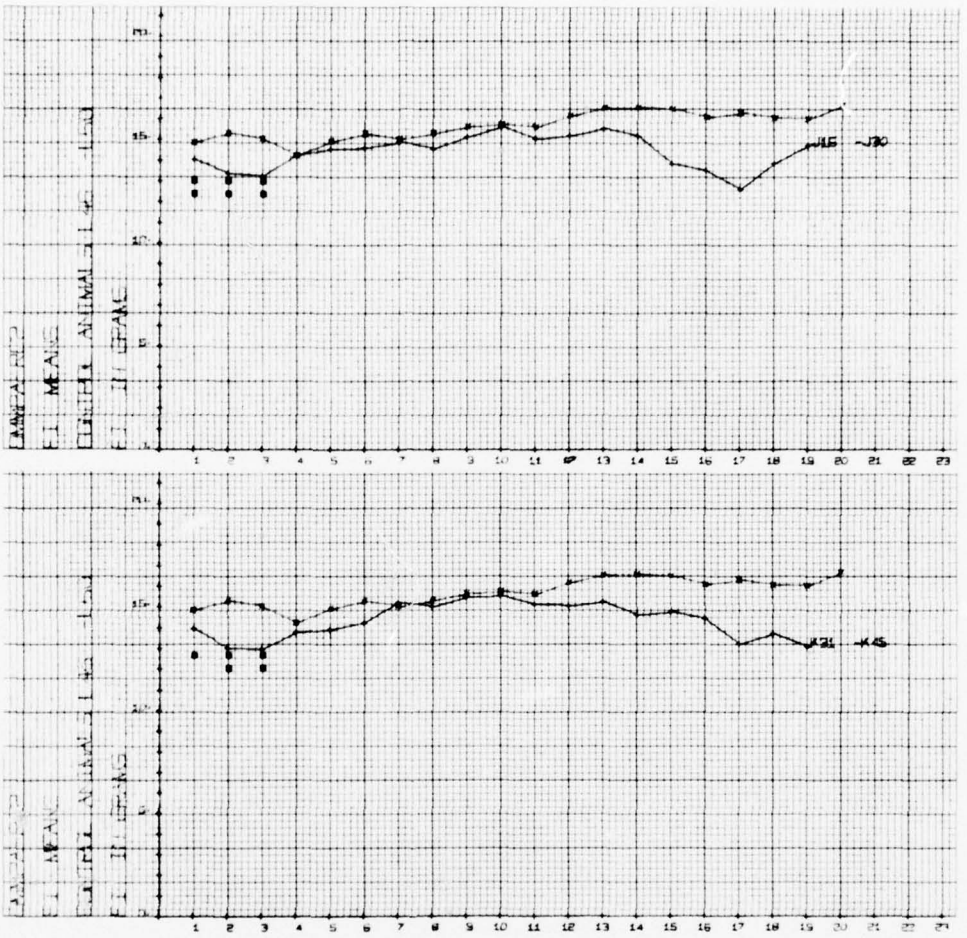

FIGURE 2C- FOOD INTAKE IN GRAMS PER ANIMAL PER DAY OF GROUP J (1.9 MG DMMPA/ANIMAL/DAY, AND GROUP K (0.9 MG DMMPA/ANIMAL/DAY). THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY WEEK INTERVAL IS PLOTTED AGAINST THE CONTROL GROUP FOR 100 DAYS OF DMMPA DOSING. TWO BLACK SQUARES INDICATES DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE SIGNIFICANT.

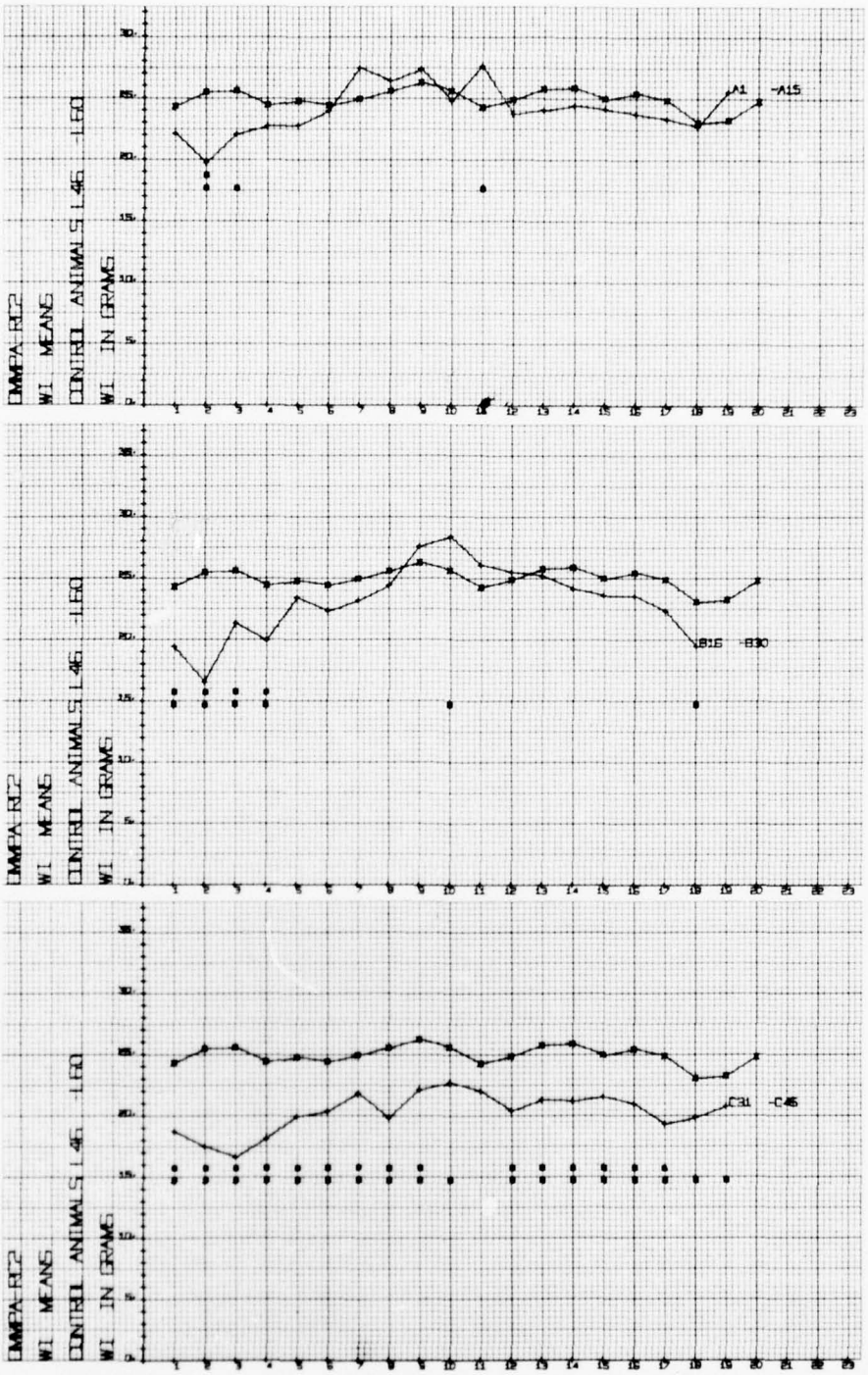

FIGURE 3A- WATER INTAKE OF RATS IN MILLILITERS PER ANIMAL PER DAY OF GROUP A (123 MG DMMPA/ANIMAL/DAY), GROUP B (62 MG DMMPA/ANIMAL/DAY) AND GROUP C (31 MG DMMPA/ANIMAL/DAY). THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING. TWO BLACK SQUARES INDICATES THE DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE IS SIGNIFICANT.

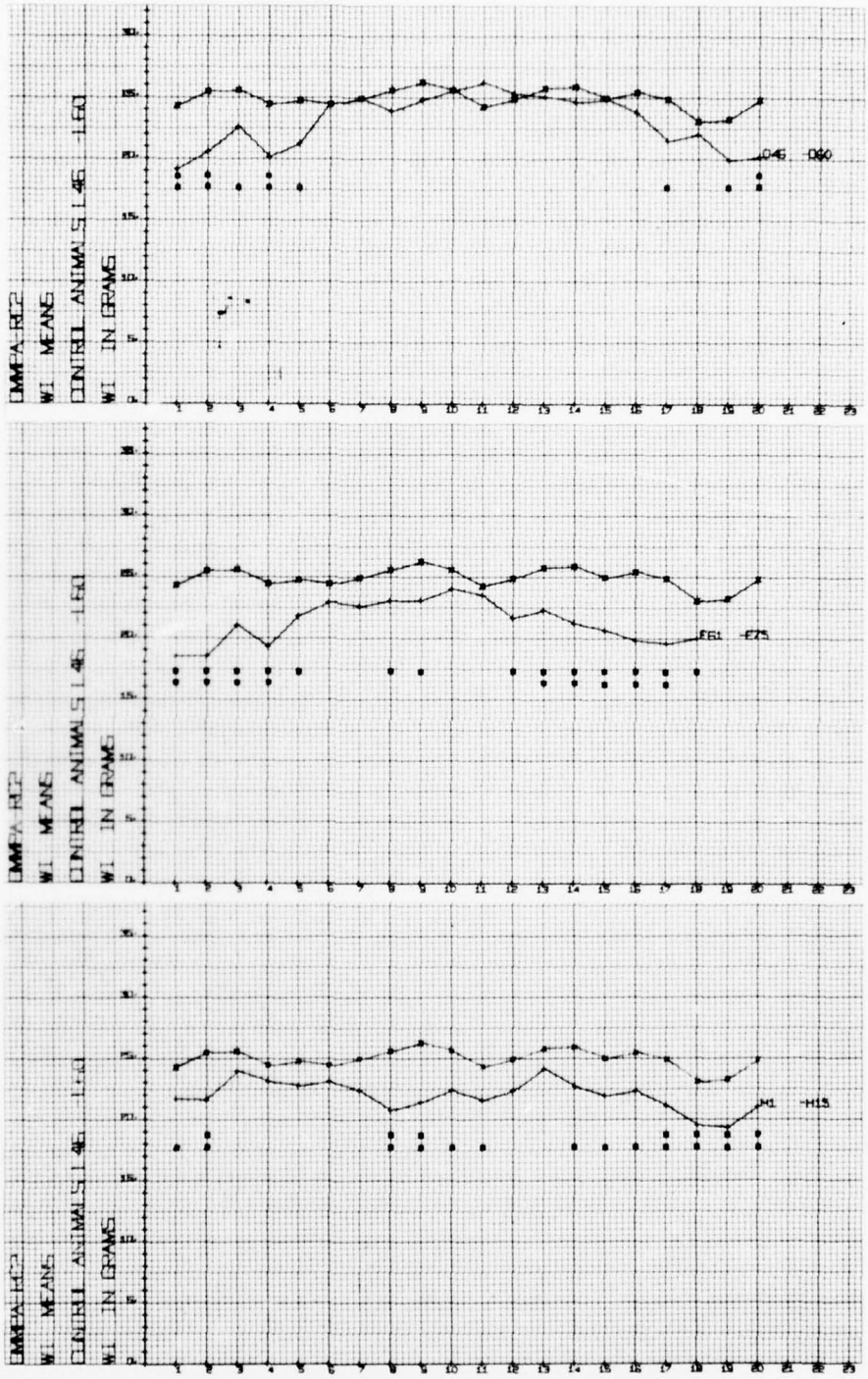

FIGURE 38- WATER INTAKE OF RATS IN MILLILITERS PER ANIMAL PER DAY OF GROUP D (15 MG DMMPA/ANIMAL/DAY), GROUP E (7·5 MG DMMPA/ANIMAL /DAY) AND GROUP H (3·8 MG DMMPA/ANIMAL/DAY)· THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING· TWO BLACK SQUARES INDICATES THE DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE INDICATES SIGNIFICANT·

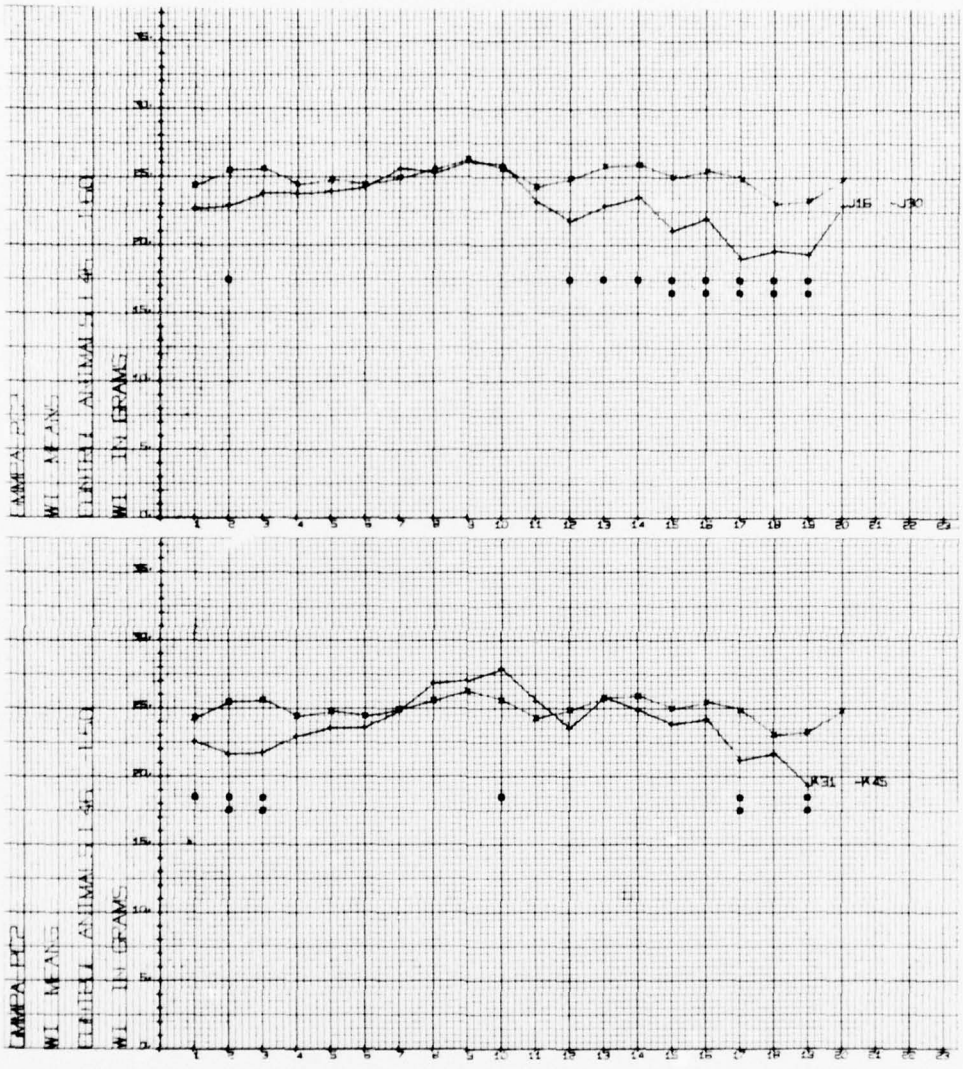

FIGURE 3C- WATER INTAKE OF RATS IN MILLILITERS PER ANIMAL PER DAY OF GROUP J (1·9 MG DMMPA/ANIMAL/DAY) AND GROUP K (0·9 MG DMMPA/ANIMAL/DAY)· THE MEAN OF EACH DOSE GROUP FOR THE 5 DAY PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING· TWO BLACK SQUARES INDICATES THE DIFFERENCE IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE CONNOTES SIGNIFICANT·

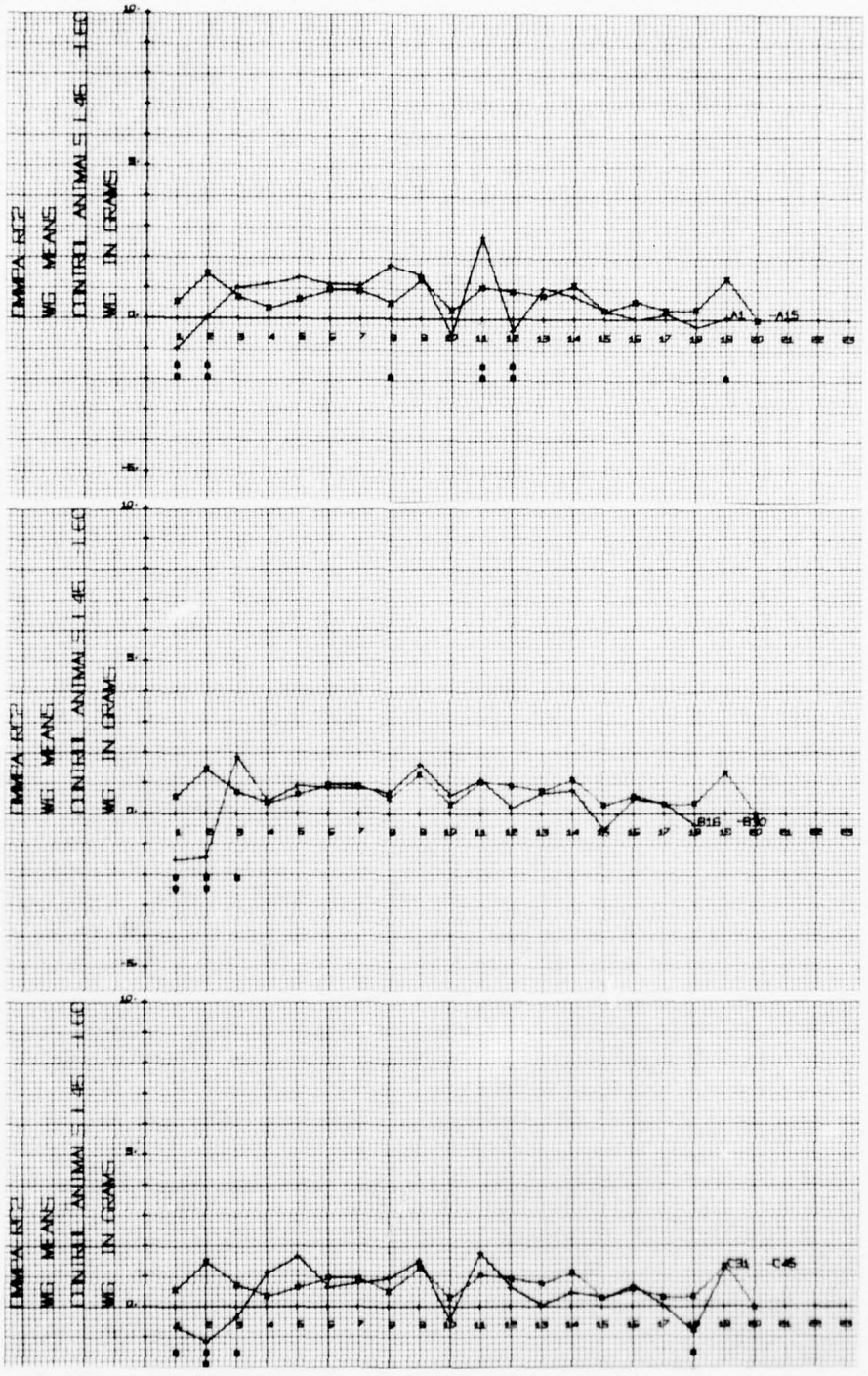

FIGURE 4A- WEIGHT GAIN IN GRAMS PER DAY OF GROUP A (123 MG/ANIMAL /DAY), GROUP B (61 MG/ANIMAL/DAY) AND GROUP C (31 MG/ANIMAL/DAY). THE MEAN WEIGHT CHANGE (POSITIVE OR NEGATIVE) FOR EACH DOSE GROUP FOR THE 5 DAY PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING.

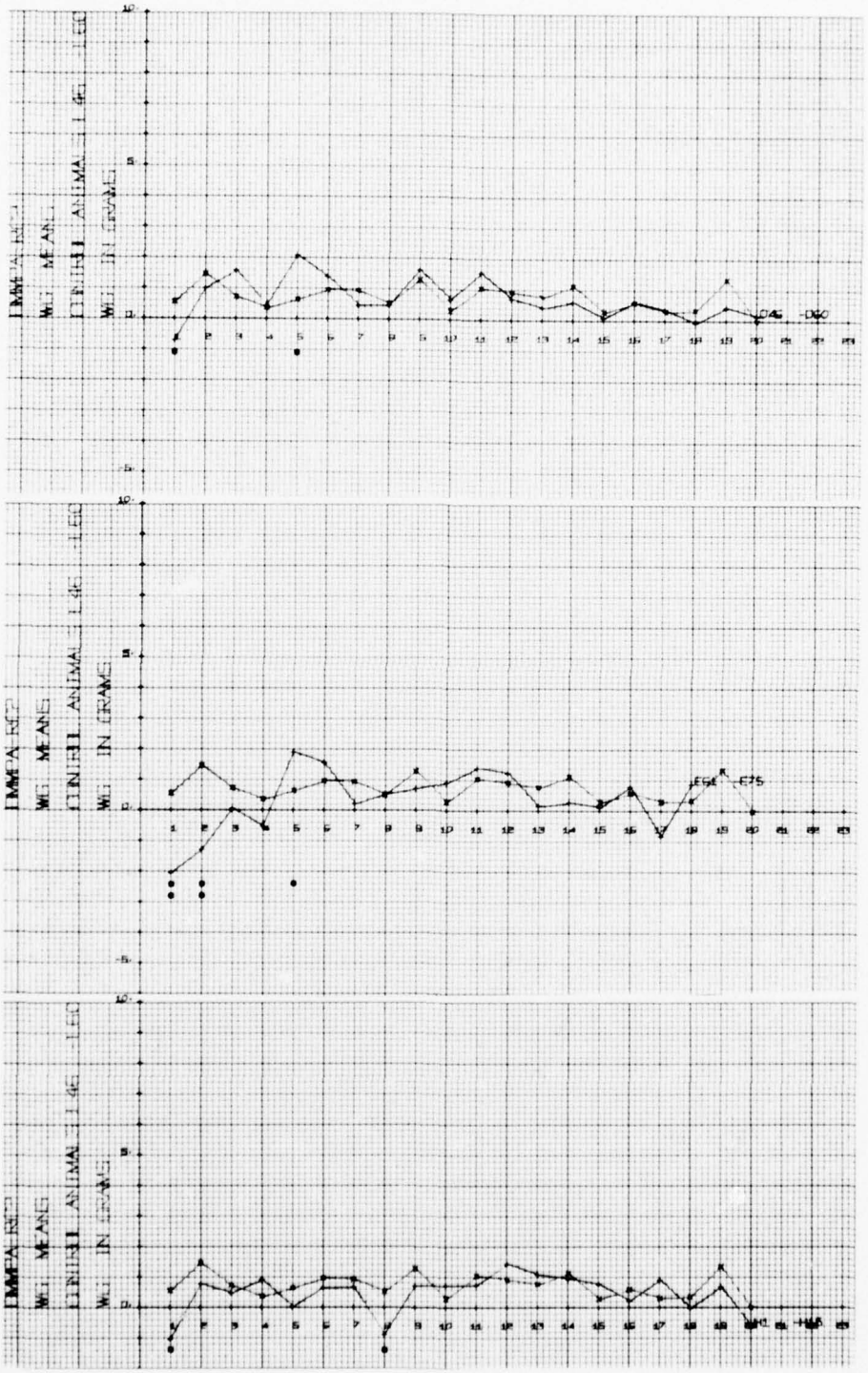

FIGURE 48- WEIGHT GAIN IN GRAMS PER DAY OF GROUP D (15 MG/ANIMAL /DAY), GROUP E (7·5 MG/ANIMAL/DAY) AND GROUP H (3·8 MG/ANIMAL/DAY). THE MEAN WEIGHT CHANGE (POSITIVE OR NEGATIVE) FOR EACH DOSE GROUP FOR THE 5 DAY PERIOD IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMPA DOSING.

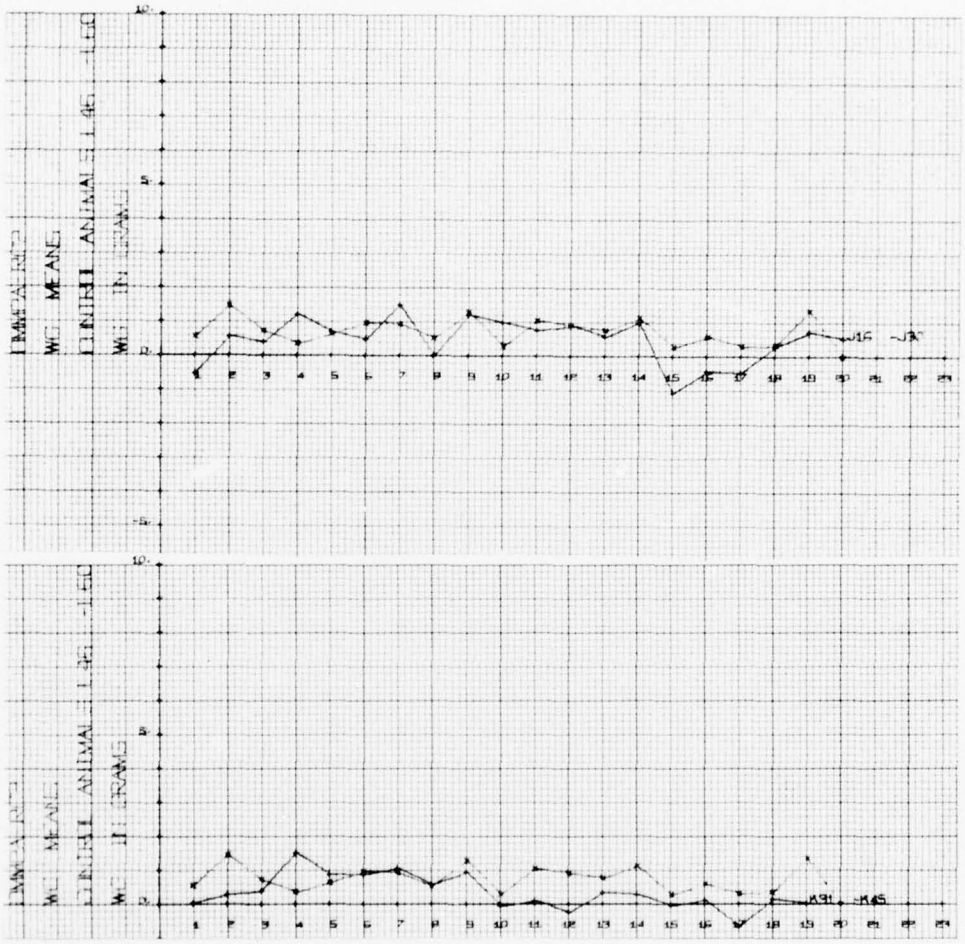

FIGURE 4C- WEIGHT GAIN IN GRAMS PER DAY OF GROUP J (1.9 MG/ANIMAL/DAY) AND GROUP K (0.9 MG/ANIMAL/DAY). THE MEAN WEIGHT CHANGE (POSITIVE OR NEGATIVE) FOR EACH DOSE GROUP IS PLOTTED AGAINST THE CONTROL GROUP FOR THE 100 DAYS OF DMMPA DOSING.

The Effect of DMMPA on Urine Components

Urine samples were analysed daily for all dose groups and controls. The analyses for blood, glucose and bilirubin were all negative with no significant change occurring in hydrogen ion concentration or urine protein.

PHYSIOLOGICAL PARAMETERS

Rectal temperature, respiration rate, heart rate and blood pressure were measured on each animal as many times as was possible during the 100 day feeding period. There were not less than 5 determinations of each parameter for each animal with some measured as many as 10 times during the run. The average was 7 times. The means and standard deviations of each dose group for each parameter as well as the mean weight of each dose group at autopsy are shown in Table III. As can be seen, there are no values of any of the parameters in the dosed animals differing significantly from the controls as assessed by "t-test". These results indicate that DMMPA has no effect on any of these parameters at doses of 123 mg/animal/day or lower.

BLOOD CELLULAR COMPONENTS

Blood collected at sacrifice of the test animals was examined for numbers of erythrocytes and leucocytes, haemoglobin concentration and haematocrit. The results with each dose group and control are shown in Table IV as the means and standard deviations. There are no values of these haematological parameters which differ significantly in the DMMPA-dosed groups from those of the controls, allowing the conclusion that DMMPA at doses of 123 mg/animal/day or lower is without effect on these blood components.

SERUM COMPONENTS

Serum samples separated from cellular components of blood taken at the time of sacrifice were analysed for creatine phosphokinase activity, glutamic-oxalic transaminase, lactic dehydrogenase and alkaline phosphatase, as well as levels of sodium and potassium. The results of each of the above

TABLE III

EFFECT OF DMMPA ON RATS ORALLY DOSED FOR PERIODS UP TO 100 DAYS

CHANGE IN PHYSICAL PARAMETERS

Dose Applied mg per animal	gm per kg	Weight at Autopsy	Rectal Temperature 0°C	Respiration Breaths per Minute	Heart Rate Beats per Minute	Blood Pressure mm Hg
123	570	246.3 (33.4)	36.4 (0.62)	170.7 (22.2)	360.9 (30.2)	114.7 (3.6)
62	280	246.3 (30.8)	36.4 (0.51)	168.6 (15.3)	366.8 (25.0)	114.8 (2.9)
31	150	237.8 (27.7)	36.4 (0.52)	170.5 (16.3)	362.6 (26.0)	114.7 (1.59)
15	80	248.4 (35.5)	36.5 (0.42)	168.6 (16.0)	368.5 (25.9)	115.2 (2.53)
75	38	245.1 (27.5)	36.5 (0.57)	171.9 (19.6)	359.4 (27.4)	113.5 (2.85)
3.8	19	244.4 (32.6)	36.5 (0.64)	173.5 (9.06)	363.3 (21.1)	112.9 (3.51)
1.9	9.0	243.3 (27.1)	36.6 (0.45)	173.8 (12.8)	352.5 (30.7)	113.4 (2.62)
0.9	5.0	238.3 (25.1)	36.5 (0.45)	170.8 (14.0)	363.4 (20.7)	113.2 (2.50)
Control		246.3 (33.4)	36.4 (0.62)	170.7 (22.2)	360.9 (30.2)	114.7 (3.61)

Table shows means and standard deviations for parameters as well as degree of significance of difference from the control animals where value of "t" greater than that for the degrees of freedom noted.

TABLE IV

EFFECT OF DMMPA ON RATS ORALLY DOSED FOR PERIODS UP TO 100 DAYS

HAEMATOLOGICAL PARAMETERS

Dose Applied			Erythrocytes x 10^6 per mm^3		Leucocytes x 10^6 per mm^3		Haematocrit Percent Cell Volume		Haemoglobin gm per 100 ml	
Group	mg per animal	gm per kg								
A	123	570	8.08	0.91	6.91	1.69	42.8	2.68	17.5	1.48
B	62	280	7.92	0.53	7.14	1.49	44.1	1.17	16.1	1.30
C	31	150	8.86	1.46	7.57	1.47	43.7	1.72	16.8	1.41
D	15	80	8.14	0.90	7.48	1.74	43.9	2.70	16.4	1.87
E	7.5	38	8.52	0.66	7.43	2.18	43.0	4.14	16.4	0.79
H	3.8	19	8.06	2.57	7.68	2.78	43.2	2.15	17.2	2.61
J	1.9	9.0	8.71	1.78	7.53	1.49	43.6	2.41	16.6	1.96
K	0.9	5.0	7.71	1.01	6.86	1.88	43.1	3.10	16.3	2.00
Control			8.24	0.76	7.48	2.34	43.1	1.94	16.4	1.66

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components for each dose group and the control are shown in Table V. Again there is no evidence that DMMPA dosing at levels as high as 123 mg/animal/day had any significant effect on any of the above parameters.

Analyses for total protein, albumin, globulin, urea nitrogen and glucose as shown in Table VI likewise indicate that DMMPA dosing at these levels per day for 100 days has no effect.

Analyses of phosphate, chloride and bilirubin contents of the serum as reported in Table VII are also indicative that DMMPA dosing does not alter the serum levels of any of these diagnostic components at doses as high as 123 mg/animal/day.

ORGAN WEIGHT AND WATER CONTENT OF ORGANS

Lung, heart, liver, spleen and kidney were isolated at the time of autopsy and, after blotting, weighed. The mean and standard deviation of each organ weight expressed as a percentage of body weight is shown for each dose group in Table VIII. In Table IX, the percentage weight of water of the wet weight of each of the above tissue plus skeletal muscle and brain are shown. There is no evidence in either of these tables that DMMPA dosing as high as 123 mg/animal/day causes significant change in organ weight or water content of the tissue of rats dosed up to 100 days.

AUTOPSY FINDINGS

DMMPA at doses to 123 mg/animal/day produced very sparse results visible on autopsy. Only at the highest dose were there any recognizeable lesions, these occurring in the lung as mild congestion, sometimes coupled with a small number of haemorrhagic spots. This was found in 5/12 animals receiving 123 mg/animal/day. At the next lowest dose of 62 mg/animal/day, only 2/13 animals had lung lesions. Below these doses no regular dose-related lesions were recorded.

TABLE V

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied			Creatine Phosphatase Int./ml		Glutamic-oxalic Transaminase Int./ml		Lactic Dehydro-genase Int./ml		Alkaline Phosphatase Int./ml		Sodium MEQ/liter		Potassium MEQ/liter	
Group	mg per animal	mg per kg												
A	123	570	66.4	6.0	30.4	2.3	217	78	68.6	11.0	146	22.6	5.5	0.78
B	62	280	62.3	7.9	28.8	4.9	205	26.5	68.8	13.4	143	34.8	5.3	1.52
C	31	150	68.5	9.5	30.7	3.1	224	46	66.2	17.6	146	20.3	5.9	0.71
D	15	80	65.6	6.5	30.3	2.3	211	32	67.9	10.9	150	31.9	6.0	1.67
E	7.5	38	70.5	6.0	30.6	3.4	201	24.2	61.1	14.6	149	50.9	5.0	1.86
H	3.8	19	70.1	8.7	31.4	4.5	235	9.6	70.5	9.2	147	23.1	5.5	1.10
J	1.9	9.0	68.1	12.2	33.2	5.5	265	23.2	64.1	15.5	146	23.1	5.8	0.68
K	0.9	5.0	69.5	15.1	29.4	2.9	263	22.6	61.1	16.5	146	18.9	5.9	0.77
Control			68.7	8.8	29.5	6.3	248	8.1	64.5	18.9	154.8	19.6	5.9	0.89

TABLE VI

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied		Total Protein g/100 ml		Albumen g/100 ml		Globulin g/100 ml		Blood Urea Nitrogen mg/100 ml		Glucose mg/100 ml	
Group	mg per animal	mg per kg									
A	123	570	7.31	0.76	4.20	0.62	3.1	0.75	8.8	0.92	114.6	7.05
B	62	280	7.03	0.51	3.89	0.78	3.17	0.63	8.6	0.92	127.0	16.6
C	31	150	7.12	0.56	4.11	1.16	3.01	1.03	6.9	0.81	126.6	21.1
D	15	80	7.21	0.46	4.33	0.46	2.87	0.38	10.9	0.97	126.5	16.5
E	7.5	38	7.35	0.84	4.46	0.48	2.90	0.76	9.14	0.92	129.1	20.7
H	3.8	19	7.29	0.88	4.28	1.14	3.02	1.24	11.5	0.91	135.0	10.0
J	1.9	9.0	7.06	0.73	4.00	0.83	3.07	0.73	9.33	0.89	134.4	15.8
K	0.9	5.0	7.13	0.77	4.00	1.18	3.14	1.05	10.2	0.96	141.2	7.81
Control			7.17	0.91	4.10	1.07	3.04	0.71	10.0	0.93	132.6	13.5

TABLE VII

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied			Phosphinate mg/100 ml		Chloride MEQ/liter		Bilirubin mg/100 ml	
Group	mg per animal	mg per kg						
A	123	570	5.56	1.45	119.3	9.17	0.110	0.012
B	62	280	5.00	1.26	120.9	6.69	0.118	0.013
C	31	150	6.00	1.61	120.9	7.70	0.11	0.013
D	15	80	5.43	1.31	121.2	6.89	0.11	0.014
E	7.5	38	5.34	1.59	122.9	6.04	0.088	0.009
H	3.8	19	5.78	1.97	120.3	7.81	0.116	0.012
J	1.9	9.0	6.02	1.51	121.3	8.10	0.107	0.011
K	0.9	5.0	5.79	1.88	122.4	6.94	0.134	0.013
Control			5.80	1.70	121.8	7.96	0.123	0.011

TABLE VIII

EFFECTS OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

Dose Applied			Organ Weights Given as Percentage of Body Weight									
Group	mg per animal	mg per kg	Lung		Heart		Liver		Spleen		Kidney	
A	123	570	0.54	0.057	0.35	0.022	4.07	0.45	0.23	0.042	0.43	0.035
B	62	280	0.55	0.070	0.33	0.032	3.64	0.41	0.22	0.040	0.41	0.046
C	31	150	0.62	0.061	0.34	0.035	3.57	0.30	0.20	0.028	0.42	0.029
D	15	80	0.53	0.053	0.33	0.051	3.59	0.20	0.21	0.030	0.41	0.035
E	7.5	38	0.58	0.060	0.34	0.030	3.48	0.20	0.21	0.019	0.41	0.015
H	3.8	19	0.61	0.065	0.33	0.023	3.50	0.24	0.22	0.040	0.42	0.021
J	1.9	9.0	0.58	0.022	0.34	0.030	3.75	0.19	0.22	0.024	0.42	0.018
K	0.9	5.0	0.58	0.010	0.32	0.020	3.62	0.31	0.21	0.037	0.42	0.047
Control			0.50	0.073	0.31	0.020	3.54	0.23	0.20	0.022	0.39	0.025

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TABLE IX

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

WATER CONTENT OF TISSUES

Dose Applied			Lung		Heart		Liver		Spleen		Kidney		Skeletal Muscle		Brain	
Group	mg per animal	mg per kg														
A	123	570	80.9	1.49	77.2	1.05	71.1	1.41	76.4	2.13	76.6	3.32	75.8	0.89	78.5	0.94
B	62	280	81.6	2.13	78.3	1.14	72.6	3.72	76.7	2.30	76.9	1.86	76.1	1.29	78.8	0.89
C	31	150	81.9	4.01	77.7	0.49	71.3	1.44	77.6	1.26	78.8	2.13	76.2	1.0.	78.4	0.65
D	15	80	81.1	1.26	77.8	0.83	71.9	2.38	76.6	1.82	77.4	2.53	76.2	0.89	78.2	0.89
E	7.5	38	80.1	1.67	77.4	0.84	72.7	2.65	77.5	1.39	77.8	2.01	76.2	1.09	78.2	0.90
H	3.8	19	79.5	1.95	77.2	0.83	71.0	1.18	76.7	1.27	76.7	3.58	75.9	0.47	78.7	0.61
J	1.9	9.0	79.5	2.15	77.5	0.66	71.4	1.04	76.2	2.12	77.5	1.71	76.9	0.43	78.3	0.96
K	0.9	5.0	79.9	2.02	77.9	1.29	71.6	1.96	76.2	1.32	77.0	3.09	75.4	1.28	78.4	1.26
Control			80.3	1.59	77.4	0.65	70.7	1.77	76.6	1.34	77.4	3.82	75.9	1.35	78.3	0.83

SECTION C:

EFFECTS OF CHRONIC APPLICATIONS OF DMMPA TO RATS

II. EFFECTS OF HIGH DOSE - 100 DAY APPLICATIONS - MALE RATS

One hundred and fifty male rats were separated into matching weight groups of 15 rats each and allowed to become adjusted to the circular individual metabolism cages for a period of two weeks. At the conclusion of this period, the rats were weighed and distributed into groups such that there was no significant difference in the mean weight of each group. DMMPA was then given orally by stomach tube daily (at 1300 hours) seven days per week for a period of 100 days. The dose pattern used was that shown in Table X. Animals were sacrificed on the 12th, 19th, 26th, 33rd, 40th, 47th, 54th, 61st, 68th, 75th, 83rd, 90th and 97th day such that 1 animal was taken from each dose group and control group at each sacrifice time. Procedures used for handling, sacrifice and analysis of parameters are those described in the Annex of reference (1), but essentially, this experiment paralleled that described in the previous section, except that the dose of DMMPA was raised and the male animals replaced the females.

NUTRITIONAL PARAMETERS

Water intake, food intake and weight gain were measured daily. In Figure 5 the comparison of the two control groups is made for the three parameters indicating that there were times during the 100 days when highly significant and significant level differences arose between the control groups, although the only difference between them was the dose of 1.0 ml of water given to one of the groups. As in the case of the female experiment, there is no apparent explanation for these differences.

The effect of DMMPA dosing on the food intake over the experimental period is shown in Figure 6 and Figure 7. Although there are many occasions where the food intake of the control group 'G' is highly significantly lower than the 'L' group's intake, the failure to demonstrate any evidence

TABLE X

DMMPA DOSES APPLIED DAILY TO MALE RATS

Group	mg per animal	mg per kg
A	489	2520
B	245	1260
C	122	630
E	61.2	315
H	30.5	157
J	15.3	78.6
K	7.6	39.6

G Control given 1.0 ml water

L Control handled as above, but without oral dose

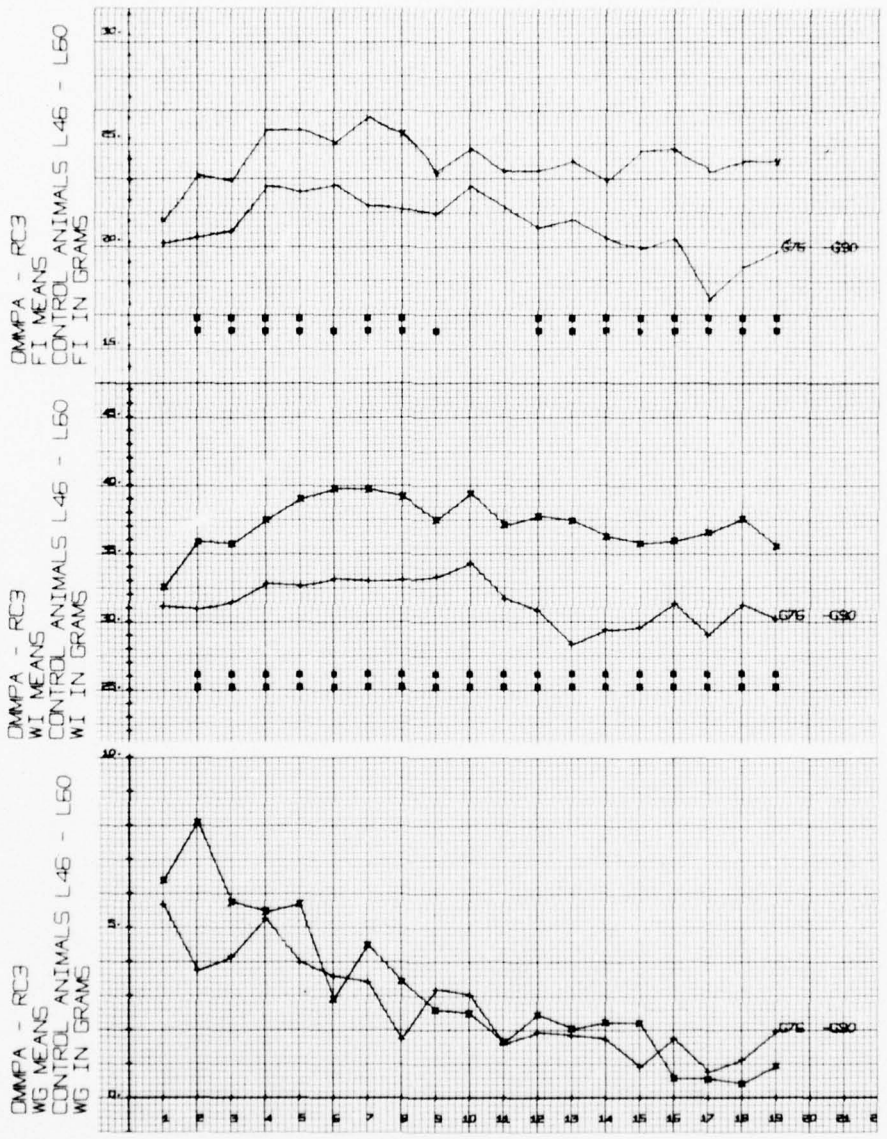

FIGURE 5- COMPARISON OF THE TWO CONTROL GROUPS L AND G FOR FOOD INTAKE. (TOP CURVES), WATER INTAKE (MIDDLE CURVES) AND WEIGHT GAIN (BOTTOM CURVES). A HIGHLY SIGNIFICANT DIFFERENCE IS MARKED WITH TWO BLACK SQUARES; A SIGNIFICANT DIFFERENCE WITH ONE BLACK SQUARE.

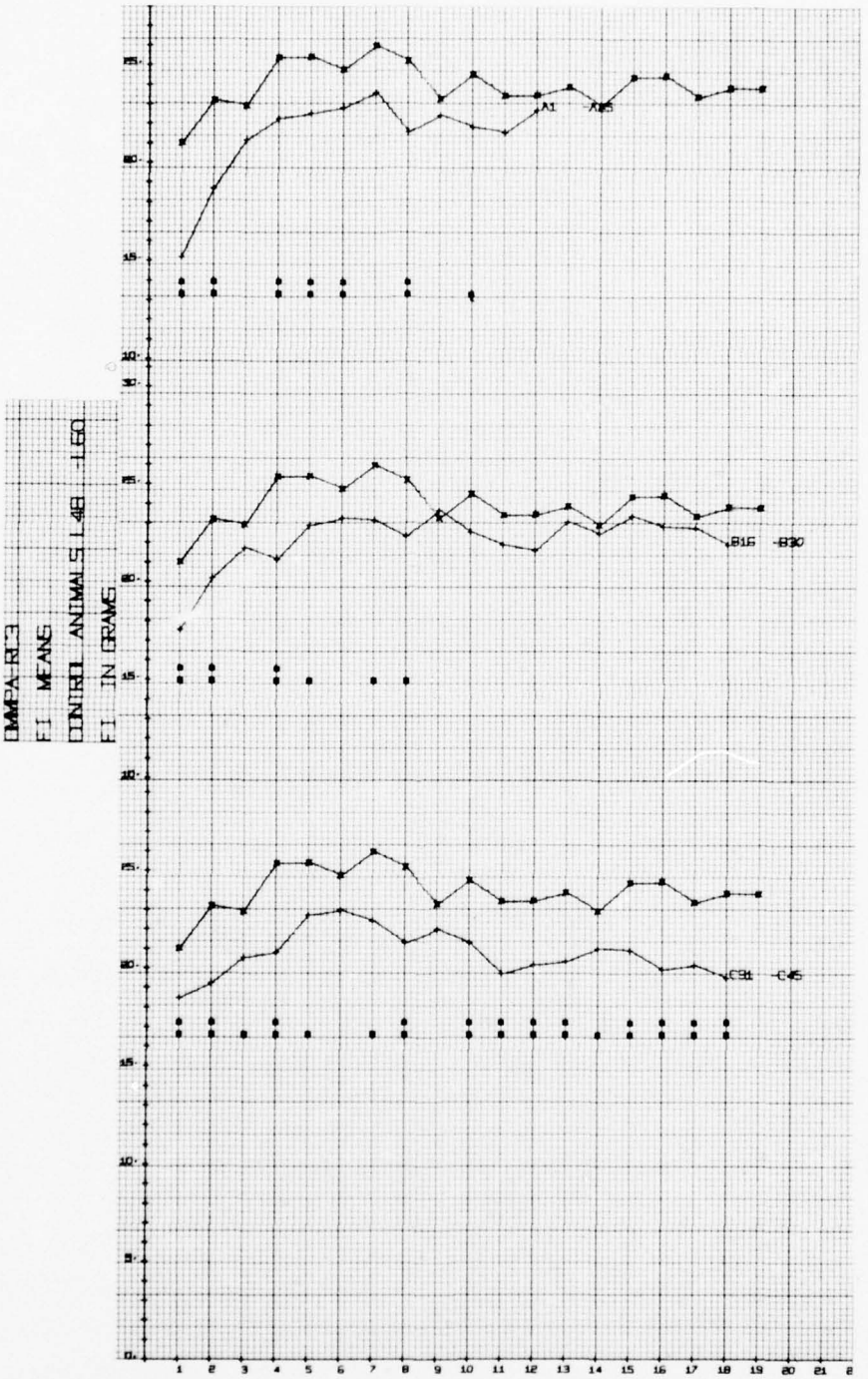

FIGURE 6- COMPARISON OF FOOD INTAKE IN ANIMALS DOSED DAILY WITH DMMPA FOR PERIODS UP TO 100 DAYS. MEAN VALUES OF EACH DOSE GROUP FOR A 5 DAY INTERVAL ARE COMPARED FOR THE ENTIRE EXPERIMENTAL PERIOD . GROUP A RECEIVED 489 MG/ANIMAL/DAY, GROUP B 245 MG/ANIMAL/DAY AND GROUP C 122 MG/ANIMAL/DAY. TWO BLACK SQUARES INDICATES DIFFERENCE OF TEST AND CONTROL IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE INDICATES DIFFERENCE IS SIGNIFICANT.

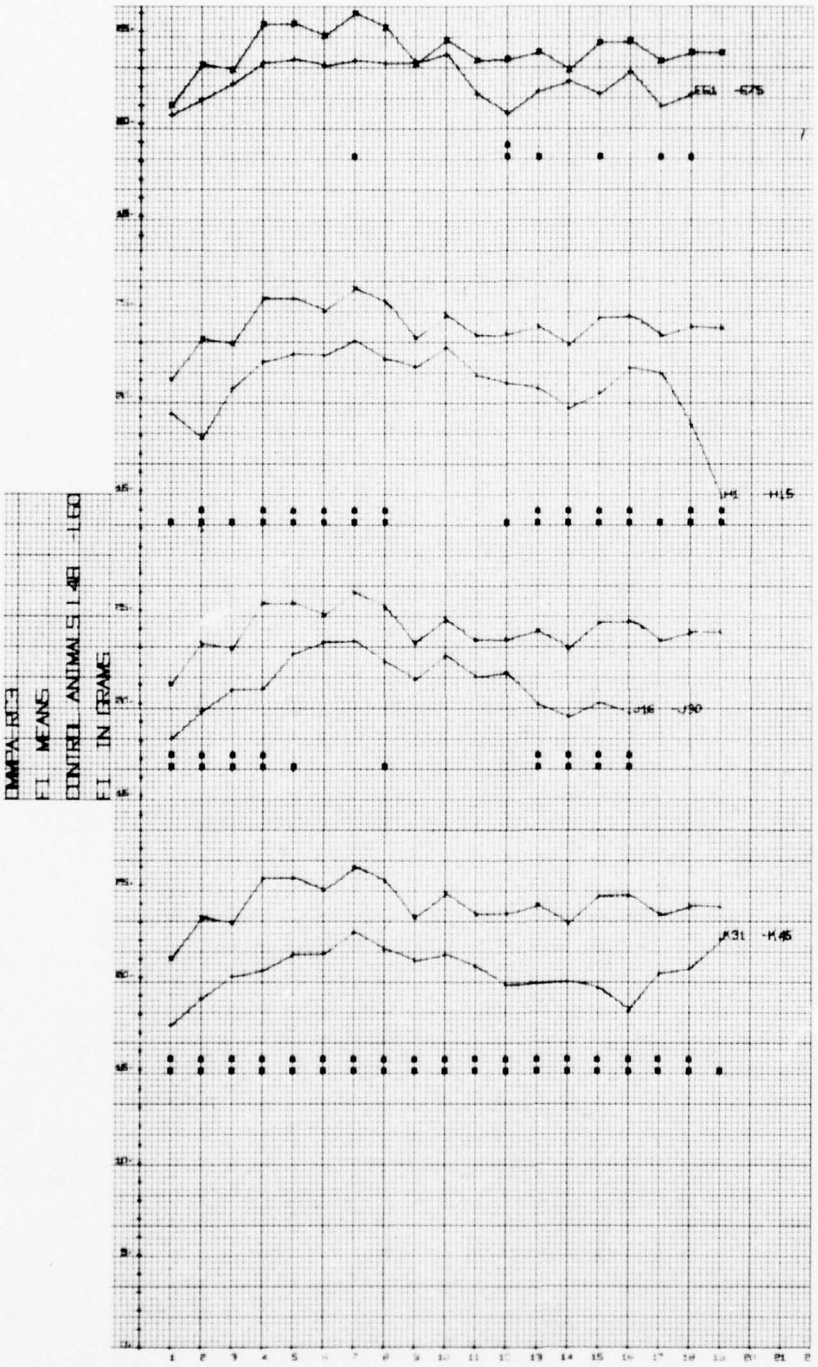

FIGURE 7- COMPARISON OF FOOD INTAKE WITH ANIMALS DOSED WITH DMMPA . GROUP E RECEIVED 61 MG/ANIMAL/DAY, GROUP H 30.5 MG/ANIMAL/DAY, GROUP J 15.3 MG/ANIMAL/DAY AND GROUP K 7.6 MG/ANIMAL/DAY. MEAN VALUES OF FOOD INTAKE FOR THE DOSE GROUP FOR A 5 DAY INTERVAL ARE PLOTTED AGAINST THE CONTROL GROUP FOR THE SAME PERIOD. TWO BLACK SQUARES INDICATES DIFFERENCE IS HIGHLY SIGNIFICANT; ONE SQUARE CONNOTES SIGNIFICANT DIFFERENCE.

of change in food intake in proportion to the dose applied leads to the conclusion that the results are mostly due to the same factors producing the difference in the control groups and not due to DMMPA.

The results of recording water intake with decreasing doses of DMMPA, as shown in Figure 8 and Figure 9, lead to the same conclusion as above, namely, that the differences are due to uncontrolled factors in the experimental design and not due to DMMPA.

The change in weight gain (see Figure 10 and Figure 11) with varying DMMPA dose, as with the controls, shows less tendency for uncontrolled variation with the 'L' control with the results leading to the conclusion that DMMPA dosing even to levels of 2.5 gm/kg does not interfere with the rats' normal ability to gain weight.

Urine collection was also made daily on the animals. Analyses indicated completely negative results for the presence of blood, glucose and bilirubin; normal levels of protein; and no significant change in urine pH with any dose of DMMPA applied.

PHYSIOLOGICAL PRAMETERS

Body weight, rectal temperature, respiration rate, heart rate and blood pressure are shown in Table XI.

Body weight in the DMMPA-treated animals at time of autopsy was compared with that of the controls. The remaining parameters were measured at least five times on each animal during the test period so that each mean and standard deviation shown represent a minimum of 80 determinations at each dose level. However, none of these parameters varied significantly from the control values, leading to the conclusion that DMMPA has no consistent effect on any of these physiological measures of the health of test animals.

BLOOD CELLULAR COMPONENTS

The effects of high daily dose application on haematological parameters are shown in Table XII, giving the mean and standard deviation for each dose group. There is no observable change in erythrocytes, leucocytes, haemoglobin or haematocrit after these doses of DMMPA, indicating that the drug has little effect on the blood cellular composition. Although only

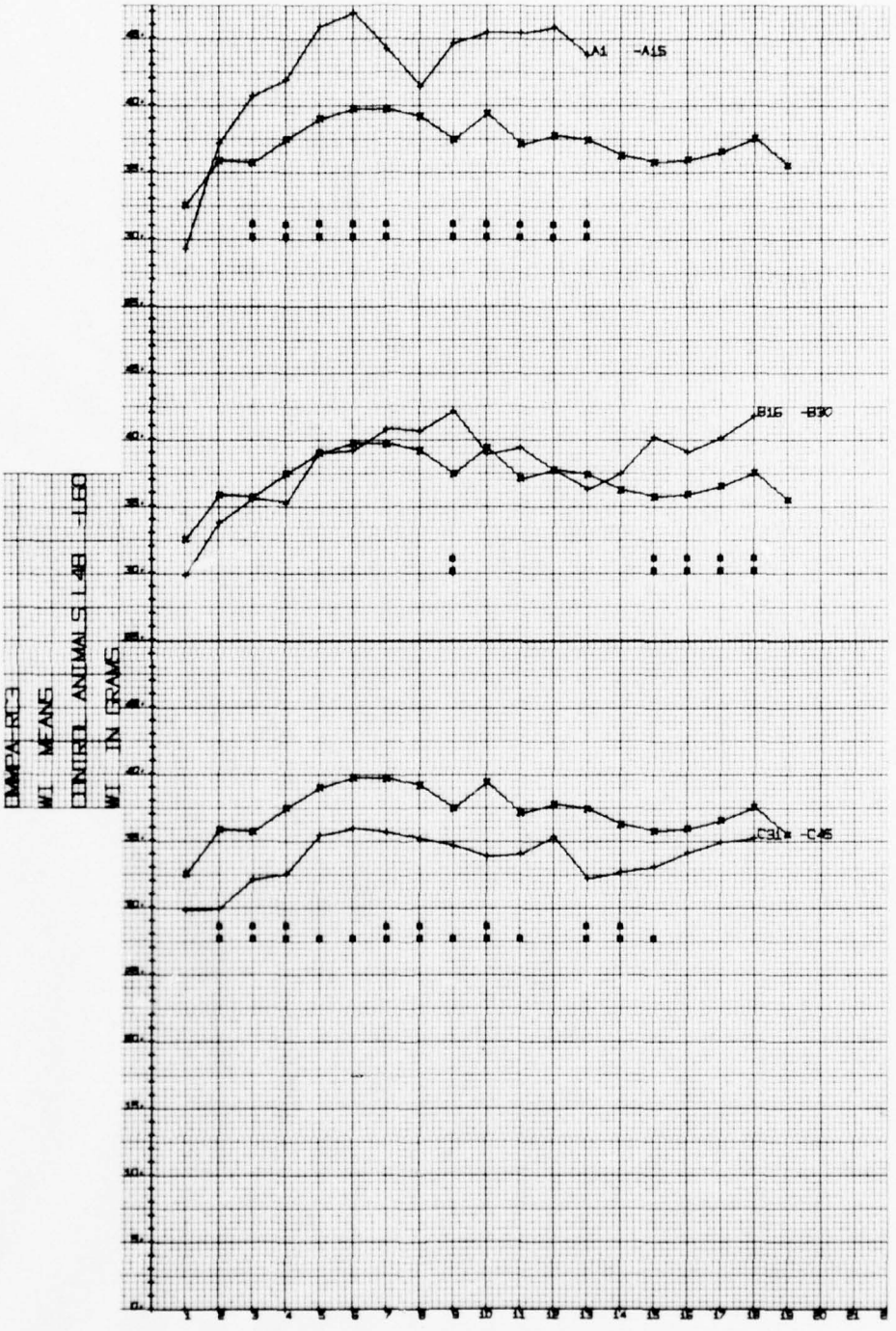

FIGURE 8- COMPARISON OF WATER INTAKE WITH ANIMALS DOSED DAILY WITH DMMPA. MEAN VALUES OF EACH DOSE GROUP FOR A 5 DAY PERIOD ARE PLOTTED AGAINST CONTROLS FOR THE SAME TIME. GROUP A RECEIVED 489 MG/ANIMAL/DAY, GROUP B 245 MG/ANIMAL/DAY AND GROUP C 122 MG/ANIMAL /DAY.

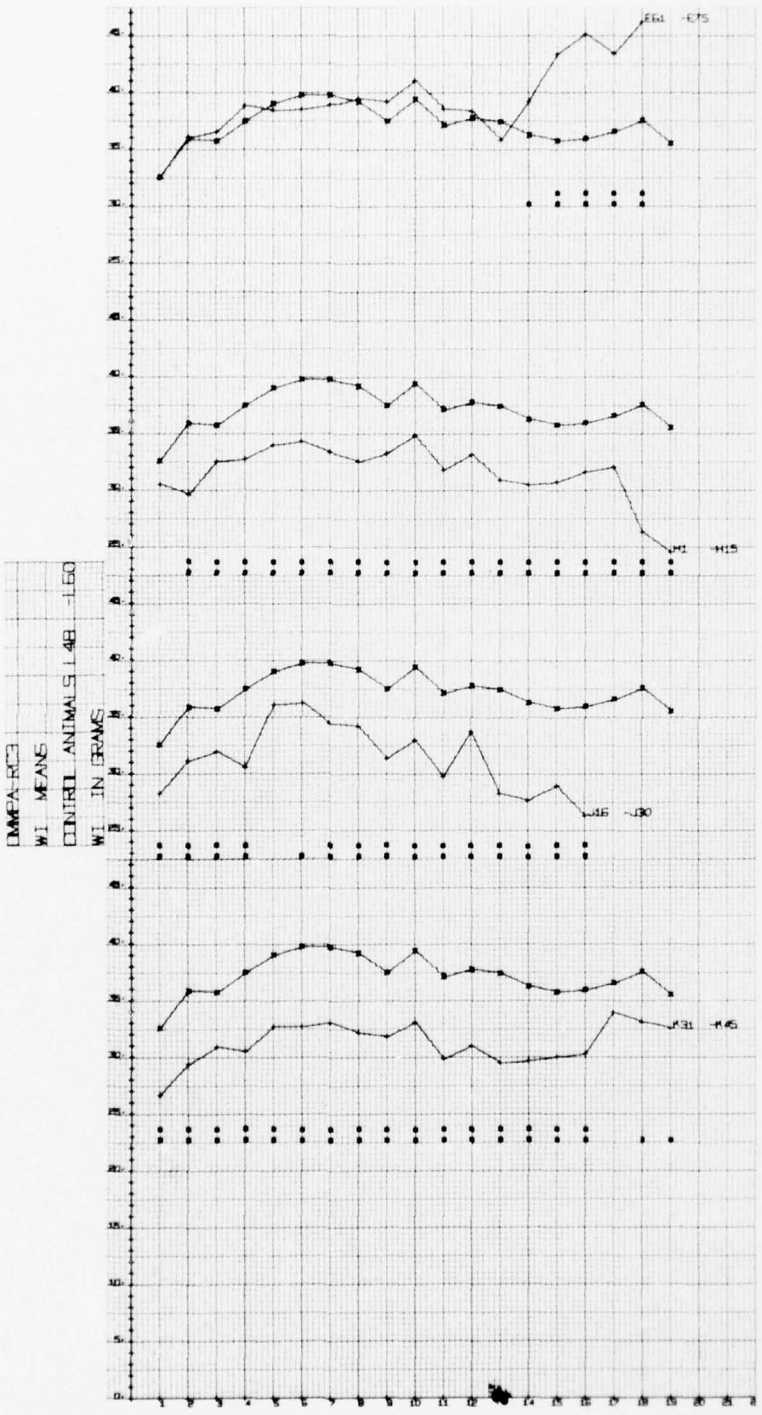

FIGURE 9- COMPARISON OF WATER INTAKE WITH ANIMALS DOSED DAILY WITH DMMPA. MEAN VALUES FOR EACH DOSE GROUP FOR THE 5 DAY PERIOD ARE PLOTTED AGAINST CONTROL VALUES FOR THE SAME PERIOD. GROUP E RECEIVED 61 MG/ANIMAL/DAY, GROUP H 30.5 MG/ANIMAL/DAY, GROUP J 15.3 MG/ANIMAL/DAY AND GROUP K 7.6 MG/ANIMAL/DAY. TWO BLACK SQUARES INDICATES THE DIFFERENCE BETWEEN MEAN VALUE OF TEST AND THAT OF CONTROL IS HIGHLY SIGNIFICANT; ONE SQUARE CONNOTES SIGNIFICANT LEVEL OF DIFFERENCE.

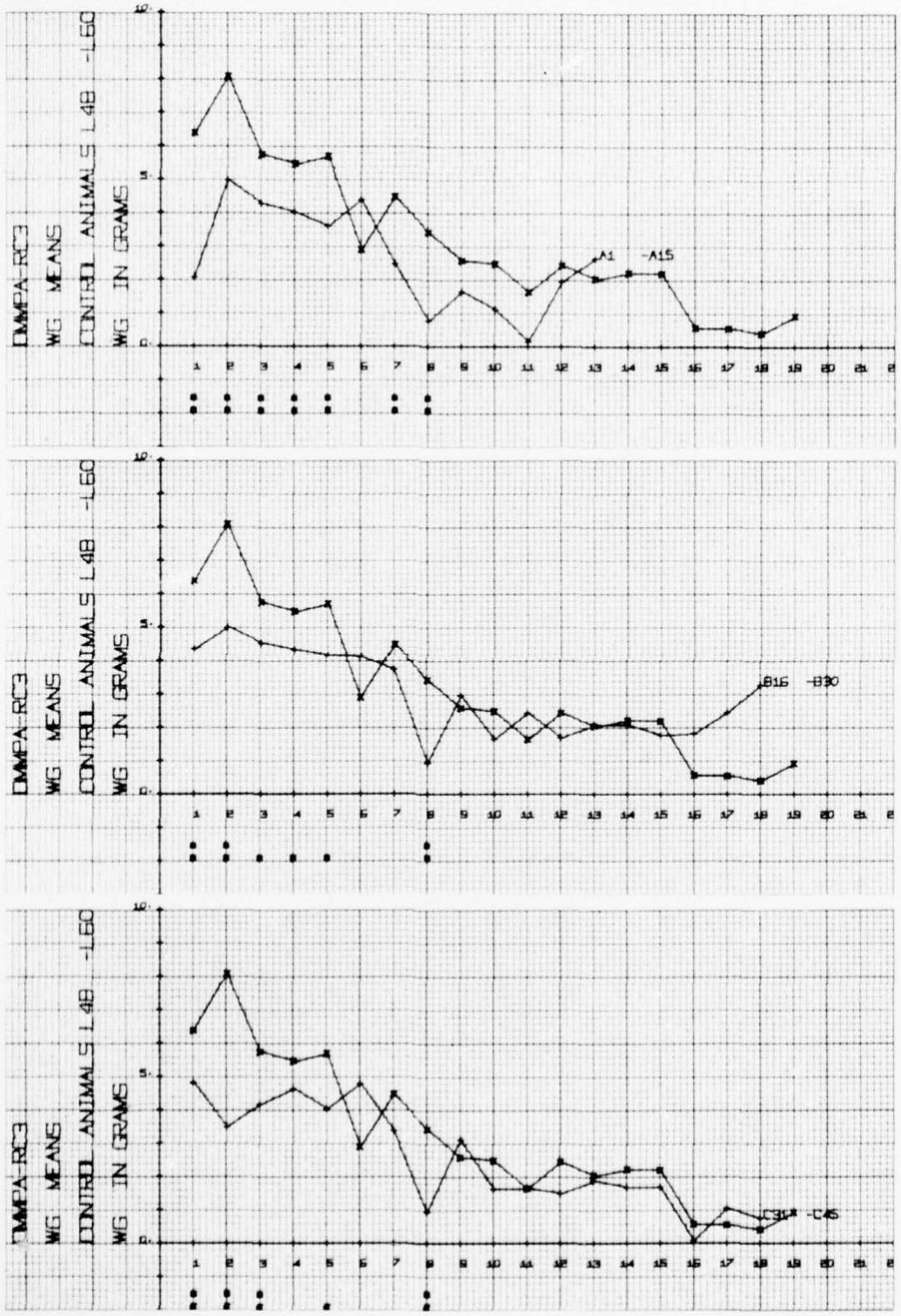

FIGURE 10- COMPARISON OF THE WEIGHT GAINED IN ANIMALS DOSED DAILY WITH DMPA. MEAN VALUES FOR EACH DOSE GROUP FOR THE 5 DAY PERIOD ARE PLOTTED AGAINST CONTROL VALUES FOR THE SAME PERIOD. GROUP A RECEIVED 489 MG/ANIMAL/DAY, GROUP B 245 MG/ANIMAL/DAY AND GROUP C 122 MG/ANIMAL/DAY. TWO BLACK SQUARES INDICATES DIFFERENCE OF MEAN IS HIGHLY SIGNIFICANT; ONE BLACK SQUARE CONNOTES SIGNIFICANT LEVELS OF DIFFERENCE.

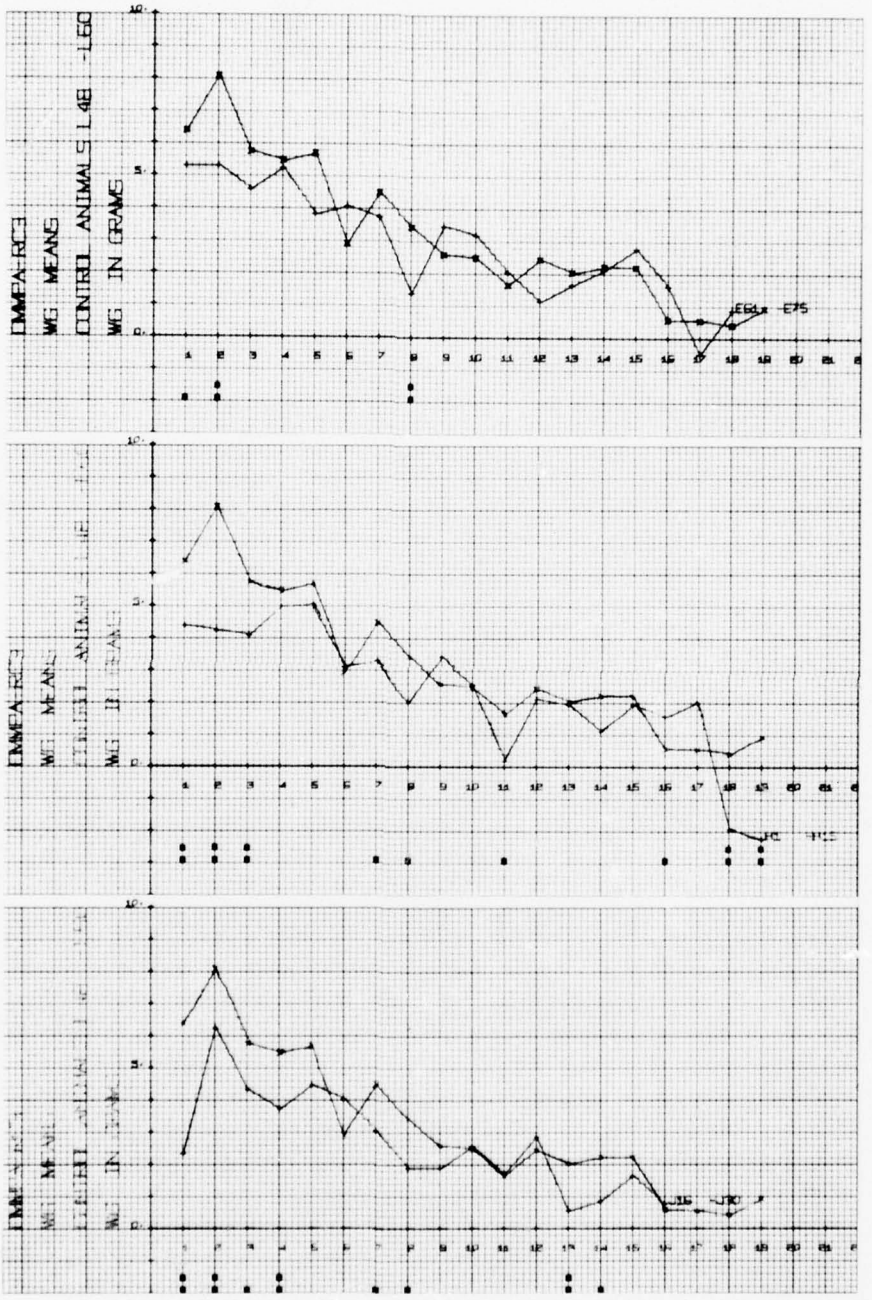

FIGURE 11- COMPARISON OF WEIGHT GAIN IN ANIMALS DOSED DAILY WITH DMMPA. MEAN VALUES FOR EACH DOSE GROUP FOR THE 5 DAY PERIOD ARE PLOTTED AGAINST CONTROL VALUES FOR THE SAME PERIOD. GROUP E RECEIVED 61 MG/ANIMAL/DAY, GROUP H 30.5 MG/ANIMAL/DAY, GROUP J 15.3 MG/ANIMAL/DAY AND GROUP K 7.6 MG/ANIMAL/DAY. TWO BLACK SQUARES CONNOTE HIGHLY SIGNIFICANT DIFFERENCE IN THE MEANS; ONE BLACK SQUARE INDICATES DIFFERENCE BETWEEN MEAN AND CONTROL IS AT SIGNIFICANT LEVEL.

TABLE XI

EFFECTS OF DMMPA AT HIGH DOSAGE ON RATS FED DAILY UP TO 100 DAYS

EFFECTS ON PHYSIOLOGICAL PARAMETERS

Dose Applied			Body Weight gm		Rectal Temperature °C		Respiration Breaths per minute		Heart Rate Beats per minute		Blood Pressure mm Hg	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2520	393.0	90.8	35.9	0.55	202.2	13.0	355	25.3	112.7	8.07
B	245	1260	342.0	94.6	35.8	0.41	211.7	11.5	358	29.9	117.3	6.62
C	122	630	339.2	97.3	35.9	0.44	208.9	11.2	362	27.2	117.4	5.39
E	61	315	362.0	92.0	35.9	0.50	213.5	7.07	357	29.7	116.7	5.01
H	30.5	157	356.0	75.9	35.8	0.52	210.5	7.59	352	24.0	116.8	6.45
J	14.3	78.6	345.0	74.4	35.1	5.58	208.0	10.4	352	27.5	115.5	5.38
K	7.6	39.6	355.0	76.7	35.8	0.59	209.0	7.14	353	22.8	117.4	5.05
Control			349.3	88.1	35.9	0.54	209.0	10.0	354	29.8	115.7	7.43

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TABLE XII

EFFECTS OF DMMPA AT HIGH DOSAGE ON MALE RATS FED DAILY UP TO 100 DAYS

EFFECT ON HAEMATOLOGICAL PARAMETERS

Dose Applied			Erythrocytes Cells x 10^6 per mm^3		Leucocytes Cells x 10^3 per mm^3		Haemoglobin gm/100 ml		Haematocrit % Cell Volume	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2520	8.3	1.4	10.3	3.7	16.7	2.5	42.8	1.5
B	245	1260	8.2	1.06	8.5	2.0	15.2	2.1	44.6	1.7
C	122	630	8.3	1.6	9.3	2.4	15.4	1.7	44.0	1.8
E	61	315	8.7	1.4	8.9	2.8	15.8	1.9	45.5	1.5
H	30.5	157	8.7	0.86	10.6	2.1	16.3	2.3	45.6	1.8
J	15.3	78.6	8.7	0.97	8.7	2.5	15.6	1.8	45.0	1.8
K	7.6	39.3	8.8	1.2	8.8	3.1	16.1	1.9	45.4	3.2
Control			8.6	1.1	8.2	2.6	15.6	1.9	45.2	2.4

total leucocytes were counted, blood smears were made for animals from each dose group and examined for the presence of abnormal cells (Wright's stain). There was no evidence of abnormal numbers of any of the leucocyte cell groups.

BLOOD SERUM COMPONENTS

The effects of high dosage of DMMPA on the blood serum components of rats dosed daily for periods up to 100 days are shown in Tables XIII, XIV and XV as the means and standard deviations of each group and are compared with the control animals. All values of the means were tested for significance by the "t-test" and were found to be insignificantly different from the controls. There is a tendency for the lactic dehydrogenase and alkaline phosphatase values of Group A to be high, likely reflecting the degree of liver congestion evident in these animals at autopsy, although even these values were not found significant. It is worthy of note that other indicators of liver damage, namely, creatine phosphokinase and SGOT were both within normal range, indicating DMMPA does not produce liver damage. Normal range values of sodium, potassium and chloride indicate DMMPA does not interfere with electrolyte balance. Blood urea nitrogen values were within normal ranges and likewise indicate that kidney damage is not produced at the levels of DMMPA used. Blood glucose values are high, but are consistently so with the controls, and although the range found in both DMMPA-treated groups is wide, it is likewise wide in the control groups. Since animals were not fasting for periods longer than five hours before sacrifice, values are as close as can be expected. The constancy of blood bilirubin values indicates DMMPA has little effect on the hepato-biliary tract.

ORGAN WEIGHTS AND WATER CONTENT OF TISSUES

Lung, heart, liver, kidney and spleen were removed from each animal at autopsy and immediately weighed. The values of the mean weight of each organ at each dose level are given in Table XVI and compared with the weights of organs taken from the control groups. The significance of the difference between mean values of dosed animals and those of controls was tested by the "t-test". Highly significant differences are shown as H.S. ($P \leq 0.01$), and

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TABLE XIII

EFFECTS OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied			Creatine Phosphokinase Int.		Glutamic-oxalic Transaminase Int.		Lactic Dehydrogenase Int.		Alkaline Phosphatase Int.		Sodium - milli-equivalents per liter	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	454	2520	79.4	24.1	23.3	9.6	435	121.8	89.1	38.9	158.7	12.2
B	245	1260	75.4	23.3	20.4	6.8	350	167	84.3	52.6	154.5	12.1
C	122	630	76.2	21.2	21.9	7.3	381	157	68.2	26.2	151.7	11.0
E	61	315	71.5	21.6	22.2	7.9	367	140.5	72.2	31.8	147.2	10.1
H	30.5	157	75.6	28.9	23.6	5.9	374	154	61.6	19.0	144.7	10.1
J	15.3	78.6	73.8	27.0	23.9	8.1	310	60.1	64.7	17.3	144.7	11.2
K	7.6	39.6	70.4	27.0	22.2	7.8	321	79.9	63.2	18.2	144.3	13.2
Control			75.9	29.0	21.9	6.8	348	98.4	59.5	13.6	145.3	10.6

TABLE XIV

EFFECTS OF DMMPA ON RATS DOSED DAILY FOR PERIODS UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied			Potassium MEQ/liter		Total Protein gm/100 ml		Albumen gm/100 ml		Globulin gm/100 ml		Phosphate mg/100 ml	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	459	2520	8.7	1.01	7.34	0.67	4.8	1.04	2.5	0.63	6.5	1.5
B	245	1260	6.4	0.57	7.27	0.92	4.21	0.96	3.07	0.41	3.89	1.3
C	122	630	6.4	0.63	7.12	0.83	4.77	1.12	2.35	0.58	5.74	1.01
E	61	315	6.1	0.61	7.22	0.61	4.48	1.04	2.74	0.84	5.66	0.76
H	30.5	157	5.9	0.44	7.43	0.69	4.80	0.59	2.63	0.73	5.74	1.13
J	15.3	78.6	5.9	0.47	7.59	0.86	5.2	1.51	2.40	0.88	5.75	1.05
K	7.6	39.6	5.6	1.16	7.2	0.85	4.9	1.0	2.33	0.54	5.5	1.2
Contol			5.9	0.48	8.08	2.65	4.60	0.74	3.48	2.6	5.84	0.99

TABLE XV

EFFECTS OF DMMPA AT HIGH DOSAGE ON RATS FED DAILY UP TO 100 DAYS

BLOOD SERUM COMPONENTS

Dose Applied			Chloride MEQ/liter		Blood Urea Nitrogen mg/100 ml		Glucose mg/100 ml		Bilirubin mg/100 ml	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	459	2520	121.7	6.60	10.5	1.73	132.7	19.2	0.13	0.08
B	245	1260	122.2	5.6	10.2	1.92	123.4	21.7	0.126	0.05
C	122	630	122.1	6.5	9.72	2.02	124.0	30.0	0.13	0.04
E	61	315	121.9	5.9	9.63	1.60	129.6	19.4	0.12	0.05
H	30.5	157	120.4	6.2	10.4	2.12	123.5	23.4	0.124	0.064
J	15.3	78.6	121.1	6.9	10.2	1.75	128.7	18.6	0.11	0.06
K	7.6	39.6	121.1	5.7	9.9	2.8	119.7	24.1	0.13	0.06
Control			121.2	5.58	10.1	1.78	133.2	13.1	0.123	0.063

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TABLE XVI

EFFECTS OF DMMPA FED TO RATS AT HIGH DOSES FOR PERIODS UP TO 100 DAYS

EFFECTS ON ORGAN WEIGHTS

Dose Applied			All tissues expressed as percentage of body weight									
Group	mg per animal	mg per kg	Lung		Heart		Liver		Spleen		Kidney	
			M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2520	0.595 H.S.	0.17	0.340 H.S.	0.06	6.57 H.S.	1.12	0.212	0.016	0.562 H.S.	0.07
B	245	1260	0.484 H.S.	0.05	0.312	0.016	4.75 H.S.	0.84	0.212	0.02	0.492 Sig	0.04
C	122	630	0.499 H.S.	0.002	0.305	0.009	4.67	1.87	0.206	0.06	0.472	0.11
E	61	315	0.445	0.07	0.298	0.04	3.92	0.69	0.192	0.04	0.419	0.06
H	30.5	157	0.437	0.06	0.302	0.02	3.96	0.46	0.196	0.03	0.428	0.03
J	15.3	78.6	0.459	0.05	0.302	0.02	4.03	0.46	0.193	0.02	0.438	0.04
K	7.6	39.6	0.483	0.06	0.318	0.02	3.89	0.51	0.20	0.04	0.423	0.04
Control			0.448	0.035	0.295	0.018	4.12	0.63	0.204	0.03	0.445	0.05

significant values ($P \leq 0.05$) as Sig. The highest dose of DMMPA used (2.5 gm/kg) caused highly significant weight increases of lung, heart, liver and kidney. A dose of 1.3 gm/kg DMMPA caused significant changes in lung, liver and kidney. The lowest dose causing significant change was 0.6 gm/kg which only caused a highly significant increase in the weight of the lung. Dose levels below this quantity caused no change in organ weights of the male rats, essentially agreeing with the findings in females.

The water content values of tissues removed from the animals at autopsy are shown in Table XVII. As can be seen, the increase in tissue weight observed with DMMPA doses 2.5, 1.3 and 0.6 gm/kg is not paralleled with increased water content in those tissues. Only the dose of 2.5 gm/kg caused a highly significant increase in water content and this effect was observed only in the lung. This suggests that the increase in weight can be attributed to the severe blood congestion observed in these tissues at autopsy and verified by histological examination.

AUTOPSY FINDINGS

With the exception of five animals in Group A which died spontaneously, all animals were sacrificed by decapitation. Group A animals received 489 mg/animal/day or 2.5 gm/kg/day. This dose caused 5 deaths by cumulative DMMPA dosing occurring after the 5th, 11th, 18th, 41st and 50th doses. These animals and the remaining 10 animals in the dose group showed extensive lung congestion and haemorrhage, with blood in the alveolar spaces and pleural cavity. As well, there were signs of extensive kidney damage occurring in the glomeruli and proximal tubules. The liver was also congested with blood. There were also signs of damage to the gut wall in which most of the mucosal layer of the ileum was eroded, leaving a flaccid and translucent wall. In this latter instance, however, 1/9 animals showed such effects among the survivors, although all which had died spontaneously had gut damage.

These findings were repeated in Group B (245 mg/animal/day or 1.3 gm/kg/day) although the degree of severity was decreased such that most animals showed damage only in lung and kidney. Animals in Group C (122 mg/animal/day or 0.6 gm/kg/day) showed only lung damage and one instance (1/14) of observable kidney damage. Doses of DMMPA below that of Group C were free from all but minor evidence of lung congestion with blood. From the autopsy finding, there

TABLE XVII

EFFECTS OF DMMPA FED TO RATS AT HIGH DOSE FOR PERIODS UP TO 100 DAYS

EFFECTS ON WATER CONTENT OF ORGANS EXPRESSED AS PERCENTAGE WET WEIGHT

Dose Applied			Lung		Heart		Liver		Spleen		Kidney		Skeletal Muscle		Brain	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2520	82.0 H.S.	1.5	78.5	1.2	72.1	2.5	77.1	1.6	76.1	2.1	77.0	1.0	78.9	0.52
B	245	1260	80.7	1.6	77.9	1.3	69.5	0.9	76.8	2.3	75.9	2.8	75.4	1.8	78.7	0.70
C	122	630	80.4	1.7	77.8	0.8	70.4	1.5	77.3	1.4	76.0	3.3	77.1	0.8	78.8	0.77
E	61	315	79.6	3.1	77.8	0.8	70.4	1.5	76.5	3.4	75.0	3.6	74.9	2.5	78.7	0.80
H	30.5	157	80.2	1.4	77.7	0.9	70.1	0.9	76.7	2.0	75.7	3.5	75.7	1.4	79.0	0.93
J	15.3	78.6	80.0	2.2	77.9	1.0	70.6	2.2	77.4	2.3	75.1	3.4	75.9	1.1	79.3	0.47
K	7.6	39.6	80.4	1.7	77.7	0.6	70.8	2.6	77.5	1.4	75.9	2.2	76.1	2.0	78.9	0.64
Control			79.9	1.5	77.6	0.87	70.3	2.1	76.2	2.5	75.8	2.5	74.3	3.0	78.8	0.83

there is a clear break in the cumulative effects of DMMPA such that doses below 122 mg/animal/day or 0.6 gm/kg/day are essentially innocuous.

SECTION C:

EFFECTS OF CHRONIC APPLICATION OF DMMPA TO RATS

III. EFFECTS OF MEDIUM DOSE - 240 DAY APPLICATION - FEMALE RATS

Attention has been previously drawn to the fact that the technique of sacrificing the animals sequentially during the 100 day dosing period used in the previous two trials resulted in only 5 animals of each dose actually receiving the total of 100 doses. The negative results of the previous two trials in most parameters studied lead to the hypothesis that this number of animals was insufficient to influence the results in the statistical methods of comparison used. Accordingly in this experiment, all animals were dosed daily, seven days per week for a period of 140 days before any sacrifice of the animals to detect change was initiated. Sacrifice occurred as before, with one animal taken from each dose and control group per week. Doses of DMMPA used were chosen purposely to bracket the level of 122 mg/kg/animal, the limit dose causing recognizable pathological signs. As well, confirmation of the cumulative deaths from a dose of 489 mg/animal /day (2.5 gm/kg/day) was sought. The doses used with all groups are shown in Table XVIII.

The female rats used were carefully selected and allowed 2 weeks to adjust to the conditions of the single metabolism cages used for each animal. At the conclusion of this period the animals were distributed in 10 groups of 15 animals, each group having the same mean weight (194.3 gm) and weight distribution. Animals were dosed with DMMPA at 1300 hours daily after the animals had been weighed and food intake, water intake and urine output recorded.

NUTRITIONAL PARAMETERS

Two control groups ('L' and 'G') were used as before. In Figure 12 the food intake, water intake and weight gain in these two untreated groups are compared. These data indicate that there is no evident difference in food intake or weight gain between the two controls over the entire

TABLE XVIII

DMMPA DOSES GIVEN DAILY TO FEMALE RATS
FOR A PERIOD FROM 140 TO 240 DAYS

Group	DMMPA mg per animal	DMMPA mg per kg
A	489	2518
B	367	1888
C	245	1246
D	184	944
E	122	623
H	92	487
J	50	252
K	37	189

G Control - 1.0 ml of water by stomach tube.

L Control - handled with insertion of stomach tube but not dosed.

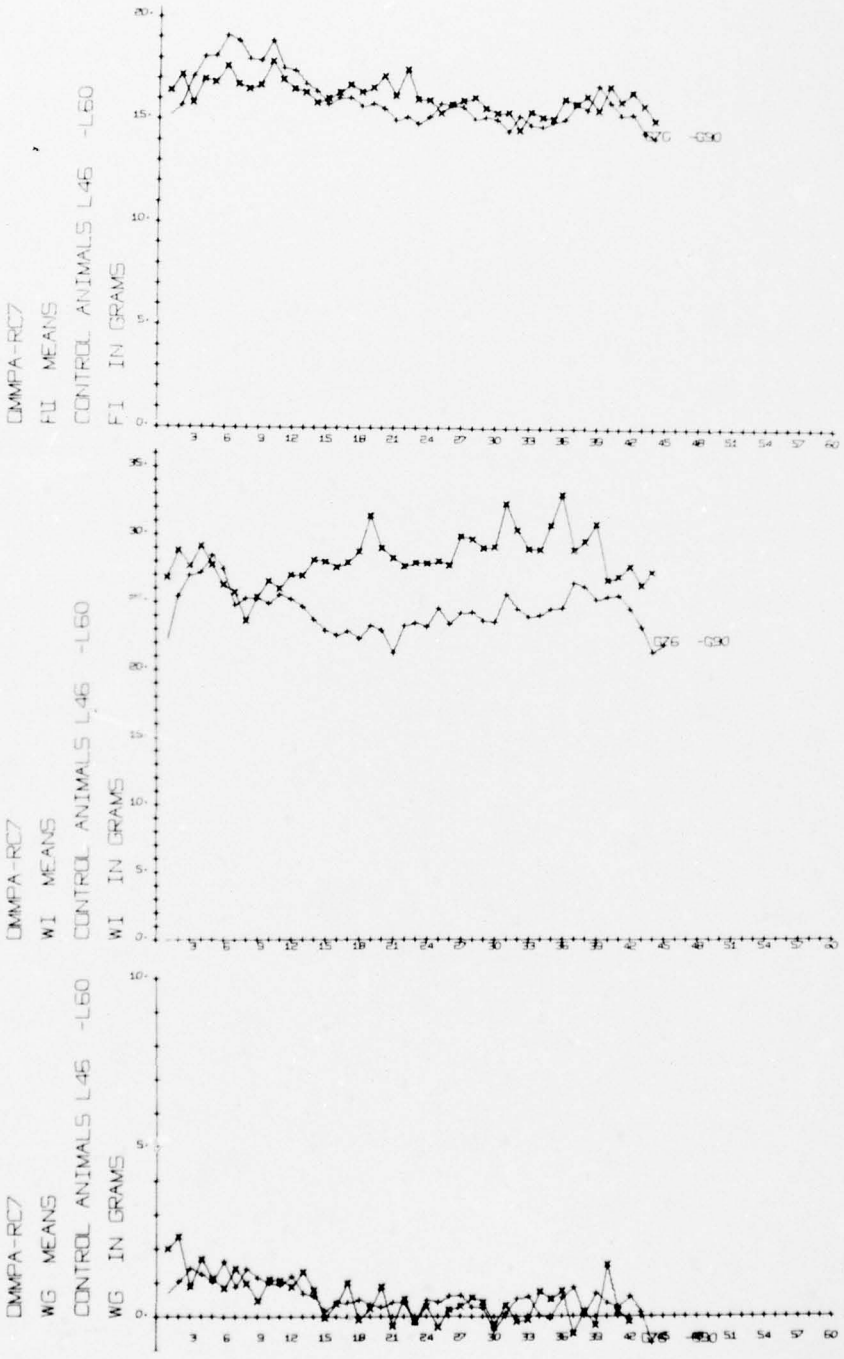

FIGURE 12- FOOD INTAKE, WATER INTAKE AND WEIGHT GAIN FOR THE TWO CONTROL GROUPS L AND G ARE COMPARED. THE MEAN WEIGHT FOR EACH ANIMAL FOR A FIVE DAY PERIOD IS COMPARED FOR THE ENTIRE 240 DAYS OF THE EXPERIMENT IN UNIT PERIODS OF 5 DAYS DURATION EACH. ONLY THE DIFFERENCE IN WATER INTAKE IS SIGNIFICANT.

experimental period. Water intake is, however, highly significantly different. There is no apparent reason why this difference should exist, since apparently all factors but the single one of a dose of 1.0 ml of water are the same for both groups.

The food intake in animals treated with DMMPA is shown in Figures 13, 14 and 15. Regardless of the dose of DMMPA given (from 489 to 37 mg/animal) there is no apparent change in food intake by tne rats. Water intake, as shown in Figures 16, 17 and 18 indicates that there is a significant to highly significant increase in water intake in the animals in all groups having daily doses from 489 to 122 mg/animal but no change in water intake for the lower doses. Because of the disagreement in the control it is difficult to decide which of the doses is the null effect dose but the choice of the lower can only be in favour of greater safety. Hence it was concluded that a dose of 122 mg/animal/day for periods of 140 to 240 days has a demonstrable adverse effect on rats such that their tendency to higher water intakes is, in some way, related to a pathological process. Weight gain comparison between the treated and untreated controls is shown in Figures 19, 20 and 21. These data indicate that weight gain is unaffected in rats dosed as high as 489 mg/animal/day.

Effect of DMMPA on Physiological Parameters

Table XIX records comparison by dose groups of weight at autopsy, rectal temperature, respiration rate, heart rate and blood pressure with the same parameters in control animals. As can be seen, there are no significant differences in any parameter but heart rate. With the latter there is a highly significant depression of heart rate at doses of 245 mg/animal/day and above. At the high dose of DMMPA (489 mg/animal/day) the depression of heart rate is seen as early as the 20th dose, with all animals responding by the 50th dose. At 367 mg/animal/day, earliest signs of heart rate decrease are seen after 50 doses, but by 100 doses all animals are showing such depression. With the lowest dose causing significant depression (245 mg/animal/day) the number of daily doses exceeded 125 before significant depression was observed

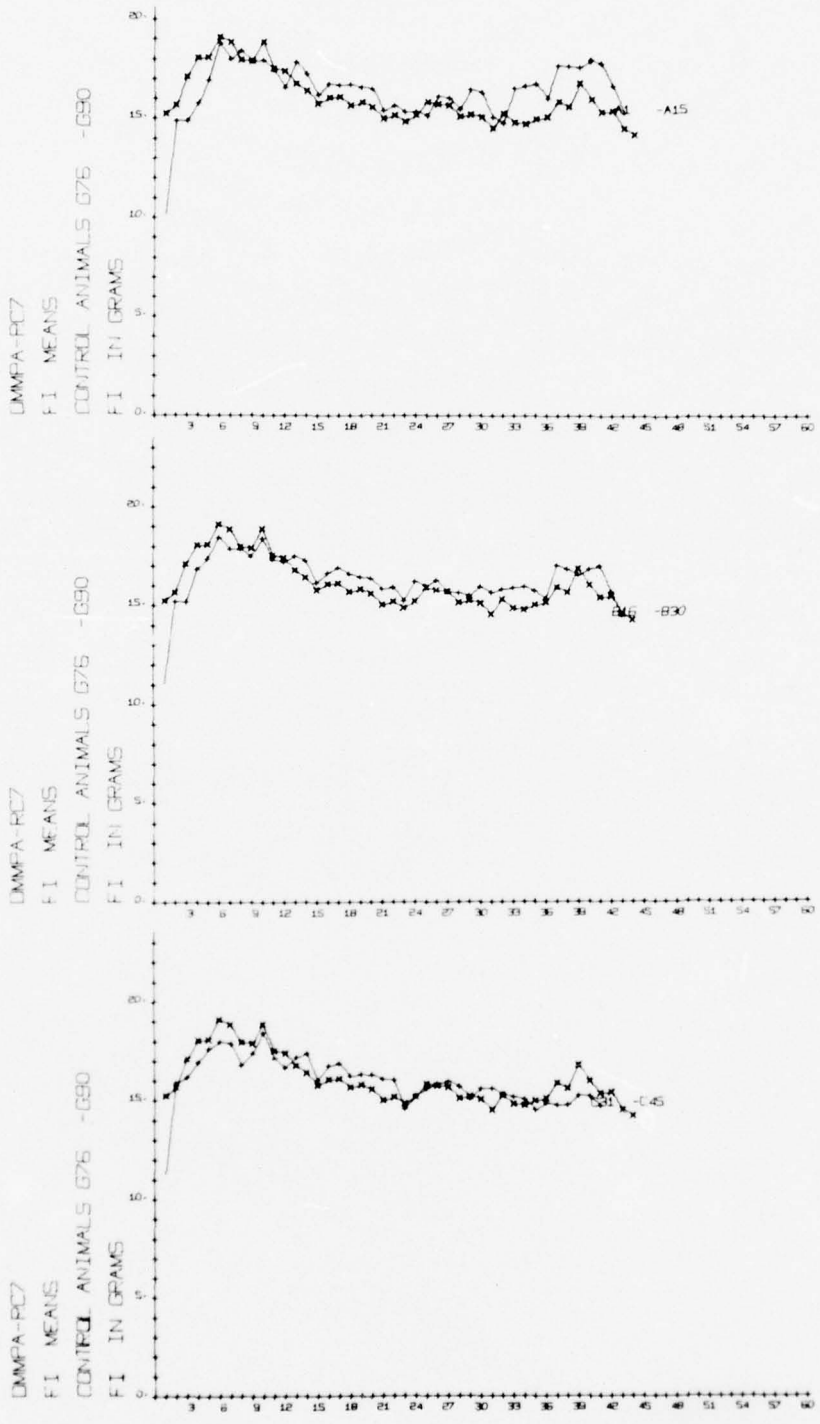

FIGURE 13- FOOD INTAKE AS MEAN WEIGHT IN GRAMS FOOD PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH THE CONTROL GROUP FOR EACH INTERVAL FOR THE ENTIRE 240 DAYS. GROUP A RECEIVED 489 MG/ANIMAL/DAY, GROUP B 367 MG/ANIMAL/DAY AND GROUP C 245 MG/ANIMAL/DAY. NONE OF THE DIFFERENCES IN THE CURVES ARE SIGNIFICANT.

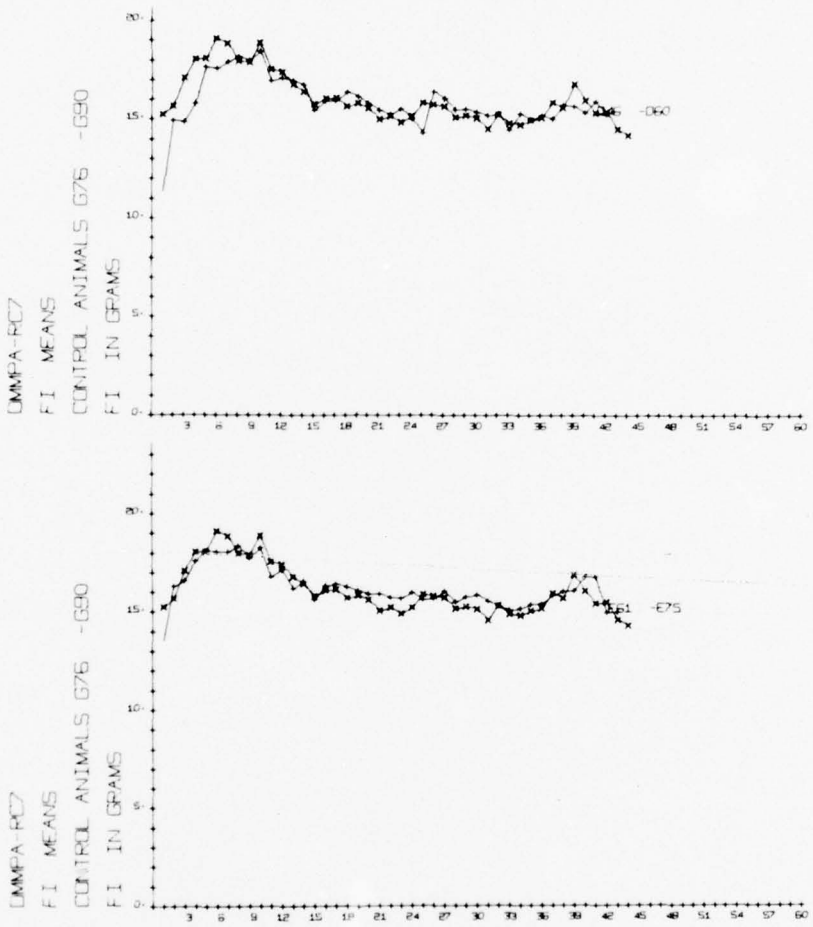

FIGURE 14- FOOD INTAKE AS MEAN WEIGHT IN GRAMS FOOD PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH THE CONTROL GROUP FOR EACH 5 DAY INTERVAL FOR 240 DAYS. GROUP D RECEIVED 184 MG/ANIMAL/DAY AND GROUP E 122 MG/ANIMAL/DAY. NONE OF THE DIFFERENCES BETWEEN THE CURVES ARE SIGNIFICANT.

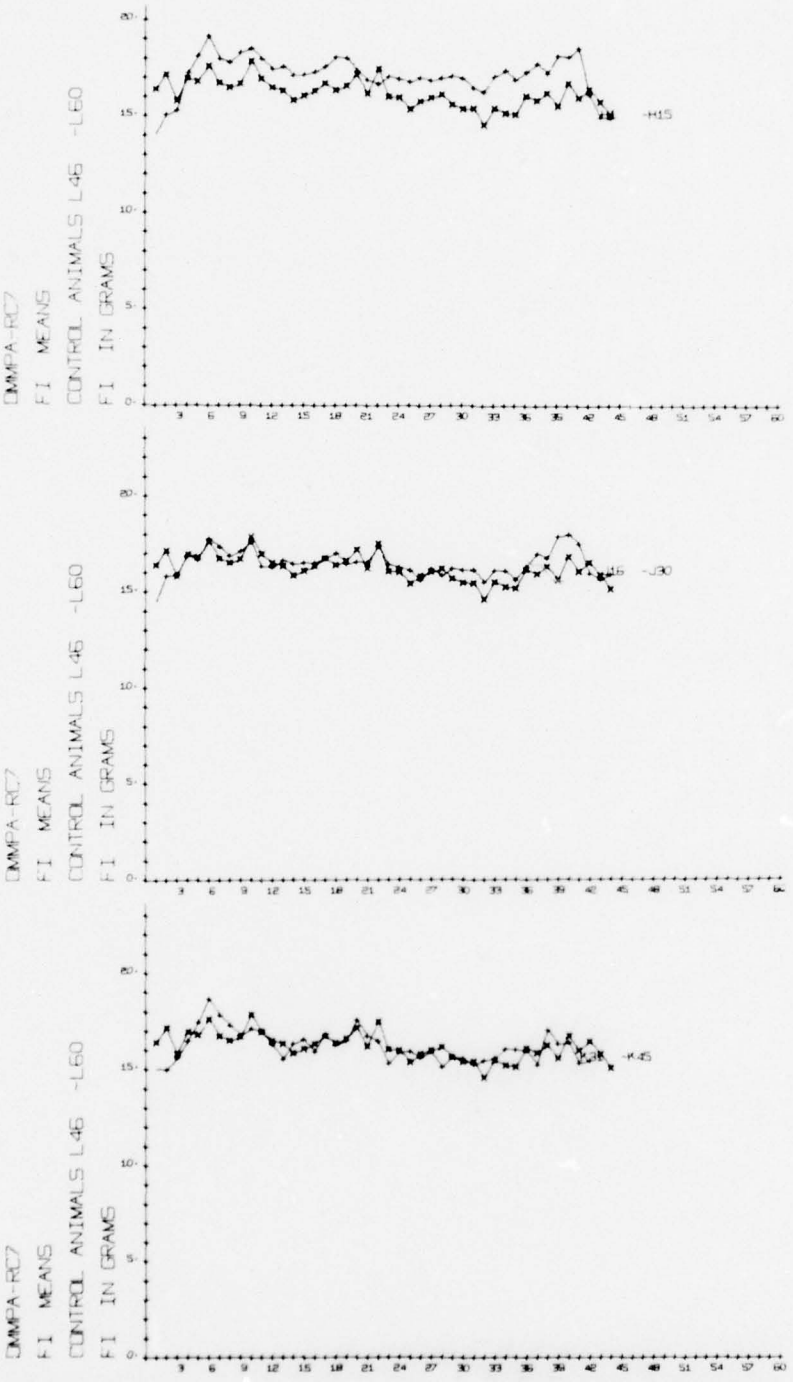

FIGURE 15- FOOD INTAKE AS MEAN WEIGHT IN GRAMS FOOD PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH THE CONTROL GROUP FOR EACH 5 DAY PERIOD FOR 240 DAYS. GROUP H RECEIVED 92 MG/ANIMAL/DAY, GROUP J 50 MG/ANIMAL/DAY AND GROUP K 37 MG/ANIMAL/DAY. NONE OF THE DIFFERENCES SHOWN ARE SIGNIFICANT.

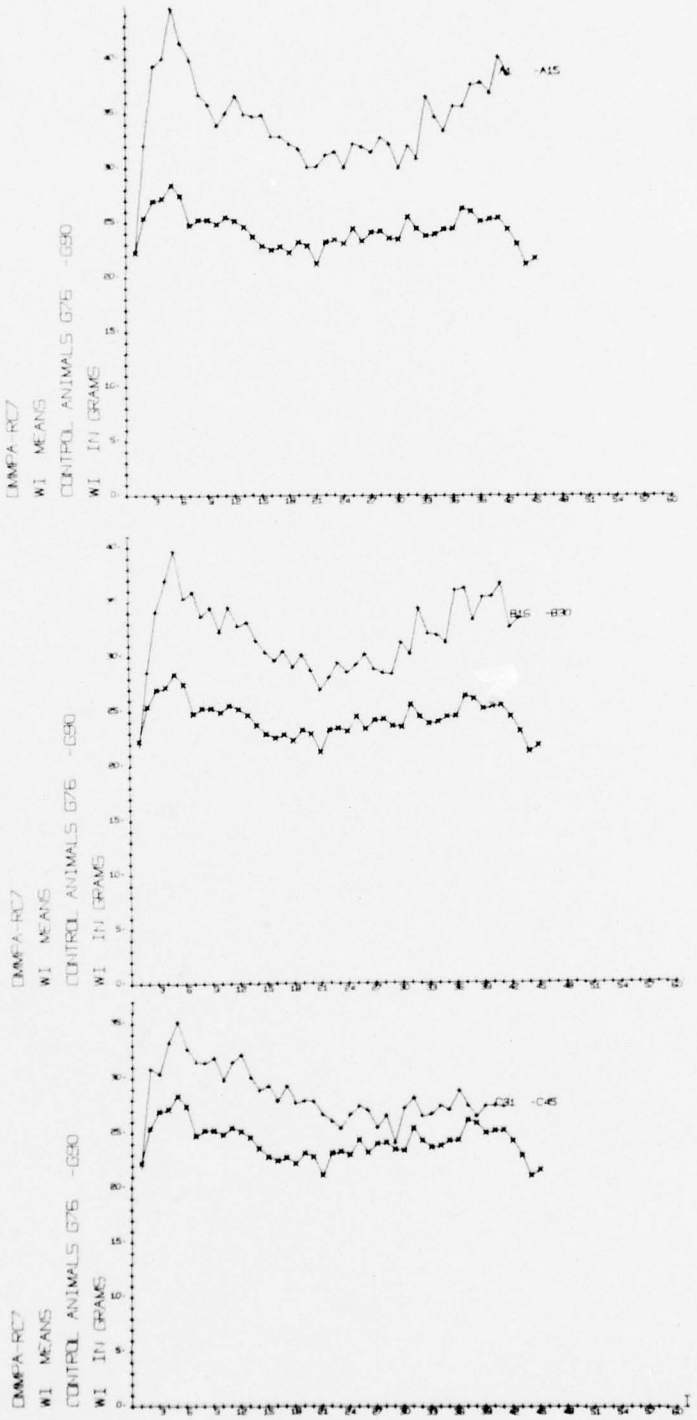

FIGURE 16- WATER INTAKE AS MEAN WEIGHT IN GRAMS WATER PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH THE CONTROL GROUP FOR EACH 5 DAY PERIOD FOR 240 DAYS. GROUP A RECEIVED 483 MG/ANIMAL/DAY, GROUP B 367 MG/ANIMAL/DAY AND GROUP C 245 MG/ANIMAL/DAY. ALL DIFFERENCES BETWEEN DOSED GROUPS AND CONTROLS ARE HIGHLY SIGNIFICANT.

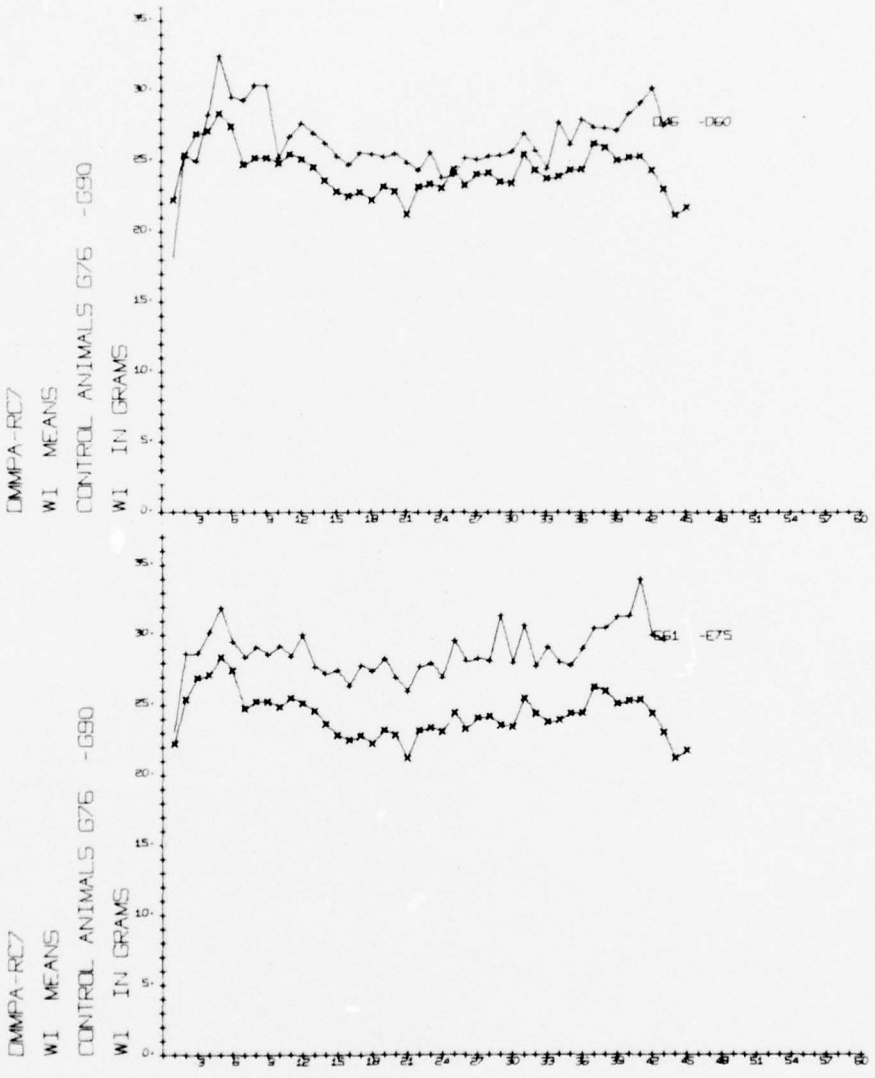

FIGURE 17- WATER INTAKE AS MEAN WEIGHT IN GRAMS WATER PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH CONTROL GROUPS FOR EACH 5 DAY PERIOD FOR 240 DAYS. GROUP D RECEIVED 184 MG/ANIMAL/DAY AND GROUP E 122 MG/ANIMAL/DAY. DIFFERENCES BETWEEN TEST AND CONTROL ARE HIGHLY SIGNIFICANT.

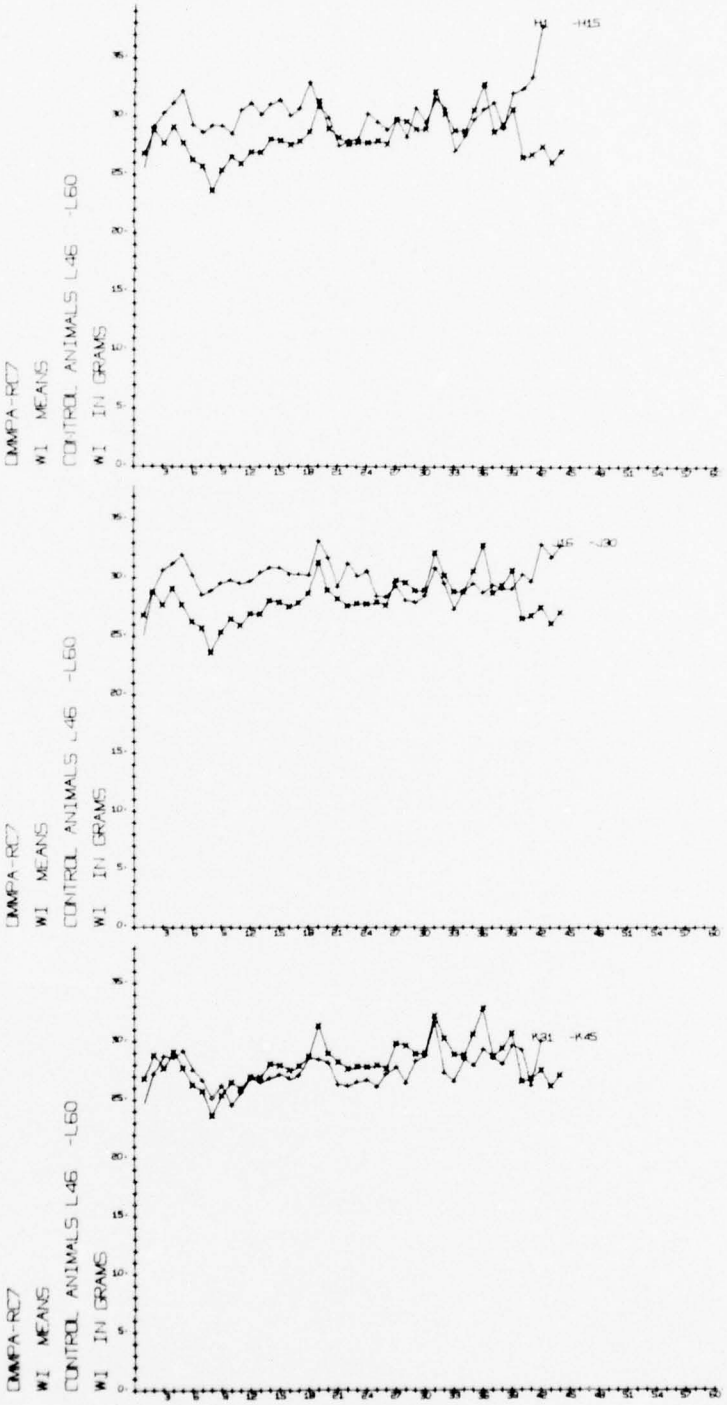

FIGURE 18- WATER INTAKE AS MEAN WEIGHT IN GRAMS WATER PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH CONTROL GROUP FOR EACH 5 DAY PERIOD FOR 240 DAYS. GROUP H RECEIVED 92 MG/ANIMAL/DAY, GROUP J 50 MG/ANIMAL/DAY AND GROUP C 37 MG/ANIMAL/DAY OF DMMPA. THE GREAT MAJORITY OF DIFFERENCES SHOWN ARE NOT SIGNIFICANT WITH ANY DOSE OF DMMPA APPLIED.

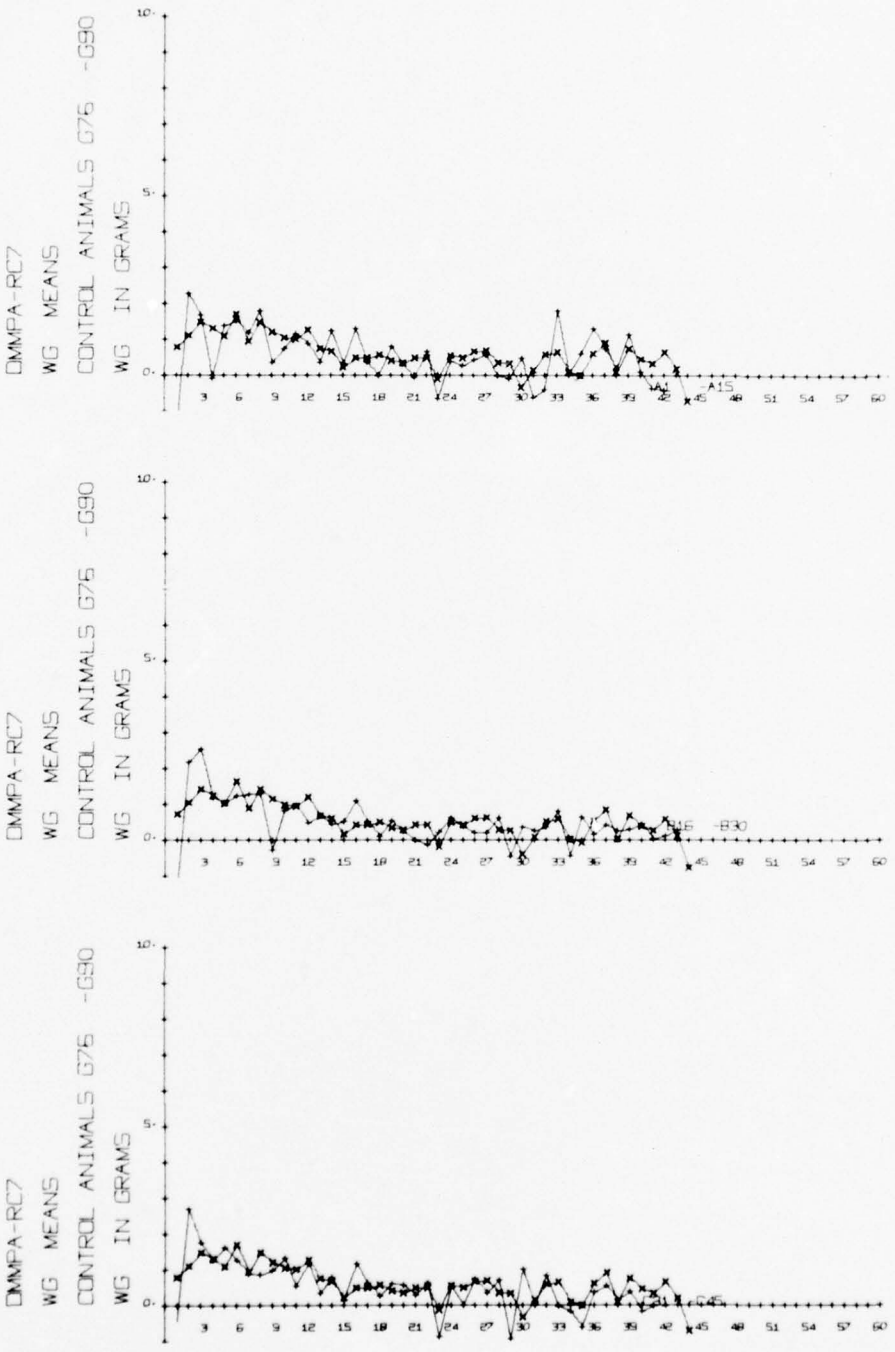

FIGURE 19- WEIGHT GAIN IN ANIMALS TREATED WITH DMMPA IN GRAMS PER DAY FOR A 5 DAY INTERVAL. THESE VALUES ARE COMPARED WITH THE EQUIVALENT VALUES IN CONTROLS FOR EACH 5 DAY PERIOD OF THE 240 DAY EXPERIMENT. GROUP A RECEIVED 489 MG/ANIMAL/DAY, GROUP B 367 MG/ANIMAL/DAY AND GROUP C 245 MG/ANIMAL/DAY. NONE OF THE DIFFERENCES BETWEEN TEST AND CONTROL ARE SIGNIFICANT.

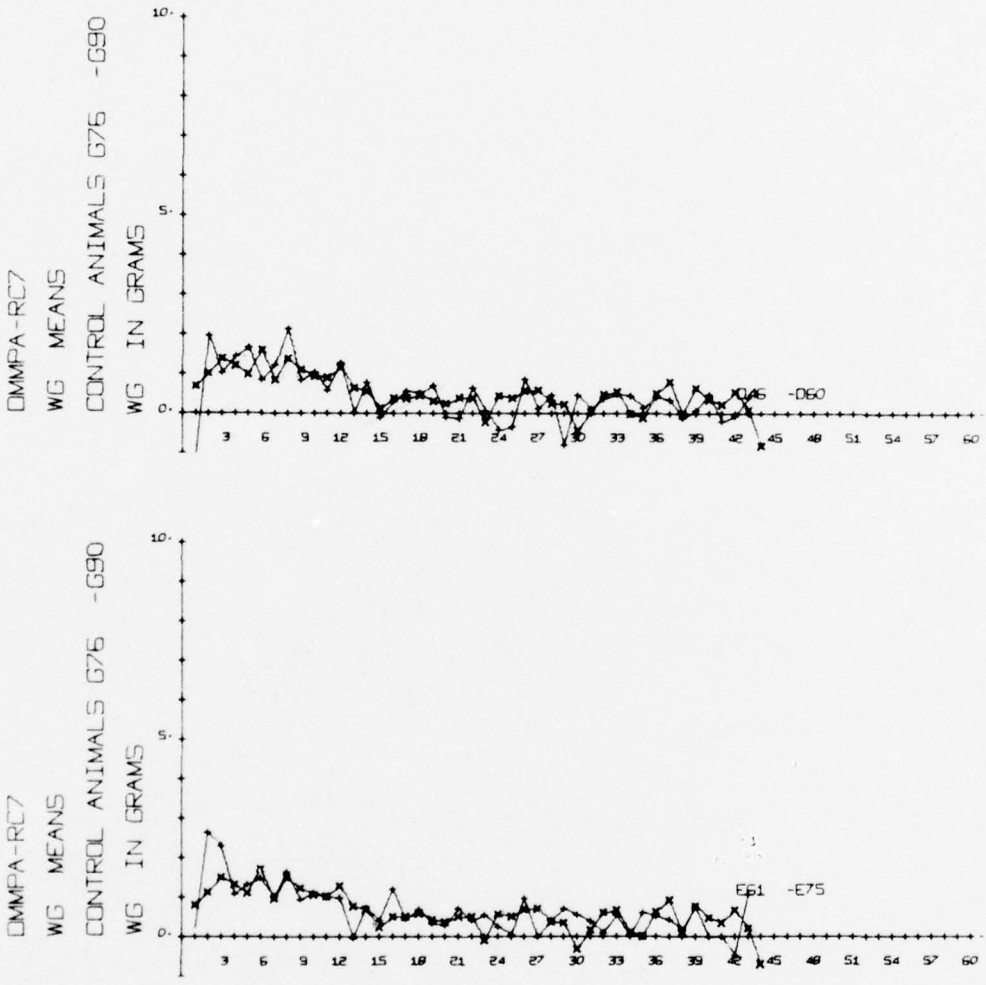

FIGURE 20- WEIGHT GAIN IN ANIMALS TREATED WITH OMMPA IN GRAMS PER DAY FOR A 5 DAY INTERVAL IS COMPARED WITH THE EQUIVALENT VALUES IN CONTROLS FOR EACH 5 DAY PERIOD OF THE 240 DAY EXPERIMENT. GROUP D RECEIVED 184 MG/ANIMAL/DAY AND GROUP E 122 MG/ANIMAL/DAY. NONE OF THE DIFFERENCES BETWEEN DOSED AND CONTROL ANIMALS ARE SIGNIFICANT.

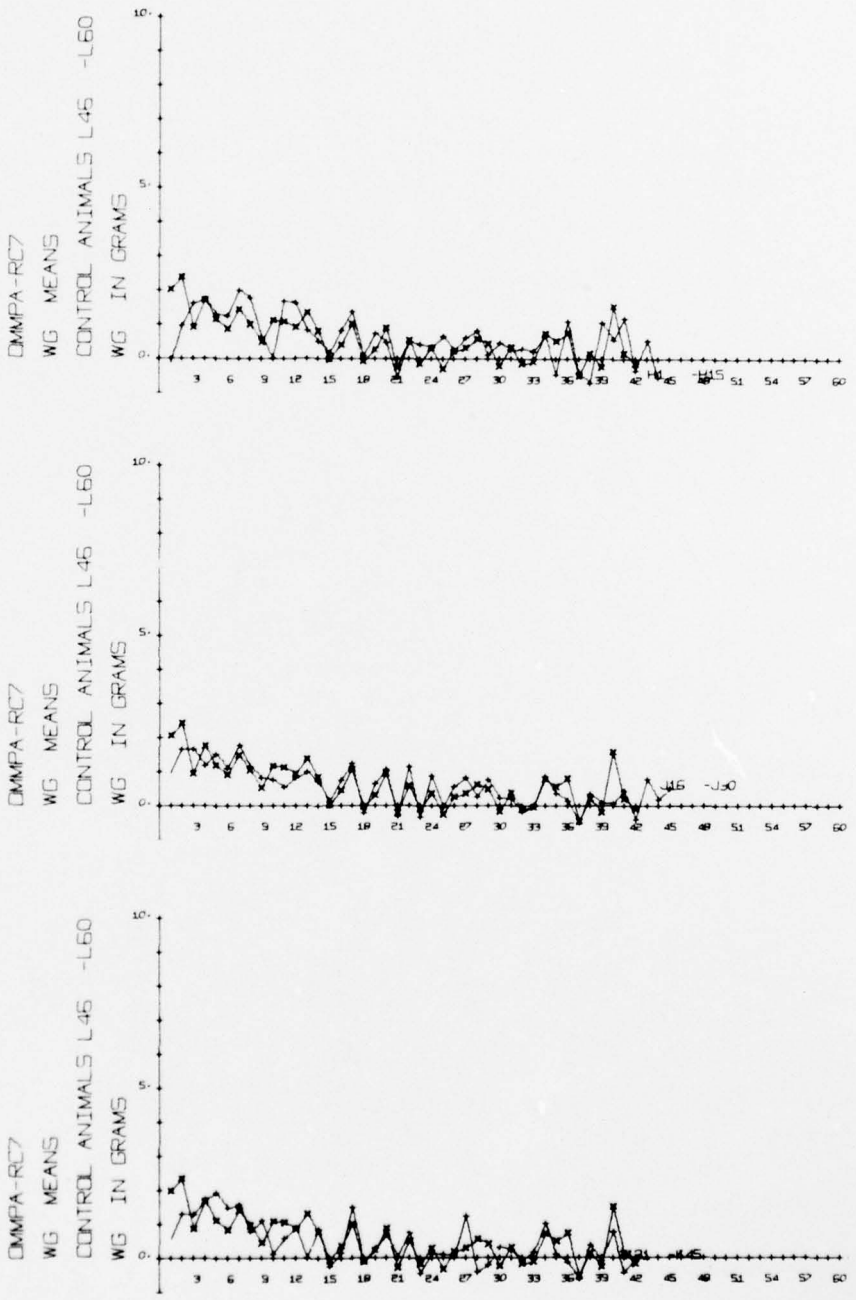

FIGURE 21- WEIGHT GAINS IN ANIMALS TREATED WITH DMMPA IN GRAMS PER DAY FOR A 5 DAY INTERVAL ARE COMPARED WITH THE EQUIVALENT VALUES IN CONTROLS FOR EACH 5 DAY PERIOD OF THE 240 DAY EXPERIMENT. GROUP H RECEIVED 92 MG/ANIMAL/DAY, GROUP J 50 MG/ANIMAL/DAY AND GROUP K 37 MG/ANIMAL/DAY OF DMMPA. NONE OF THE DIFFERENCES BETWEEN DOSED AND CONTROL ANIMALS ARE SIGNIFICANT.

TABLE XIX

THE EFFECT OF DMMPA ON PHYSIOLOGICAL PARAMETERS OF RATS FED DAILY FOR 140 TO 240 DAYS

Dose Applied			Weight at Autopsy gm		Rectal Temperature °C		Respiration Breaths per minute		Heart Rate Beats per minute		Blood Pressure mm of Hg	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2518	281	50.4	36.4	0.59	195	13.6	352 H.S.	20.9	120	2.72
B	367	1888	286	45.6	36.5	0.32	198	11.2	361 H.S.	17.3	119	2.44
C	245	1246	279	40.7	36.5	0.29	198	10.4	359 H.S.	14.9	119	2.08
D	184	944	278	46.7	36.5	0.32	199	10.2	364	14.0	119	1.96
E	122	623	294	45.6	36.5	0.24	199	9.3	365	14.3	119	2.07
H	92	487	304.7	51.0	36.6	0.24	198	9.3	369	15.9	118	2.11
J	50	252	298	50.6	36.6	0.21	199	10.5	371	16.4	119	2.02
K	37	189	289	44.3	36.6	0.26	199	10.4	367	17.4	118	2.01
Control			287	44.9	36.5	0.34	197	9.4	369	16.0	119	2.35

and even at 240 doses not all animals responded with depressed heart rates. This chronic effect would appear to be a dose dependent one, with the limiting dose just causing depression of the heart rate somewhere below 245 mg, but greater than 184 mg/animal/day.

Effects of DMMPA on Blood Cellular Components

Table XX records the mean and standard deviation of all dose groups of total erythrocytes, total leucocytes, haemoglobin concentration and haematocrit. As can be seen in this table, no dosed group varied significantly in any of these haematological parameters from the control. Thus DMMPA at doses as high as 489 mg/animal/day is without effect on blood cellular components and likely on haemopoiesis as well. No differential counts were made on the leucocytes, but random blood smears were prepared of all dose groups and after staining with Wright's were found to have normal appearing distribution of leucocytes.

Effects of DMMPA on Blood Serum Components

In Tables XXI A, B and C are given the means and standard deviations of 14 blood serum components analysed in samples taken from animals dosed with DMMPA and compared with control animals receiving no DMMPA. No significant change in the levels of creatine phosphokinase, glutamic-oxalic transaminase, lactic dehydrogenase, alkaline phosphatase, sodium, potassium, total protein, albumin, globulin phosphate, chloride, urea nitrogen, glucose or bilirubin was observed. Significant differences were calculated by "t-test". From these results it was concluded that DMMPA in doses as high as 489 mg/animal/day was without effect on blood serum components of value in diagnosing liver, heart and kidney damage, and by inference that DMMPA is without pathological effects on these organs as well.

Effects of DMMPA on Wet Weight of Tissues

At autopsy lung, heart, liver, spleen and kidney were removed, blotted dry and weighed. The mean weights of these tissues and the standard deviations for each dose group and control are shown in Table XXII. Highly

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TABLE XX

THE EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS FROM 140 TO 240 DAYS

HAEMATOLOGICAL CHANGE

Dose Applied			Erythrocytes X 10^6		Leucocytes X 10^3		Haemoglobin gm/100 ml		Haematocrit	
Group	mg per animal	mg per kg	M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.
A	489	2518	8.1	0.91	6.9	1.68	17.5	1.5	42.8	2.7
B	367	1888	7.9	0.53	7.1	1.49	16.1	1.3	44.1	1.17
C	245	1246	8.9	1.46	7.6	1.97	16.8	1.4	43.7	1.72
D	184	944	8.1	0.90	7.5	1.74	16.4	1.9	43.9	2.7
E	122	623	8.5	0.66	7.4	2.17	16.4	0.79	43.0	2.1
H	92	487	8.1	2.57	7.68	2.8	17.2	1.61	43.2	2.2
J	50	252	8.7	1.78	7.53	1.5	16.6	1.96	43.6	2.4
K	37	189	7.7	1.00	6.9	1.88	16.3	2.0	43.1	2.1
Control			8.24	0.92	7.4	2.2	16.4	1.9	42.9	3.0

TABLE XXI (A)

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS OF 140-240 DAYS

SERUM COMPONENTS

Dose Applied			Creatine Phosphokinase Int.		Glutamic-oxalic Transaminase Int.		Lactic Dehydrogenase Int.		Alkaline Phosphatase Int.		Sodium MEQ/liter	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2518	66.4	6.0	30.4	2.3	217	78.1	68.6	11.0	146.0	22.6
B	367	1888	62.4	8.0	28.8	4.9	205	26.5	68.8	13.4	143.3	34.8
C	245	1246	68.5	9.5	30.7	3.9	224	46.3	66.2	17.7	146.3	20.4
D	184	944	65.6	6.54	30.4	2.3	211	31.6	67.9	11.0	150.3	32.0
E	122	623	70.5	6.01	30.7	3.4	202	24.3	61.1	14.6	149	42.0
H	92	487	70.1	6.70	31.4	4.5	235	27.5	70.5	9.2	147	23.1
J	50	252	68.1	12.1	33.2	5.5	265	23.2	64.1	15.5	146.4	23.0
K	37	189	69.4	15.1	29.4	2.9	263	22.6	61.1	16.5	145.6	18.0
Control			68.2	10.6	36.4	5.5	235	63.8	65.4	18.0	152.0	18.5

TABLE XXI (B)

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS OF 140-240 DAYS

SERUM COMPONENTS

Dose Applied			Potassium MEQ/liter		Total Protein gm/100 ml		Albumen gm/100 ml		Globulin gm/100 ml		Phosphate mg/100 ml	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2518	5.5	0.78	7.3	0.76	4.2	0.62	3.1	0.71	5.6	1.5
B	367	1888	5.3	1.5	7.0	0.51	3.9	0.78	3.2	0.63	5.1	1.3
C	245	1246	5.9	0.71	7.1	0.56	4.1	0.15	3.0	1.03	5.9	1.6
D	184	944	6.0	1.7	7.2	0.46	4.3	0.46	2.9	0.38	5.4	1.3
E	122	623	5.9	1.9	7.3	0.84	4.5	0.48	2.9	0.76	5.3	1.6
H	92	487	5.5	1.11	7.3	0.88	4.3	1.14	3.0	1.24	5.8	1.9
J	50	252	5.8	0.68	7.1	0.73	4.0	0.83	3.1	0.73	6.0	1.5
K	37	189	5.9	0.77	7.1	0.77	4.0	1.18	3.1	1.05	5.9	1.8
Control			5.9	0.85	7.2	0.79	4.2	0.93	3.04	0.70	5.7	1.8

TABLE XXI (C)

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS FROM 140-240 DAYS

SERUM COMPONENTS

Dose Applied			Chloride MEQ/liter		Blood Urea Nitrogen mg/100 ml		Glucose mg/100 ml		Bilirubin mg/100 ml	
Group	mg per animal	mg per kg	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2518	119.3	9.17	8.8	0.92	114.7	37.1	0.11	0.012
B	367	1888	120.8	6.69	8.6	0.91	127.7	16.6	0.118	0.013
C	245	1246	120.9	7.7	8.9	0.81	126.5	21.0	0.111	0.013
D	184	944	121.2	6.9	10.9	0.97	126.5	16.5	0.113	0.014
E	122	623	122.9	6.0	9.1	0.92	119.1	20.7	0.088	0.009
H	92	487	120.2	7.8	11.5	0.91	115.9	10.0	0.116	0.012
J	50	252	121.3	8.1	9.4	0.89	124.4	15.7	0.107	0.011
K	37	189	122.3	6.9	10.2	0.97	124	7.8	0.134	0.013
Control			120.4	7.9	10.1	0.97	114	43.0	0.117	0.012

TABLE XXII

EFFECT OF DMMPA IN RATS DOSED DAILY FOR PERIODS OF 140-240 DAYS

CHANGE IN TISSUE WEIGHT

Dose Applied			Organ Weights Given as Percentage of Body Weight									
Group	mg per animal	mg per kg	Lung		Heart		Liver		Spleen		Kidney	
			M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
A	489	2518	0.64	0.25	0.38	0.08	5.52	1.2	0.228	0.07	0.474	0.06
			H.S.		H.S.		H.S.				H.S.	
B	367	1888	0.469	0.08	0.31	0.03	4.43	0.31	0.201	0.03	0.418	0.03
							H.S.					
C	245	1246	0.49	0.14	0.30	0.02	4.09	0.24	0.218	0.03	0.411	0.03
D	184	944	0.53	0.15	0.30	0.02	4.04	0.47	0.21	0.017	0.41	0.05
			Sig									
E	122	623	0.50	0.09	0.29	0.03	3.78	0.39	0.20	0.03	0.38	0.04
H	92	487	0.429	0.07	0.290	0.02	3.8	0.32	0.191	0.02	0.38	0.04
J	50	252	0.469	0.06	0.303	0.02	3.76	0.32	0.246	0.17	0.402	0.04
K	37	189	0.426	0.03	0.356	0.11	3.92	0.73	0.209	0.07	0.455	0.15
Control			0.495	0.06	0.310	0.03	3.50	0.41	0.20	0.03	0.380	0.04

significant increases in weight were observed in lung, heart, liver and kidney in animals dosed at 489 mg/animal/day; and a highly significant increase in liver weight only in animals dosed at 367 mg/animal/day. Below this dose there was no significant change in organ weights, indicating that animals dosed as high as 245 mg/animal/day were unaffected by DMMPA when dosed daily for periods not less than 140 days and not longer than 240 days.

Effects of DMMPA on the Water Content of Tissues

Samples of lung, heart, liver, spleen, kidney and skeletal muscle were removed at autopsy and the water content of these tissues determined by drying to constant weight. In Table XXIII the mean value of the water content as a percentage of wet weight is given for each dose group and the control. There were no observable changes in the water content of the above tissues in comparison with the control indicating that DMMPA at doses as high as 489 mg/animal/day for 240 days promotes neither a dehydration nor a hydration of these tissues.

AUTOPSY FINDINGS

Cumulative deaths from DMMPA occurred only with the highest dose used (489 mg/animal/day or approximately 2.5 gm/kg/day). Four of the fifteen animals died "cumulative deaths" with the remaining animals surviving until sacrifice at 140 doses or greater. Deaths occurred after the 11th, 12th, 17th and 18th doses. Autopsies on these animals showed severely damaged lungs with congestion and haemorrhage into both the pleural space and the alveolar space. Livers were distended and congested with blood. Kidneys were congested and showed haemorrhage into the glomerular space and damage to the proximal and distal tubules. Cause of death was ascribed to the lung damage.

Other spontaneous deaths occurred in the course of the 240 days of the experiment. The majority of these were found due to rupture of the trachea, oesophagus or stomach by the ball-tipped needle used for dosing. Animals dying for these mechanical reasons were removed from the groups for all calculations.

TABLE XXIII

EFFECT OF DMMPA ON RATS DOSED DAILY FOR PERIODS OF 140-240 DAYS

WATER CONTENT OF TISSUE

Dose Applied			Water Content as Percentage of Wet Weight of Tissue					
Group	mg per animal	mg per kg	Lung	Heart	Liver	Spleen	Kidney	Skeletal Muscle
A	489	2518	80.4	78.5	72.4	77.1	77.7	76.5
B	367	1888	80.4	77.6	69.8	74.9	77.3	77.0
C	245	1246	81.3	78.1	70.6	76.4	77.5	77.1
D	184	944	80.9	78.6	71.3	76.0	76.8	77.6
E	122	623	80.2	78.6	70.6	76.2	76.4	76.9
H	92	487	80.1	77.8	70.2	75.9	75.8	76.1
J	50	252	80.9	77.6	71.0	75.5	75.9	75.9
K	37	189	80.3	78.4	72.1	76.7	76.0	76.7
Control			80.3	78.0	71.2	75.7	76.0	76.9

All remaining animals were sacrificed by decapitation and autopsied within 24 hours of death. All animals were held at 5°C until autopsy.

Autopsy results on animals receiving 489, 367, 295 and 184 mg/animal/day were almost identical with those found in animals dying from accumulation of DMMPA. Lung damage was extensive at the two highest doses and decreased in severity with doses of 295 and 184 mg/animal/day. Liver and kidney also showed signs of damage at the two highest doses but such damage was rarely seen at the lower two doses. At 122 mg/animal/day DMMPA, 4/14 animals showed mild degrees of lung congestion with petechial haemorrhages. There was no evidence of haemorrhage into the alveolar spaces and no blood was found in the pleural space. At doses below 122 mg/animal/day the incidence of pathological findings either macroscopic or microscopic was no greater than that found in the controls. The pathological findings indicate a clear break in responses at or below 122 mg/animal/day, where essentially minimal results were obtained. It was concluded that doses as high as 92 mg/animal/day or 487 mg/kg/day of DMMPA were essentially without effect on the rats even when given for periods up to 240 days.

DISCUSSION

Assessment of toxicological effects on animals dosed daily for periods from 100 to 240 days was undertaken chiefly to obtain some assessment of the safety of use of the compound. This is, of course, in addition to the delineation of the effects of chronic feeding. The primary requirement is the establishment of the levels at which pathological effects occur and, subsequently, the highest daily doses of DMMPA at which these specific pathological effects do not occur.

One useful datum in determining the no-effect dose is the cumulative LD_{50} (100 day) which in the case of DMMPA proved difficult, if not impossible, to determine. All that can be said is that 2.5 gm/kg/day is the highest dose at which any cumulative deaths occurred. The nearest next lowest dose examined at which no cumulative lethality was observed was 1.8 gm/kg/day which will be a close approximation to the no-effect dose. However, in Table XXIV are summarized the highest doses at which specific tests of pathological response was

TABLE XXIV

SUMMARY TABLE OF MAXIMUM DOSE OF DMMPA
APPLIED DAILY FOR 140-240 DAYS
HAVING NO EFFECT ON SCREENING PROCEDURES

Criteria of Pathological Effect	Maximum No-Effect Dose DMMPA	
	mg/animal/day	mg/kg/day
Food Intake	489	2518
Water Intake	92	487
Weight Gain	489	2518
Urine Components	489	2518
Heart Rate	122	623
Blood Pressure	489	2518
Respiration Rate	489	2518
Body Temperature	489	2518
Total Erythrocytes	489	2518
Total Leucocytes	489	2518
Haemoglobin	489	2518
Haematocrit	489	2518
Blood Serum Components (14 entities)	489	2518
Tissue Weight	245	1246
Water Content of Tissues	489	2518
Lung Damage	92	487

assessed. From this table it will be seen that 0.5 gm/kg/day is the highest dose having no pathological response in any of the parameters tested. In fact, only water intake, heart rate and lung damage of the 30 element screening procedures were affected at all by doses of DMMPA as high as 2.5 gm/kg/day. However, the safe dose must be derived from the responsive or more sensitive criteria and is thus 0.5 gm/kg/day.

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The effects of chronic daily dosing of dimethyl morpholinophosphoramidate (DMMPA) have been assessed in rabbits for dermal application and in rats for parenteral application.

One hundred day application of DMMPA dermally to rabbits was without effect.

One hundred day application to rats, both male and female, was without effect on breeding both in the incidence of pregnancies, number of pups per litter and the number of still-born.

Three separate experiments are described in which male and female rats were dosed with DMMPA in periods from 100 to 240 days. Some thirty separate pathological screening tests were examined including nutritional, physiological, haematological, blood chemistry, tissue weight and water content procedures as well as macroscopic and microscopic pathology. Of these, only water intake, heart rate and lung damage were found to be affected. The highest dose of DMMPA having no effect on these latter parameters was 0.5 gm/kg/day.

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