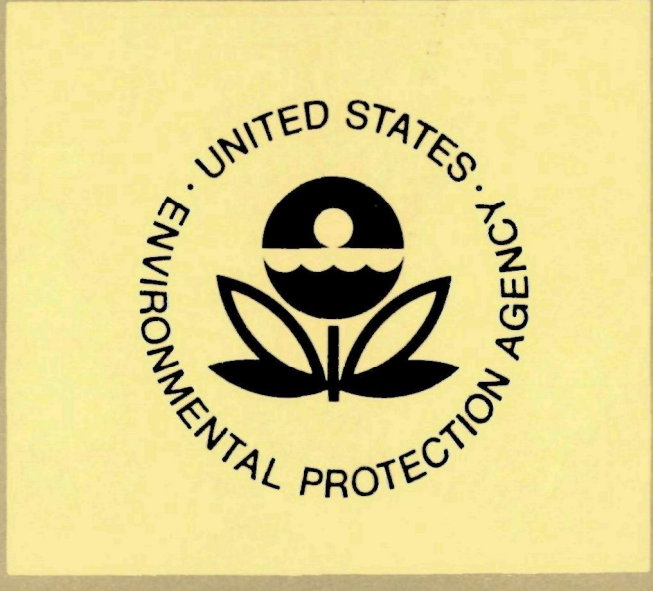
EPA-600/1-76-010a
January 1976

Environmental Health Effects Research Series

ASSESSMENT OF TOXICITY OF AUTOMOTIVE METALLIC EMISSIONS
Volume I



Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals - but always with intended application to human health measures.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/1-76-010a
January 1976

ASSESSMENT OF TOXICITY OF AUTOMOTIVE METALLIC EMISSIONS. VOLUME I:

Assessment of Fuel Additives Emission Toxicity via Selected Assays of Nucleic Acid and Protein Synthesis

By

David J. Holbrook, Jr., Ph.D.
Department of Biochemistry
School of Medicine
University of North Carolina
Chapel Hill, North Carolina 27514

Contract No. 68-02-1205

Project Officer

Ms. Frances P. Duffield
Catalyst Research Program Office
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

U. S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Various parameters of toxicity have been studied for salts of manganese, lead, palladium and platinum. Following intraperitoneal injection, the acute toxicities (LD-50 doses, 14-day observation period, doses expressed in molar quantities) in decreasing order, were: $PtCl_4$ > $Pt(SO_4)_2 \cdot 4H_2O$ (from B. F. Goldsmith) > $PdCl_2 \cdot 4H_2O$ > $Pt(SO_4)_2 \cdot 4H_2O$ (from K and K Laboratories), $MnCl_2 \cdot 4H_2O$ > $PdSO_4$ > $PtCl_2$ > $PbCl_2$. Following oral administration, the acute toxicities (LD-50 doses), in decreasing order, were: $PtCl_4$ > $Pt(SO_4) \cdot 4H_2O \geq PdCl_2 \cdot 2H_2O$, $RuCl_3$ > $MnCl_2 \cdot 4H_2O$ > $PdSO_4$, $PtCl_2$ > PtO_2, PbO, $PbCl_2$, PdO, MnO_2.

Following dietary (via drinking fluid) administration of soluble salts of Pb^{2+} ($PbCl_2$) or Pt^{4+} ($PtCl_4$ or $Pt(SO_4)_2 \cdot 4H_2O$), the highest concentrations of metallic cations occurred in the kidney, intermediate levels in the liver, and generally lower levels in the spleen, heart, testes, brain and blood. In rats which survived for 14-days following the administration of approximately the oral LD-50 and intraperitoneal LD-50, the kidneys contained approximately 16 and 37 μgPt/g wet tissue, respectively, and the livers contained approximately 2 and 34 μg Pt/g wet tissue, respectively.

Weights of five organs (liver, kidney, spleen, heart, testes) were measured in rats which had been treated with various metallic salts in the diet (either drinking fluid or solid feed). The organ weights were expressed as a percentage of body weight. Rats which received $MnCl_2 \cdot 4H_2O$ at a level of 1.6 or 3.7 g/liter (8.3 or 18.6 mmoles/liter) for 90 days did not show statistically significant changes in the weights of any organs. $PbCl_2$, at a level of 1.0 g/liter (3.7 mmole/liter) for 30 or 90 days, consistently increased the kidney weights of treated rats (17% and 23% above control in the 90-day experiments). The use of saturated solutions of $PdCl_2 \cdot 2H_2O$ (8 days) or of $PdSO_4$ (8 or 30 days) as the drinking fluid did not cause a consistent

change in any of the organ weights of the treated rats.

If $PtCl_4$ was added to the drinking fluid at 183 mg/liter (0.5 mmoles/liter) for 30 or 90 days, or at 550 mg/liter (1.6 mmoles/liter) for 8 days, no consistent changes were observed in the organ weights. If the concentration-duration of $PtCl_4$ was increased to 550 mg/liter (1.6 mmoles/liter) for 30 days or 825 mg/liter (2.4 mmoles/liter) for 9 days, the kidneys were increased in weight by approximately 6% in each of four experiments and the testes were increased by approximately 11% in each of four experiments; however, in each tissue in each individual experiments, the differences between control and metal-treated animals showed statistically significant differences ($p < 0.05$) or trends ($p < 0.10$) in only about one-half of the experiments.

After experimental rats were maintained on metal-containing diets for approximately 8, 30 or 90 days, hepatic microsomes were isolated and the following parameters related to in vitro drug metabolism were measured: yield of microsomal protein/g liver; in vitro activities of aniline hydroxylase and aminopyrine demethylase; content of cytochromes P-450 and b5/mg microsomal protein. Treatment with $MnCl_2 \cdot 4H_2O$, 1.6 g/liter (8.3 mmoles/liter) or 3.7 g/liter (18.6 mmoles/liter) for 90 days did not alter any of the studied parameters of drug metabolism. The administeration of low levels of Pd^{2+} (saturated solution of $PdCl_2 \cdot 2H_2O$ for 8 days or saturated solution of $PdSO_4$ for 8 or 30 days) in the drinking fluid resulted in somewhat decreased activities of aniline hydroxylase and aminopyrine demethylase. $PtCl_2$ (saturated solution as drinking water) did not produce consistently statistically different levels of aniline hydroxylase or aminopyrine demethylase.

A wide range of dose levels-duration of soluble salts of Pt^{4+} did not cause consistent changes in the levels of aniline hydroxylase or aminopyrine

demethylase in liver tissue; dosages and durations used included 0.5 mmoles/liter (183 mg $PtCl_4$/liter) for 30 and 90 days; 1.6 mmoles (550 mg $PtCl_4$/liter or 750 mg $Pt(SO_4)_2 \cdot 4H_2O$/liter for 8 days or for 30 days ($PtCl_4$ only).

Work has been completed on the development of a rapid and convient method for the analysis of ribosomal RNA in studies of RNA synthesis.

CONTENTS

I. INTRODUCTION

A. Contract. These studies were conducted pursuant to contract number 68-02-1205 and project number DU-73-B439 with the Enviormmental Protection Agency.

B. Importance of compounds. Lead salts are an emission product from mobile (or automotive) emission sourses due to the addition of tetraethyl lead to gasoline. Because of known toxic properties of lead salts, it has been proposed that alkyl manganese compounds be substituted as a fuel additive for tetraethyl lead. With the introduction of platinum and palladium in the catalytic converters of 1975-model year vehicles, it is of concern to determine the quantities of platinum and palladium metal and salts which will be in emission products and the biological effects of these compounds on mammalian tissues.

C. Studies undertaken. Consequently, experiments were undertaken in this laboratory to study the effects of various metal salts on the following: acute lethal dose following oral and intraperitoneal administration, growth of animals receiving the salts in feed or drinking fluid, the tissue concentration of some of the metals in various organs, the size of selected organs, the in vitro activity of two representative microsomal drug-metabolizing enzymes and the cytochrome P-450 and b5 concentrations in liver, the activity in vitro of eucaryotic DNA polymerases, and RNA synthesis in vivo.

II. MATERIALS AND METHODS

All experimental studies were conducted with male Sprague-Dawley rats. The animals were received at 3-3.5 weeks of age and were maintained for 1-1.5 weeks before use. The mean body weights were usually 100-110 g when the rats were used for the lethal-dose experiments or started on the diets.

In the lethal dose experiments, the salts were administered orally (via stomach tube) or intraperitoneally. The rats were observed through a 14-day observation period. In the completed experiments, the LD-50 values were calculated by the method of Litchfield and Wilcoxon (1).

In the diet experiments, four rats were maintained per cage. The metallic salt under study was dissolved in the drinking fluid. Animals consumed feed and drinking fluid ad libitum. Analyses for metals were performed on samples from three lots of feed (Purina Laboratory Chow). The feed contained (mean $\pm$ std. dev.): 56 ± 5 mg Mn/kg feed and 0.99 ± 0.07 mg Pb/kg feed; the analyses of the three lots for platinum were 0.09, < 0.02, and < 0.02 mg Pt/kg feed. Measurements were made of the body weights of individual rats and feed and fluid consumption per cage of four rats at 7-day intervals during the course of each diet experiment.

At the termination of the dietary experiments, samples of liver were used for the isolation of microsomes. Aniline hydroxylase was measured by the method of Imai et al. (2), modified by the addition of $HgCl_2$ (3). Aminopyrine demethylase was measured by the formation of formaldehyde (Nash reaction) (4).

The analyses of the rat tissues for platinum, lead and manganese were carried out by Yoakum, Stewart and Sterrett (5) of Stewart Laboratories, Inc. by an emission spectrochemical method.

III. LETHAL DOSE STUDIES

The rats used in these studies usually had a mean body weight of 100-110 grams. The LD-50 values and the 95% confidence limits were determined by the method of Litchfield and Wilcoxin (1).

A. Oral administration. The summary of the lethel dose studies following oral administration are given in Table 1.

In terms of the LD-50 (dose lethal to 50% of the animals within the 14-day observation period), the acute toxicities (expressed in molar quantities), in decreasing order, were: $PtCl_4 > Pt(SO_4)_2 \cdot 4H_2O \geq PdCl_2 \cdot 2H_2O$, $RuCl_3 > MnCl_2 \cdot 4H_2O > PdSO_4$, $PtCl_2 > PtO_2$, PbO, $PbCl_2 > MnO_2$, PdO.

Thus, the two soluble Pt^{4+} salts were found to be the most toxic salts following oral administration. As anticipated, the poorly absorbed, insoluble salts, namely $PbCl_2$, $PtCl_2$, PbO, PtO_2, PdO and MnO_2, were the least toxic. In the latter named cases, doses could not be increased sufficiently to attain 50% lethality in the experimental rats and still maintain the volume administered to 2% or less of the body weight.

In each of these cases, the LD-50 dose is greater than the 5,000 mg/kg body weight which the National Institute for Occupational Safety and Health uses as a criterion for inclusion as a toxic substance in the Toxic Substances List, 1972 edition;[7] thus, in terms of the acute LD-50, the salts $PbCl_2$, PbO, PdO, PtO_2, and MnO_2 would be considered "non-toxic" by this standard.

Also included in Table 1 are the LD-10 and LD-90, i.e., the doses which cause the death of 10% and 90%, respectively, of the rats in the 14-days after oral administration. In those cases where data are available, the order of toxicities for the oral LD-10, "the minimal lethal dose", is the same as that given above for the order of the LD-50 values.

The slopes of the toxicity curves can be compared by the ratio of the LD-90 to LD-10 (Table 1). For example, $MnCl_2 \cdot 4H_2O$ is relative non-toxic and the oral LD-10 dose is 6.3 mmoles/kg body weight. However, if the dose was increased 1.4-fold to 9.0 mmoles/kg, 90% of the treated rats died. In contrast, $PtCl_4$ was orally the most toxic of the salts tested; the ratio of the LD-90 to LD-10 was approximately 5.0. The other soluble or partially-

Table 1.

Lethal Doses Following Oral Administration

Compound	Unit (per kg body weight)	LD-50 (95% confidence limits)	LD-10	LD-90	LD-90 / LD-10
$PtCl_4$	mmoles	0.70 (0.51-0.96)	0.31	1.57	5.1
	mg salt	240 (171-320)	104	530	
	mg cation	136 (99-188)	60	310	
$Pt(SO_4)_2 \cdot 4H_2O$	mmoles	2.2 (1.57-3.1)	1.37	3.5	2.6
	mg salt	1010 (720-1400)	630	1620	
	mg cation	430 (310-600)	270	690	
$PdCl_2 \cdot 2H_2O$	mmoles	2.7 (2.2-3.4)	1.56	4.8	3.1
	mg salt	590 (470-730)	330	1030	
	mg cation	290 (240-360)	166	520	
$RuCl_3$	mmoles	3.2 (2.4-4.0)	1.78	5.4	3.0
	mg salt	650 (500-830)	370	1130	
	mg cation	310 (240-400)	180	550	
$MnCl_2 \cdot 4H_2O$	mmoles	7.5 (7.0-8.1)	6.3	9.0	1.4
	mg salt	1490 (1380-1610)	1260	1780	
	mg cation	410 (380-450)	350	490	
$PdSO_4$	mmoles	>7.5	--	--	--
	mg salt	>1500	--	--	
	mg cation	>790	--	--	

Table 1 (continued)

Compound	Unit (per kg body weight)	LD-50 (95% confidence limits)	LD-10	LD-90	$\frac{\text{LD-90}}{\text{LD-10}}$
$PtCl_2$	mmoles	>8	--	--	--
	mg salt	>2,000	--	--	
	mg cation	>1,400	--	--	
PtO_2	mmoles	>35	--	--	--
	mg salt	>8,000	--	--	
	mg cation	>6,900	--	--	
PbO	mmoles	>45	<30		
	mg salt	>10,000	<6,700	--	
	mg cation	>9,300	<6,200	--	
$PbCl_2$	mmoles	>>35	21-35	--	--
	mg salt	>>9,600	5,800-9,600	--	
	mg cation	>>7,200	4,300-7,200	--	
PdO	mmoles	>> 82	--	--	--
	mg salt	>> 10,000	--	--	
	mg cation	>> 8,700	--	--	
MnO_2	mmoles	(est.) 135	(est.) 60	--	--
	mg salt	(est.) 12,000	(est.) 5,200	--	
	mg cation	(est.) 7,400	(est.) 3,300	--	

soluble salts tested had intermediate LD-90 to LD-10 ratios of 2.5-3.1.

If the acute toxicities following oral administration are expressed as mg of cation/kg of body weight, the LD-50 values were in the following decreasing order: $PtCl_4$ > $PdCl_2 \cdot 2H_2O$, $RuCl_3$ > $MnCl_2 \cdot 4H_2O$, $Pt(SO_4)_2 \cdot 4H_2O$ > $PdSO_4$ > $PtCl_2$ > MnO_2, PtO_2, $PbCl_2$, PbO, PdO.

Likewise, if the LD-10 values are expressed in terms of mg of cation/kg, the acute toxicities are in the following decreasing order: $PtCl_4$ > $PdCl_2 \cdot 2H_2O$, $RuCl_3$ > $Pt(SO_4)_2 \cdot 4H_2O$ > $MnCl_2 \cdot 4H_2O \gg MnO_2$, $PbCl_2$, PbO.

B. Intraperitoneal injection. The summary of the lethal dose studies following intraperitoneal injection are given in Table 2. Values are given for the LD-50, LD-10 and LD-90 and each parameter is expressed in terms of mmoles/kg body weight, mg salt/kg body weight, and mg cation/kg of body weight.

In terms of the LD-50, the acute toxicities (expressed in molar quantities) in decreasing order, were: $PtCl_4$ > $Pt(SO_4)_2 \cdot 4H_2O$ (Goldsmith) > $PdCl_2 \cdot 2H_2O$ ≥ $Pt(SO_4)_2 \cdot 4H_2O$ (K and K Laboratories), $MnCl_2 \cdot 4H_2O$ > $PdSO_4$ > $PtCl_2$ > $PbCl_2$.

On a molar basis, the LD-10 are in the following decreasing order: $PtCl_4$ > $Pt(SO_4)_2 \cdot 4H_2O$ (Goldsmith) > $PdCl_2 \cdot 2H_2O$ > $MnCl_2 \cdot 4H_2O$, $Pt(SO_4)_2 \cdot 4H_2O$ (K and K) > $PdSO_4$ > $PbCl_2$. This was exactly the same order as the molar LD-50 values.

The limiting dosages differentiating toxic and nontoxic substances used for inclusion of a substance in the Toxic Substances List (7) is 2,000 mg/kg following an intraperitoneal injection in rats. According to this standard, all of the tested compounds are "toxic" after intraperitoneal injection.

If the LD-50 following intraperitoneal injection is expressed in terms of mg cation/kg body weight, the acute toxicities of the compounds decrease in the following order: $PtCl_4$ > $MnCl_2 \cdot 4H_2O$ > $PdCl_2 \cdot 2H_2O$, $Pt(SO_4)_2 \cdot 4H_2O$ (Goldsmith) > $Pt(SO_4)_2 \cdot 4H_2O$ (K and K Lab), $PdSO_4$ > $PtCl_2$ > $PbCl_2$.

The intraperitoneal LD-10 values, expressed in mg cation/kg body weight, are in essentially the same sequence as the eight most toxic compounds listed for the intraperitoneal LD-50 values.

C. Duration of survival. The rapidity of death in non-surviving rats following oral administration varied widely. For example, rats receiving approximately the oral LD-50 survived for <1.0 day, $PtCl_4$, $Pt(SO_4)_2 \cdot 4H_2O$; 1-2.5 days, $RuCl_3$; 3.5-4 days, $MnCl_2 \cdot 4H_2O$; or 5 days, $PdCl_2 \cdot 2H_2O$. For rats which received approximately the intraperitoneal LD-50, the non-surviving rats lived for <1.0 day, $MnCl_2 \cdot 4H_2O$; 3.0-3.5 days, $Pt(SO_4)_2 \cdot 4H_2O$, $PdCl_2 \cdot 2H_2O$; and 4.0-4.5 days, $PdSO_4$.

Table 2.

Lethal Doses Following Intraperitoneal Injection

Compound	Unit (per kg body weight)	LD-50 (95% confidence limits)	LD-10	LD-90	LD-90 / LD-10
$PtCl_4$	mmoles	0.11 (0.09-0.15)			
	mg salt	38 (29-50)			
	mg cation	22 (17-29)			
$Pt(SO_4)_2 \cdot 4H_2O$	mmoles	0.68 (0.60-0.76)	0.56	0.82	1.5
	mg salt	310 (280-350)	260	380	
(K and K Lab)	mg cation	132 (117-149)	110	160	
$MnCl_2 \cdot 4H_2O$	mmoles	0.70 (0.61-0.80)	0.56	0.87	1.6
	mg salt	138 (120-159)	111	172	
	mg cation	38 (33-44)	31	48	
$Pt(SO_4) \cdot 4H_2O$	mmoles	0.3-0.4	0.2-0.3	0.4-0.6	2
(Goldsmith)	mg salt	138-184	92-138	184-280	
	mg cation	59-78	39-59	78-117	
$PdCl_2 \cdot 2H_2O$	mmoles	0.57 (0.45-0.72)	0.39	0.82	2.1
	mg salt	121 (95-154)	84	175	
	mg cation	60 (48-77)	42	87	
$PdSO_4$	mmoles	1.42 (1.11-1.81)	(est.) 0.77	1.8	2.4
	mg salt	290 (220-370)	(est.) 156	370	
	mg cation	151 (118-193)	(est.) 82	195	
$PbCl_2$	mmoles	8.5 (5.0-14.4)	1.6	16.8	10
	mg salt	240 (1400-4000)	440	4700	
	mg cation	1760 (1050-3000)	330	3500	
$PtCl_2$	mmoles	2.5 (1.58-4.0)			
	mg salt	670 (420-1060)			
	mg cation	490 (310-770)			

In rats which survived for 7 to 14 days after administration of doses which were two-thirds or less of the oral or intraperitoneal LD-50, significant lack of weight gain was noted during days 0-7 in rats receiving most of the compounds. However, weight gain, (expressed in grams) was approximately the same in the treated and control rats during days 7-14 of the observation period.

Two samples of $Pt(SO_4)_2 \cdot 4H_2O$ were tested in the intraperitoneal lethal dose studies; one was purchased from ICN-K and K Laboratories and a second sample from D. F. Goldsmith Chemical and Metal Corp. The two samples differed in their acute toxicities by approximately 2-fold. It has not been possible to identify the cause of the differences.

Detailed data on the acute toxicities are given in the attached Appendix. The data include the duration of survival by all animals and non-surviving animals and the weight gain during the two weeks following metal administration to the surviving animals. Also included are the plots of the probits versus log dose which were used in the method of Litchfield and Wilcoxon (1) to evaluate the LD-10 and LD-90 values.

IV. METAL CONTENT OF VARIOUS TISSUES

Analyses for lead, manganese and platinum were conducted by Yoakum, Stewart and Sterrett (5). In a series of rats treated for 90-91 days, the control rats ingested approximately 0.15 g of manganese (from the solid feed). The tissue concentration of Mn was 1.4 and 1.0 μg Mn/g wet tissue in the liver and kidney, respectively. In Mn-treated rats, which received 8.3 mM $MnCl_2.4H_2O$ as the drinking fluid and ingested approximately 2.3 g of Mn per rat during the 90-91 day interval, the concentration of Mn was somewhat increased, namely 2.8 and 1.6 μg Mn/g of wet tissue in the liver and kidney, respectively. The Mn concentration in spleen, heart, testes and blood was not increased in the tissues of Mn-treated rats.

A second group of rats received 3.6 mM $PbCl_2$ in the drinking water for 90-91 days and ingested approximately 3 g of lead per rat during the interval; control rats ingested < 0.01 g of Pb in the solid feed during the same interval. Kidney showed a marked accumulation of Pb (to 11.1 μg Pb/g of wet tissue) in the lead-treated rats; in the same rats the concentration in liver was 1.2 μg Pb/g of wet tissue. The corresponding levels in the control rats were approximately

0.3 μg Pb/g of wet tissue in both kidney and liver. The other tissues -- spleen, heart, testes and blood -- did not exhibit appreciably higher levels of Pb in the Pb-treated rats.

Soluble Pt^{4+} salts were included in the drinking fluid of rats for 8-9 day intervals. The approximate total Pt intake (mg Pt per rat) and data on the tissue concentration of Pt in various tissues are presented in Table 3. Although the Pt concentrations in tissues of untreated control rats often attain levels measurable by the technique used by Stewart Laboratories, Inc., the levels are low and are generally less than 0.1 μg Pt/g of wet tissue. For the higher levels of Pt^{4+} intake in the Pt-treated rats, the highest tissue concentrations of Pt occurred in the kidney and ranged from 4.5-5 μg Pt/g of wet tissue. High levels, ranging from 0.7-2.5 μg Pt/g, also occurred in the liver. In contrast, brain showed only a very low level of Pt which may reflect a contribution from the blood. Separate experiments were conducted on the tissue concentrations of Pt in rats which received a saturated solution of $PtCl_2$ as the drinking fluid for 30-31 days. In the $PtCl_2$-treated rats, the mean Pt concentration for liver, kidney and spleen were < 0.08 μg Pt/g of wet tissue.

In Table 4 are presented the Pt concentration of tissues removed from rats which had survived for the 14-day observation period in lethal-dose experiments. The doses of $Pt(SO_4)_2.4H_2O$ administered by both the oral and intraperitoneal routes were approximately 90% of the LD-50 values by the respective routes.

Table 3. Pt content of tissues of rats maintained on drinking fluid containing Pt salts.

Diet (drinking fluid)	Control	$Pt(SO_4)_2.4H_2O$		$PtCl_4$
Pt salt concn. (mg Pt/lit)	-	106	319	319
Duration of diet (days)	-	8	9	8
Total Pt intake	< 0.01	26	80	60
Tissue	**Tissue concentration of Pt (µg Pt/g wet tissue)**			
Liver	< 0.02 ± 0.02	0.07 (0.04-0.09)	0.85 (0.73-0.97)	2.2 (2.0-2.5)
Kidney	< 0.23 ± 0.45	0.26 ± 0.05	4.6 (4.5-4.7)	4.8 ± 0.5
Spleen	< 0.08 ± 0.08	0.02 (0.01-0.01)	0.13	0.24
Heart	< 0.02 ± 0.01	0.02	0.25	-
Testes	< 0.014 ± 0.010	0.04 ± 0.05	-	-
Brain	-	-	0.015 ± 0.002	-
Blood	0.10 ± 0.13	0.05	0.22 (0.09-0.36)	0.23 (0.19-0.27)

Control rats are those from diet experiments after approximately 8 or 30 days; 5-7 values for blood, spleen and heart, 13-16 values for liver, kidney and testes. ±, standard deviation is given for means with 4 values; ranges are indicated in parentheses for means of 2 values.

Table 4. Pt concentration in rat tissues following the administration of single high doses of $Pt(SO_4)_2.4H_2O$

Treatment	Controls	$Pt(SO_4)_2.4H_2O$	
Route	Oral	Oral	I.p.
Dose of Pt (mg Pt/kg)	-	382	113
Tissue	Tissue Concentration of Pt (µg Pt/g wet weight of tissue)		
Liver	< 0.01 (0.004-0.006)	2.3 (1.2-3.5)	34 (30-38)
Kidney	< 0.008 (0.004-0.004)	16 (13-19)	37 (28-46)
Spleen	< 0.013 (0.007-0.011)	3.3 (2.3-4.2)	16 (12-20)
Heart	0.02	0.8	3.0
Testes	0.011 (0.009-0.013)	0.5 (0.4-0.6)	1.2 (0.9-1.5)
Brain	0.01	0.10 (0.07-0.14)	0.6 (0.07-1.1)
Blood	< 0.008	3.3	1.0

Range of 4 values for control liver, kidney and spleen, and range of 2-3 values of all other tissues are given in parentheses. Control values are the mean values of Pt concentration in 2 rats which received orally NaCl.

During the two-week observation period, the rats gained weight at a rate from one-third to three-fourths the rate of the control rats. In the orally treated rats, the highest concentration of Pt occurred in the kidney (approximately 16 μg Pt/g) and appreciable levels of Pt also occurred in liver and spleen (range, 1-4 μg Pt/g of wet tissue). In the intraperitoneably treated rats, the kidney, liver and spleen showed very high levels of Pt in the range of 10-40 μg of Pt/g of wet tissue.

In a comparable lethal dose experiment, rats were treated orally with a dose of $MnCl_2.4H_2O$ equivalent to 100% of the oral LD-50 value and the tissues were analyzed in surviving rats at the end of the 14-day observation period. In contrast to the finding with the Pt salt, the oral administration of a single, large but nonlethal dose of $MnCl_2.4H_2O$ to rats did not result in the retention after 14 days of excess concentrations of Mn in any of the tissues analyzed (Table 5). Due to low levels of absorption and/or a high capacity for excretion of the Mn, the tissue Mn levels of the experimental rats were approximately equal to the levels found in control rats.

These studies show that in rats treated with soluble Pt^{4+} salts, appreciable levels of the metal can be found in the kidney, liver and spleen. Further studies will be necessary to determine the effects of the Pt and other metals on various biochemical reactions.

V. ORGAN WEIGHTS

Weights of five organs (liver, kidney, spleen, heart and testes) were measured in rats which had been treated with various metallic

Table 5. Mn concentrations in rat tissues following the oral administration of a single large dose of $MnCl_2.4H_2O$

Treatment	Controls[a]	$MnCl_2.4H_2O$
Dose of Mn (mg Mn/kg)	–	416
Tissue	Tissue Concentration of Mn (μg Mn/g wet weight of tissue)	
Liver	1.60 ± 0.87	1.9 (1.3-2.5)
Kidney	0.75 ± 0.50	1.3 (1.0-1.5)
Spleen	1.46 ± 1.99	1.3 (1.1-1.5)
Heart	0.55 ± 0.35	0.7
Testes	0.44 ± 0.35	0.5 (0.4-0.5)
Brain	0.3	0.03
Blood	0.86 ± 0.44	0.4 (0.2-0.6)

Control values are from rats treated orally with NaCl, and rats on diet experiments for approximately 8 or 30 days. Means ± standard deviations are given for 6-7 samples of spleen, heart and blood and for 13-18 samples of liver, kidney and testes from control rats; ranges are given in parentheses where two values are available from Mn-treated rats.

salts in the diet (either drinking fluid or solid feed). The organ weights were expressed as the percentage of the body weight. In the discussions which follow, no consistent changes in organ weights occurred unless specifically noted. In general, following treatment with most metallic salts (at the doses used) weight changes were not observed in liver, spleen and heart; several metals changed the kidney weight and a few salts changed the testes weight.

$MnCl_2 \cdot 4H_2O$. $MnCl_2 \cdot 4H_2O$, in the drinking fluid at 8.3 or 18.6 mmoles/liter (1640 or 3690 mg/liter) for 90 days did not bring about major changes in organ weights, although there was some enlargement (12, 13, and 8% above control; not statistically significant) in the spleen of all three experimental groups of animals. None of the other organs of rats receiving $MnCl_2 \cdot 4H_2O$ in the drinking fluid showed consistent changes.

$PbCl_2$. In rats which received 3.7 mmoles/liter (1022 mg/liter) for 30 days or for 90 days, the size of the liver was increased (6-12% above control; only one statistically significant) in three of the four experiments. In contrast, the kidney size was increased in both the 30-day dietary experiments (7% and 6% above control; not statistically significant) and the 90-day experiments (17% and 23% above control; each statistically significant.) Consistent changes in organ weights were not noted in the cases of spleen, heart and testes although spleen showed increased size in two of four experiments. In a single experiment, $PbCl_2$ (8.3 mmoles/liter or 2300 mg/liter) for 30 days caused 18% and 25% (neither statistically significant) increases in kidney and spleen size, respectively. The increase in kidney size due to treatment with Pb^{2+} is consistent with the data by Hirsch (6) and by others.

$PdCl_2$ (anhydrous) and $PdCl_2 \cdot 2H_2O$. Although the addition of $PdCl_2$ (anhydrous) to the feed (13.2 mmoles/kg or 2345 mg/kg; 30-days) caused

changes in several organ weights, the pattern was not consistent to that found in a second experiment. The use of a saturated solution of $PdCl_2 \cdot 2H_2O$ as the drinking fluid (8-days) also did not cause a consistent change in any of the organ weights.

$PdSO_4$. The use of a saturated solution of $PdSO_4$ as the drinking fluid for 8 or 30 days did not cause a consistent pattern of changes in the organ weights of the experimental rats. In a single experiment in which solid $PdSO_4$ was added for 30-days to the feed at a level of 5.9 mmoles/kg feed (1.19 g salt/kg feed), no changes were observed in organ weights; each rat received a mean of 3.8 mmoles of Pd salt (0.40 g of Pd) during the total diet period.

$PtCl_4$. When this salt was added to the drinking fluid at 0.5 mmoles/liter (183 mg salt/liter) for 30 days or 90 days, or at 1.6 mmoles/liter (550 mg salt/liter) for 8 days, no consistent changes were detected in the weights of the five organs. If the concentration was increased to 1.6 mmoles/liter (550 mg salt/liter) for 30 days or to 2.4 mmoles/liter (825 mg salt/liter) for 9 days, the weight of the kidneys were increased by approximately 6% in each of 4 experiments but only two experiments gave $p < 0.1$.

In addition, in rats treated at the higher levels (1.6 mmoles/liter for 30 days or 2.4 mmoles/liter for 9 days), the testes were increased in weight by approximately 11% in each of the 4 experiments but the difference was statistically significant ($p < 0.05$) in only one experiment.

$Pt(SO_4)_2 \cdot 4H_2O$. At a concentration of 1.6 mmoles/liter (750 mg salt/liter) in the drinking fluid in 8-9 day experiments, increased kidney weight was observed in only one of 2 experiments and increased testes weight (approximately 8%) was found in each of the two experiments but none of the differences were statistically significant.

VI. DRUG METABOLISM IN VITRO

Selection of substrates. A wide variety of substrates are metabolized by the NADPH-dependent mixed function oxidase system of hepatic microsomes. The interaction of substrates with the cytochrome P-450 may produce one of several types of spectral changes. Various substrates produce a type I spectrum which is characterized by a peak at 385-390 nm and a trough at 419-425 nm; alternatively, other substrates produce a type II spectrum (trough at 390-405 nm; peak at 426-435 nm) upon interaction with cytochrome P-450 (8-10). In the current study, it was considered desirable to select one representative substrate for each type of interaction with cytochrome P-450. Consequently, in vitro drug metabolism studies by isolated hepatic microsomes were conducted with two substrates: the N-demethylase of aminopyrine, a type I substrate, and the p-hydroxylase of aniline, a type II substrate (11, 12). However, the removal of one N-methyl group from aminopyrine yields a metabolite which has a type II spectrum and which is N-demethylated (11).

The yield of microsomal protein (mg/g liver) found in this study is appreciably greater than the yield of approximately 20 mg/g liver reported in various publications for liver of fed rats. The difference can be attributed predominantly to the fasting period of approximately 14 hours (range 13-15 hours) used before the removal of rat tissues in these studies. For example, McLean and Day (13), using male Wistar rats weighing approximately 150-250 g, the levels of microsomal protein (mg/g liver) were 25 ± s.d. 2 and 38± s.d. 10 in fed and 18-hour-fasted rats, respectively; the latter value is in close agreement with the yield of 40 ± s.d. 5 (15 groups) found in the livers of 14-hour-fasted, Sprague-Dawley rats in this study. The control values obtained in these experiments for the activities of aniline hydroxylase and aminopyrine demethylase and for the content of cytochrome P-450 and cytochrome b5 are within the range of control values reported for male rats in various studies.

After experimental rats were maintained on metal-containing diets for approximately 8, 30 or 90 days, hepatic microsomes were isolated and parameters related to drug metabolism were measured. Data on presented in Table 6. Microsomal protein was expressed as mg microsomal protein/g liver; aniline hydroxylase activity, nmoles p-aminophenol produced/mg microsomal protein/20 min; aminopyrine demethylase, nmoles formaldehyde produced/mg microsomal protein/10 min; cytochrome P-450 and cytochrome b5, nmoles/mg microsomal protein.

Treatment with $MnCl_2 \cdot 4H_2O$ for approximately 90 days at either 8.3 mmoles (1.6 g salt)/liter or 18.6 mmoles (3.7 g salt)/liter did not appear to consistently change any of the parameters (Table 6A). Treatment with $PbCl_2$ at 3.7 mmoles (1.0 g salt)/liter for 90 days or 8.3 mmoles (2.3 g salt)/liter for approximately 30 days did not affect recovery of microsomal protein or the activity of aniline hydroxylase; for the other three parameters, it was not possible to detect a trend since data were obtained for only one experiment each.

$PdCl_2 \cdot 2H_2O$, a slightly soluble salt, was administered to rats as a saturated solution as the drinking fluid for 8 days and a marked decrease was noted in the activities of aniline hydroxylase and aminopyrine demethylase (Table 6B). However, caution must be exercised in the interpretation of this trend. $PdSO_4$, which is also a slightly soluble salt, was administered as a saturated solution as the drinking fluid for 8 days or for approximately 30 days. In each of these experiments no trend of changes was observed in the activities of the two enzymes (Table 6C). At this time, it is not possible to explain the apparent differences as a result of the administration the two slightly soluble salts of Pd^{2+}.

Table 6A.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean metal consumption /rat/ diet period	Microsomal protein (mg/g liver)	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$MnCl_2 \cdot 4H_2O$	58 Control, paired		93	2.6 mmoles	45.9 ±2.8	14.5 ±1.4	62.2 ±5.0	0.870 ±0.046	0.369 ±0.026
	59 Mn-treated	8.29 (1640) /liter	93	40.3 mmoles (2.2g Mn)	50.3 ±3.4 m 109%	13.9 ±0.4 ns	56.5 ±6.9 ns	0.812 ±0.088 ns	0.326 ±0.028 * 88%
$MnCl_2 \cdot 4H_2O$	62 Control, Paired		88	2.8 mmoles	36.4 ±0.8	22.5 ±1.1	73.7 ±6.0	0.750 ±0.098	0.389 ±0.039
	63 Mn-treated	18.65 (3690) /liter	88	71.8 mmoles (3.9g Mn)	36.2 ±2.1 ns	24.0 ±4.0 ns	75.0 ±8.1 ns	0.710 ±0.060 ns	0.395 ±0.037 ns

Table 6B.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean metal consumption /rat/ diet period	Microsomal protein (mg/g liver	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$PdCl_2 \cdot 2H_2O$	36 Control, paired		8		45.4 ±2.6	14.2 ±2.4	57.8 ±1.0	nm	nm
	37 Pd-treated	(satd. soln.)	8		51.6 ±4.1 m 113%	11.0 ±1.8 m 77%	52.0 ±3.4 * 90%	nm	nm
	31 Control, paired		8		43.6 ±3.6	14.5 ±0.5	67.7 ±4.9	nm	nm
	30 Pd-treated	(satd. soln.)	8		41.8 ±1.4 ns	9.7 ±2.6 * 67%	44.9 ±8.9 ** 66%	nm	nm

Table 6C.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean metal consumption /rat/ diet period	Microsomal protein (mg/g liver	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$PdSO_4$	68 Control, paired		8		42.1 ±1.9	15.0 ±1.5	56.3 ±3.6	nm	nm
	69 Pd-treated	(satd. soln.)	8		42.5 ±3.5 ns	16.4 ±2.1 ns	49.4 ±6.9 ns 88%	nm	nm
$PdSO_4$	60 Control, paired		31		48.4 ±2.3	19.1 ±1.0	76.8 ±8.4	0.587 ±0.083	0.282 ±0.017
	61 Pd-treated	(satd. soln.)	31		47.2 ±3.3 ns	17.3 ±4.0 ns 90%	73.6 ±15.0 ns	0.632 ±0.049 ns	0.280 ±0.023 ns

$PtCl_2$, an "insoluble" salt of Pt^{2+}, was administered to rats as a saturated solution as the drinking fluid for approximately 30 days. No consistent changes were observed in the activities of aniline hydroxylase and aminopyrine demethylase although the former enzyme showed decreased activity in two of the three experiments (Table 6D).

The Pt^{4+} salts, $PtCl_4$ and $Pt(SO_4)_2 \cdot 4H_2O$, are very soluble and were included in the drinking water. A wide range of dosages and durations were used in the dietary experiments: 0.54 mmoles (183 mg $PtCl_4$)/liter for 30 and 90 days, and 1.6 mmoles (550 mg $PtCl_4$ or 750 mg $Pt(SO_4)_2 \cdot 4H_2O$/liter) for 8 days and for 30 days ($PtCl_4$ only). Under none of the conditions were consistent changes observed in the level of the drug metabolizing enzymes (aniline hydroxylase or aminopyrine demethylase), or in the recovery of microsomal protein in the liver (Tables 6E, 6F).

It is apparent that the activities of the two representative drug metabolizing enzymes and amounts of cytochromes P-450 and b5 are not extremely sensitive to lower levels of dietary Pt^{4+} and Pd^{2+}. In order to administer in the diet sufficient Pt^{4+} and Pd^{2+} to affect these parameters of drug metabolism, it will be essential or preferable to administer the metallic salts in the solid feed rather than in the drinking fluid. Obviously, the use of higher doses of the insoluble or slightly soluble salts will require administration in the solid feed. Such studies are currently in progress.

Table 6D.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean metal consumption /rat/ diet period	Microsomal protein (mg/g liver	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$PtCl_2$	7 Control, paired		31		46.0 ±3.2	16.3 ±2.2	64.3 ±20.7	nm	nm
	8 Pt-treated	(satd. soln.)	31		43.9 ±3.0 ns	13.5 ±1.4 m 83%	63.9 ±17.6 ns	nm	nm
	9 Control, paired		30		55.0 ±11.3	11.4 ±1.3	55.9 ±14.3		
	11,12 Pt-treated	(satd. soln.)	30		43.1 ±3.0 m 78%	8.3 ±1.4 * 73%	81.6 ±13.7 * 146%	nm	nm
	29 Control, paired		29		46.5 ±3.1	16.0 ±3.1	80.1 ±16.9	nm	nm
	28 Pt-treated	(satd. soln.)	29		44.6 ±1.4 ns	15.9 ±2.3 ns	73.1 ±7.9 ns	nm	nm

Table 6E.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean Metal consumption /rat/ diet period	Microsomal protein (mg/g liver	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$PtCl_4$	22 Control, paired		30	0.00 mmoles	41.1 ±4.4	16.5 ±3.5	74.0 ±16.1	nm	nm
	23 Pt-treated	0.544 (183) /liter	30	0.54 mmoles (105 mg Pt)	40.2 ±3.1 ns	15.5 ±0.5 ns	76.3 ±11.2 ns	nm	nm
	20 Control, paired		29	0.00 mmoles	41.7 ±3.7	12.8 ±3.0	64.8 ±10.4	nm	nm
	21 Pt-treated	0.544 (183) /liter	29	0.47 mmoles (91 mg Pt)	40.8 ±3.2 ns	13.6 ±3.7 ns	70.6 ±22.0 ns	nm	nm
$PtCl_4$	52 Control, paired		91	0.00 mmoles	49.1 ±4.9	11.6 ±1.2	55.7 ±10.6	0.790 ±0.140	0.324 ±0.044
	53 Pt-treated	0.544 (183) /liter	91	1.97 mmoles (384 mg Pt)	46.7 ±3.9 ns	14.3 ±2.1 m 123%	63.4 ±3.4 ns 114%	0.763 ±0.128 ns	0.348 ±0.050 ns

Table 6F.

Metallic Salt	Dietary Group	Dietary concn., mmoles /liter or kg (mg salt /liter or kg)	Days on diet	Mean metal consumption /rat/ diet period	Microsomal protein (mg/g liver	Aniline hydroxylase	Aminopyrine demethylase	Cytochrome P-450	Cytochrome b5
$PtCl_4$	46 Control, paired		29	0.00 mmoles	39.5 ±3.6	27.3 ±2.0	105.7 ±9.5	0.478 ±0.031	0.326 ±0.027
	47 Pt-treated	1.63 (550) /liter	29	1.63 mmoles (317 mg Pt)	42.1 ±2.7 ns	25.3 ±1.6 ns	104.3 ±9.4 ns	0.575 ±0.107 ns 120%	0.324 ±0.011 ns
	38 Control, paired		29	0.00 mmoles	45.6 ±1.2	25.4 ±2.5	85.8 ±6.6	nm	nm
	39 Pt-treated	1.63 (550) /liter	29	1.34 mmoles (261 mg Pt)	42.6 ±8.4 ns	23.4 ±2.6 ns	86.2 ±12.9 ns	nm	nm
	27 Control, paired		29	0.00 mmoles	47.9 ±3.4	14.0 ±1.3	76.6 ±5.9	nm	nm
	26 Pt-treated	1.63 (550) /liter	29	1.27 mmoles (248 mg Pt)	45.6 ±2.6 ns	15.0 ±1.4 ns	77.7 ±7.7 ns	nm	nm

VII. STUDIES ON RNA SYNTHESIS IN VIVO

Two major rRNA-containing peaks resulted from centrifugation of resuspended hepatic polysomes on a sucrose gradient containing 0.1 M NaCl and 0.001 M EDTA. The peaks had sedimentation coefficients of approximately 27S and 43S. A_{260}/A_{280} ratios of the isolated peaks indicated a greater content of protein than in corresponding peaks prepared from phenol-SDS or SDS-extracted rRNA. When compared with profiles of phenol-SDS or SDS-extracted rRNA, material treated with only EDTA and NaCl exhibited greater homogeniety and/or tighter conformation. Increases in gradient NaCl concentration (0.25 M or 0.5 M) resulted in additional peaks, all having S values greater than 27S. As indicated by labeling studies for 1 h and 24 h, the EDTA-NaCl procedure was comparable to the SDS extraction procedure for observation of rRNA. Ribosomal RNA from RNase-treated polysomes (5 mg or 11 mg RNA/0.1 µg RNase) showed greater structural integrity following EDTA-NaCl treatment than following SDS extraction. When compared with diethylpyrocarbonate as a means of improving resolution of rRNA following RNase degradation of polysomes, the EDTA-NaCl procedure gave equally satisfactory results with significantly greater convenience. A manuscript describing this study has been prepared.

VIII. REFERENCES

1. Litchfield, J. T., and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99 (1949).

2. Imai, Y., Ito, A., and Sato, R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J. Biochem. (Tokyo) 60: 417 (1966).

3. Chhabra, R. S., Gram, T. E., and Fouts, J. R. A comparative study of two procedures used in the determination of hepatic microsomal aniline hydroxylation. Toxicol. Appl. Pharmacol. 22: 50 (1972).

4. Schenkman, J. B., Remmer, H., and Estabrook, R. W. Spectral studies of drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 3: 113 (1967).

5. Yoakum, A. M., Stewart, P. L., and Sterrett, J. E. Method development and subsequent survey analysis of biological tissues for platinum, lead and manganese content. Environ. Health Perspect. (1975)

6. Hirsch, G. H. Effect of chronic lead treatment on renal function. Toxicol. Appl. Pharmacol. 25: 84 (1973).

7. Christensen, H. E. (ed.), National Institute for Occupational Safety and Health Toxic Substances List, 1972 Edition (1972).

8. Schenkman, J. B., Remmer, H. and Estabrook, R. W. Mol. Pharmacol., 3, 113-123 (1967).

9. Mannering, G. J., Sladek, N. E., Parli, C. J. and Shoeman, D. W. In Microsomes and Drug Oxidations (J. R. Gillette et al., ed.). Academic Press (1969).

10. Hayes, J. R. and Campbell, T. C. Biochem. Pharmacol., 23, 1721-1731 (1974).

11. Correia, M. A. and Mannering, G. J. Mol. Pharmacol., 9, 470-485 (1973).

12. Flynn, E. J., Lynch, M. and Zannoni, V. G. Biochem. Pharmacol., 21, 2577-2590 (1972).

13. McLean, A. E. M. and Day, P. A. Biochem. Pharmacol., 23, 1173-1180 (1974).

X. APPENDIX; DATA ON LETHAL DOSE LEVELS

Values are the means (or weighted means) ± (upper) standard deviations ± (lower) standard errors; the number of values is given in parentheses. NA, not applicable or not applied. In the column of "percentage survival", the underlined values were used for the determination of the LD-50, LD-10 and LD-90 doses by the method of Litchfield and Wilcoxon. The standard deviations and standard error in the percentage survival column are calculated from the products of the percentage survival and the number of rats in each experiment in which that dose was tested. Statistical analyses (student's t-test) were applied only to weekly weight gains: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; m, $0.05 < p < 0.10$; no marking used were $p > 0.10$. Percentage changes of the weight gains are indicated only where $p < 0.10$ or where the percentage was less than 90% or more than 110% of the control values.

[a]"Corrected value from Table 1 of Litchfield and Wilconon for measured 0% or measured 100% survival.

[b]"Expected value" was <0.01% or >99.99% by method of Litchfield and Wilcoxon and not used in determination of lethal doses.

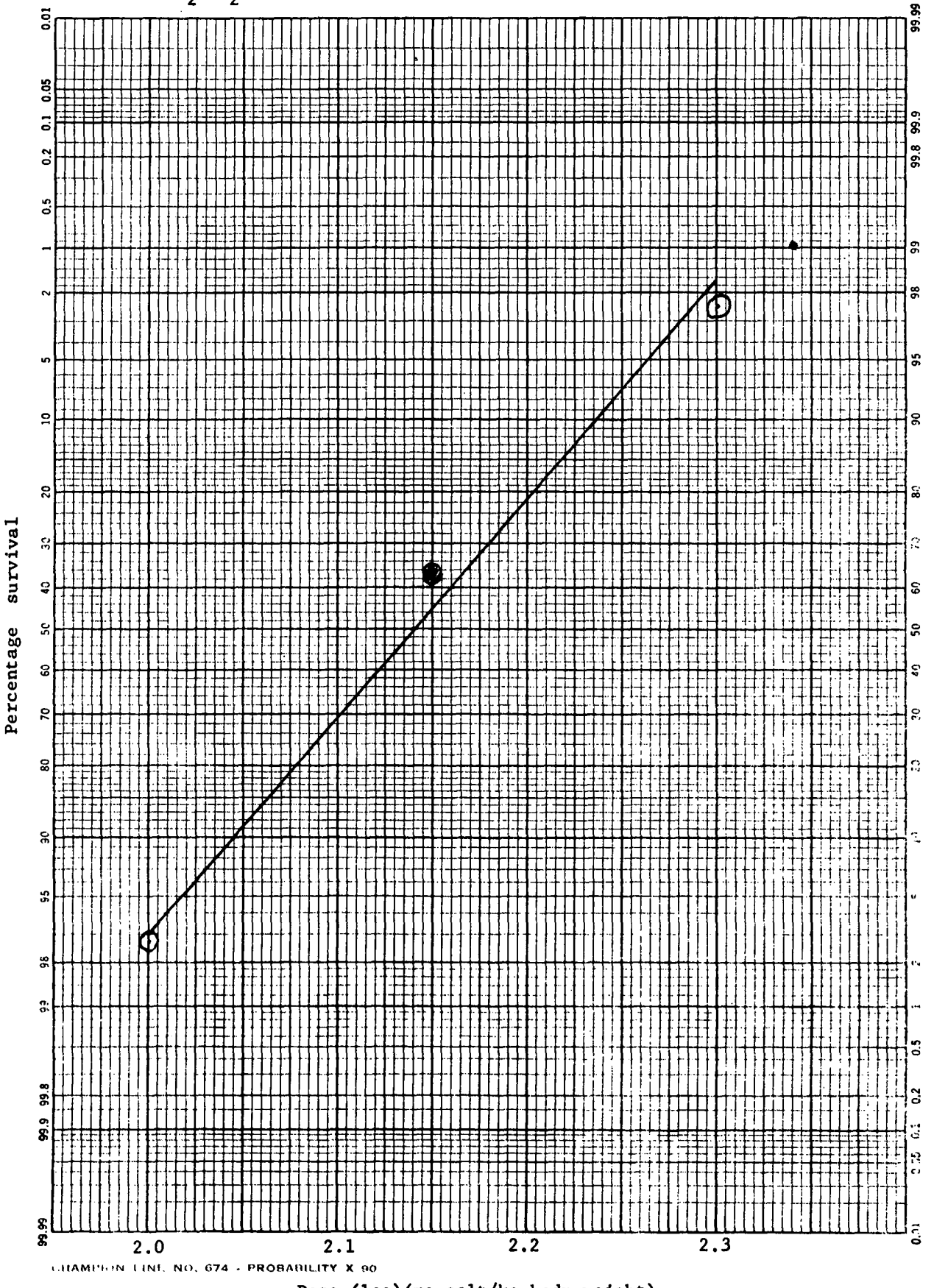
[c]Value not used in determination of lethal doses.

[d]Value approximated by assumption of 0.25 survivors/number tested where 0% survival was measured, and 0.25 non-survivors/number tested where 100% survival was measured.

$MnCl_2 \cdot 4H_2O$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	21,25, 32,34	10/10	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (10)	NA	117 ±8 ±3 (10)	63 ±11 ±3 (10)	110 ±13 ±4 (10)	48 ±9 ±3 (10)
400	2,21, 25	0/11	NA[b] 0.0 ±0.0 ±0.0	2 ±2 ±1 (11)	2 ±2 ±1 (11)	112 ±12 ±4 (11)	NA	NA	NA
200	2,21, 25	0/10	2.5[d] 0.0 ±0.0 ±0.0	8 ±4 ±1 (10)	8 ±4 ±1 (10)	112 ±9 ±3 (10)	NA	NA	NA
141.4	32,34	4/12	33.3 33.3 ±0.0 ±0.0	120 ±159 ±46 (12)	12 ±3 ±1 (8)	114 ±13 ±4 (12)	18 ±34 ±17 (4) * 29%	56 ±57 ±28 (4) m 51%	38 ±25 ±12 (4) ns 79%
100	2,21, 25	11/11	97.7[d] 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	NA	112 ±8 ±2 (11)	33 ±19 ±6 (9) *** 52%	81 ±21 ±7 (9) ** 74%	48 ±12 ±4 (9) ns
50	21,25	11/11	NA[b] 100.0 ±0.0 +0.0	336 ±0 ±0 (11)	NA	112 ±8 ±2 (11)	45 ±11 ±3 (11) ** 71%	96 ±15 ±4 (11) * 87%	51 ±12 ±4 (11) ns

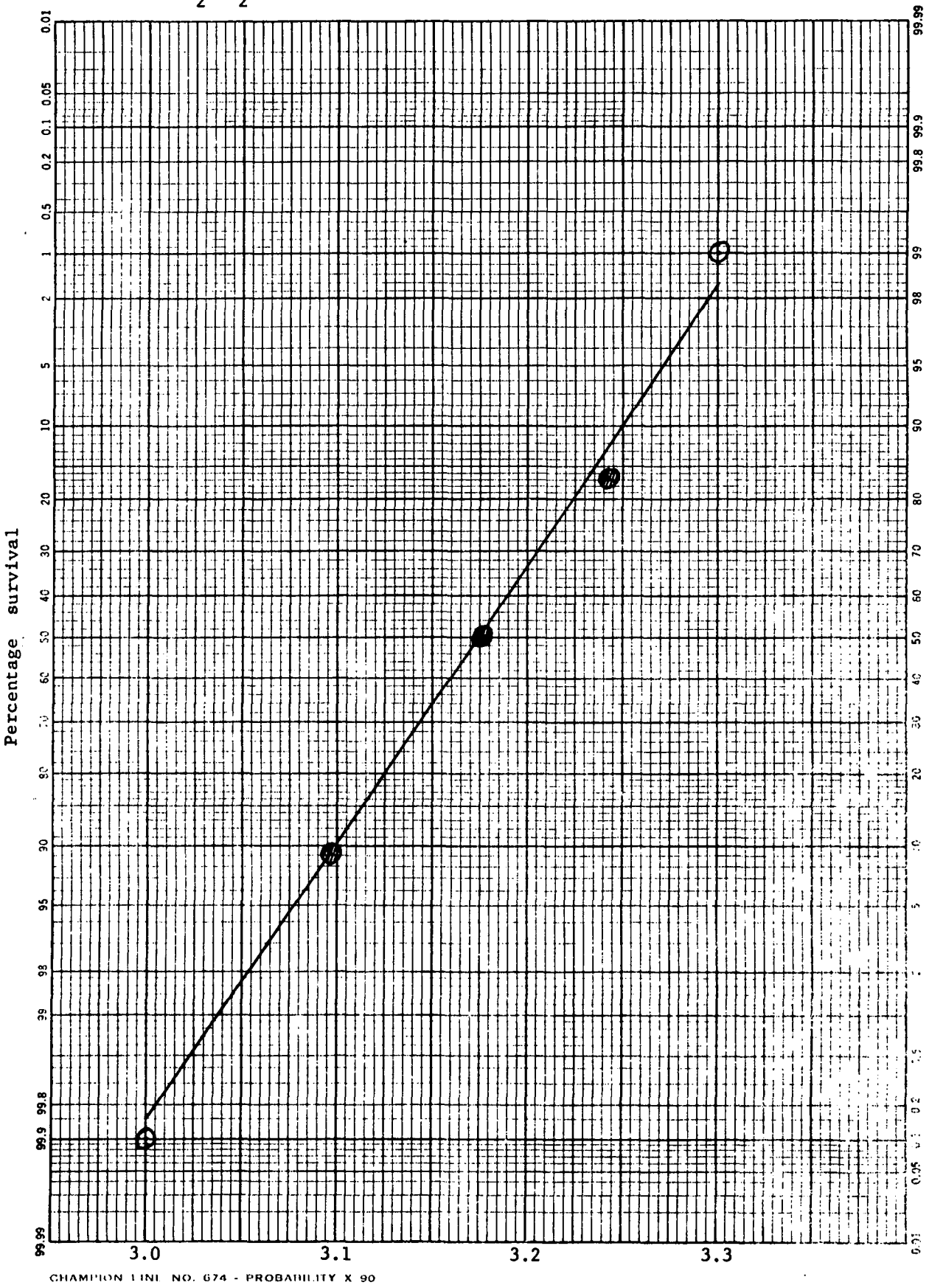
$MnCl_2.4H_2O$ / Intraperitoneal injection



$MnCl_2 \cdot 4H_2O$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr) all animals	Duration of survival (hr) non-survivors	Body weight (g) on day 0	Weight gain (g) days 0-7	Weight gain (g) days 0-14	Weight gain (g) days 7-14
Controls	5,8, 14,23	14/14	NA	336 ±0 ±0 (14)	NA	107 ±10 ±3 (14)	51 ±10 ±3 (14)	100 ±31 ±8 (14)	49 ±23 ±6 (14)
2500	1,5, 14,23	1/12	NA[c] 8.3 ±15.1 ±4.3	40 ±94 ±27 (12)	13 ±11 ±3 (11)	109 ±9 ±2 (12)	NA	NA	NA
2000	5,8, 14,23	0/12	1.0[a] 0.0 ±0.0 ±0.0	22 ±23 ±7 (12)	22 ±23 ±7 (12)	103 ±7 ±2 (12)	NA	NA	NA
1750	8,14, 23	2/12	16.7 16.7 ±14.7 ±4.2	106 ±114 ±33 (12)	60 ±43 ±14 (10)	109 ±8 ±2 (12)	NA	NA	NA
1500	5,8, 14,23	6/12	50.0 50.0 ±28.9 ±8.3	213 ±133 ±38 (12)	90 ±48 ±19 (6)	103 ±14 ±4 (12)	22 ±22 ±9 (6) ** 43%	84 ±26 ±10 (6) ns 84%	62 ±9 ±3 (6) m 127%
1250	1,8, 14,23	10/11	90.9 90.9 ±12.6 ±3.8	307 ±97 ±29 (11)	NA	111 ±10 ±3 (11)	39 ±25 ±9 (7) ns 76%	103 ±26 ±10 (7) ns	64 ±10 ±4 (7) m 131%
1000	5,8, 14,23	12/12	99.9[a] 100.0 ±0.0 ±0.0	336 ±0 ±0 (12)	NA	105 ±7 ±2 (12)	40 ±19 ±5 (12) m 78%	99 ±20 ±6 (12) ns	59 ±14 ±4 (12) ns 120%

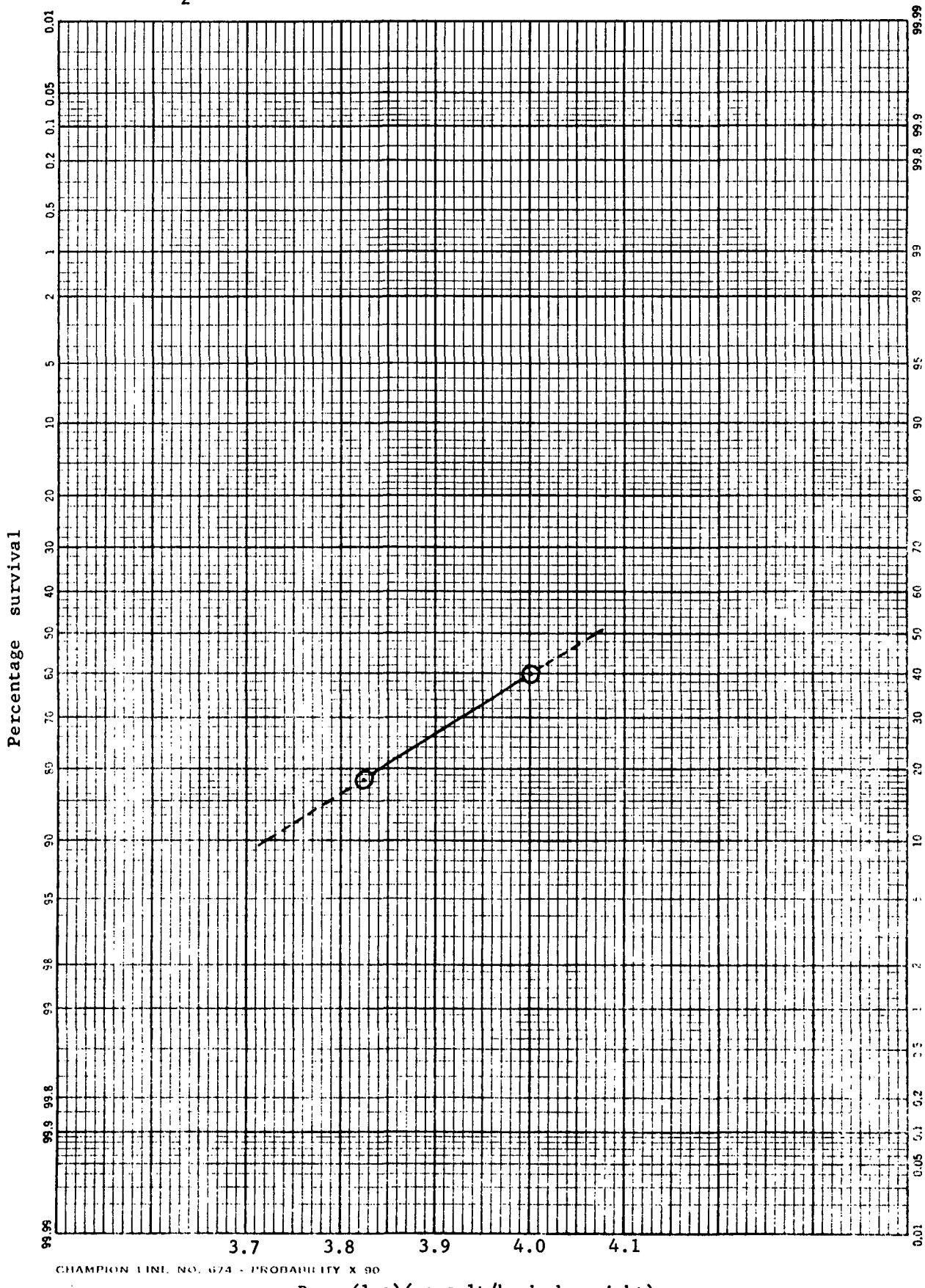
$MnCl_2.4H_2O$ / Oral administration



MnO_2 / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	33,38	6/6	NA 100.0 ±0.0 ±0.0	336 ±0 ±0	NA	99 ±9 ±4 (6)	68 ±11 ±4 (6)	128 ±12 ±5 (6)	60 ±7 ±3 (6)
10,000	33,38	6/10	60.0 60.0 ±0.0 ±0.0	206 ±168 ±53 (10)	11 ±8 ±4 (4)	103 ±10 ±3 (10)	58 ±15 ±6 (6) ns 85%	120 ±16 ±6 (6) ns	62 ±6 ±2 (6) ns
6,666	38	9/11	81.8 81.8	285 ±113 ±34 (11)	NA	99 ±10 ±3 (11)	51 ±18 ±6 (9) * 75%	102 ±44 ±15 (9) ns 80%	50 ±28 ±9 (9) ns 83%

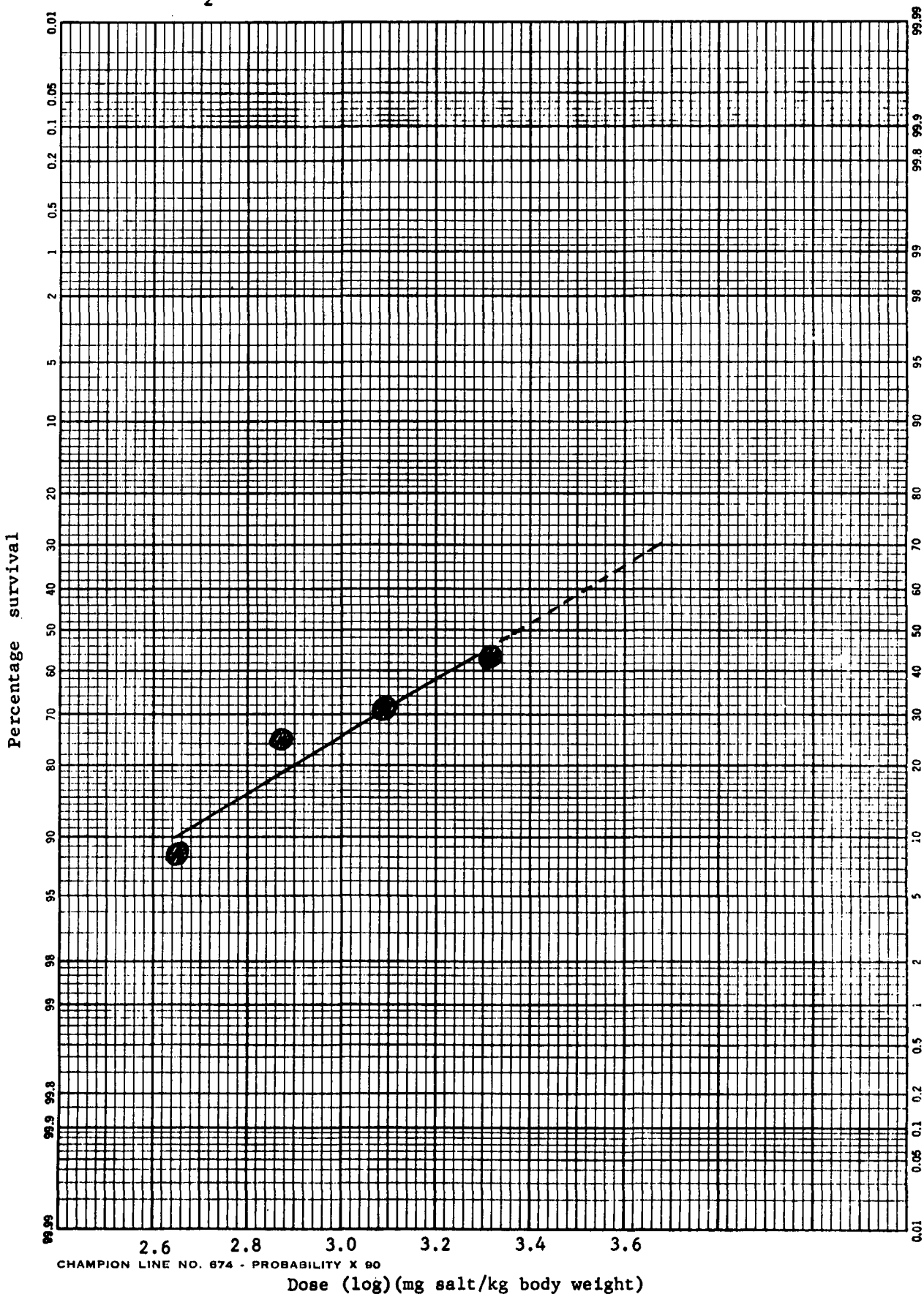
MnO_2 / Oral administration



$PbCl_2$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	12,21, 28,41, 48	13/13	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (13)	NA	111 ±9 ±3 (13)	55 ±19 ±5 (13)	125 ±17 ±5 (10)	62 ±11 ±4 (10)
2083	28,41, 48	9/16	56.2 56.2 ±14.3 ±3.6	227 ±130 ±33 (16)	87 ±39 ±15 (7)	101 ±6 ±2 (16)	30 ±24 ±8 (10) * 55%	76 ±36 ±12 (9) ** 61%	41 ±21 ±7 (9) * 66%
1250	21,28, 41,48	11/16	68.8 68.8 ±27.1 ±6.8	257 ±126 ±31 (16)	82 ±63 ±28 (5)	110 ±9 ±2 (16)	31 ±18 ±5 (12) ** 56%	63 ±37 ±11 (11) *** 50%	31 ±30 ±9 (11) ** 50%
750	12,21, 28,41, 48	12/16	75.0 75.0 ±29.8 ±7.5	304 ±74 ±18 (16)	206 ±101 ±50 (4)	106 ±9 ±2 (16)	27 ±18 ±5 (15) *** 49%	57 ±37 ±11 (11) *** 46%	32 ±16 ±5 (11) *** 52%
450	12,21, 28,41	11/12	91.7 91.7 ±12.3 ±3.6	325 ±39 ±11 (12)	NA	111 ±6 ±2 (12)	29 ±25 ±7 (12) ** 53%	54 ±44 ±16 (8) *** 43%	28 ±24 ±9 (8) ** 45%
270	21,28	7/7	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (7)	NA	116 ±4 ±2 (7)	29 ±16 ±6 (7) ** 53%	74 ±30 ±11 (7) ** 59%	44 ±16 ±6 (7) * 71%

$PbCl_2$ / Intraperitoneal injection



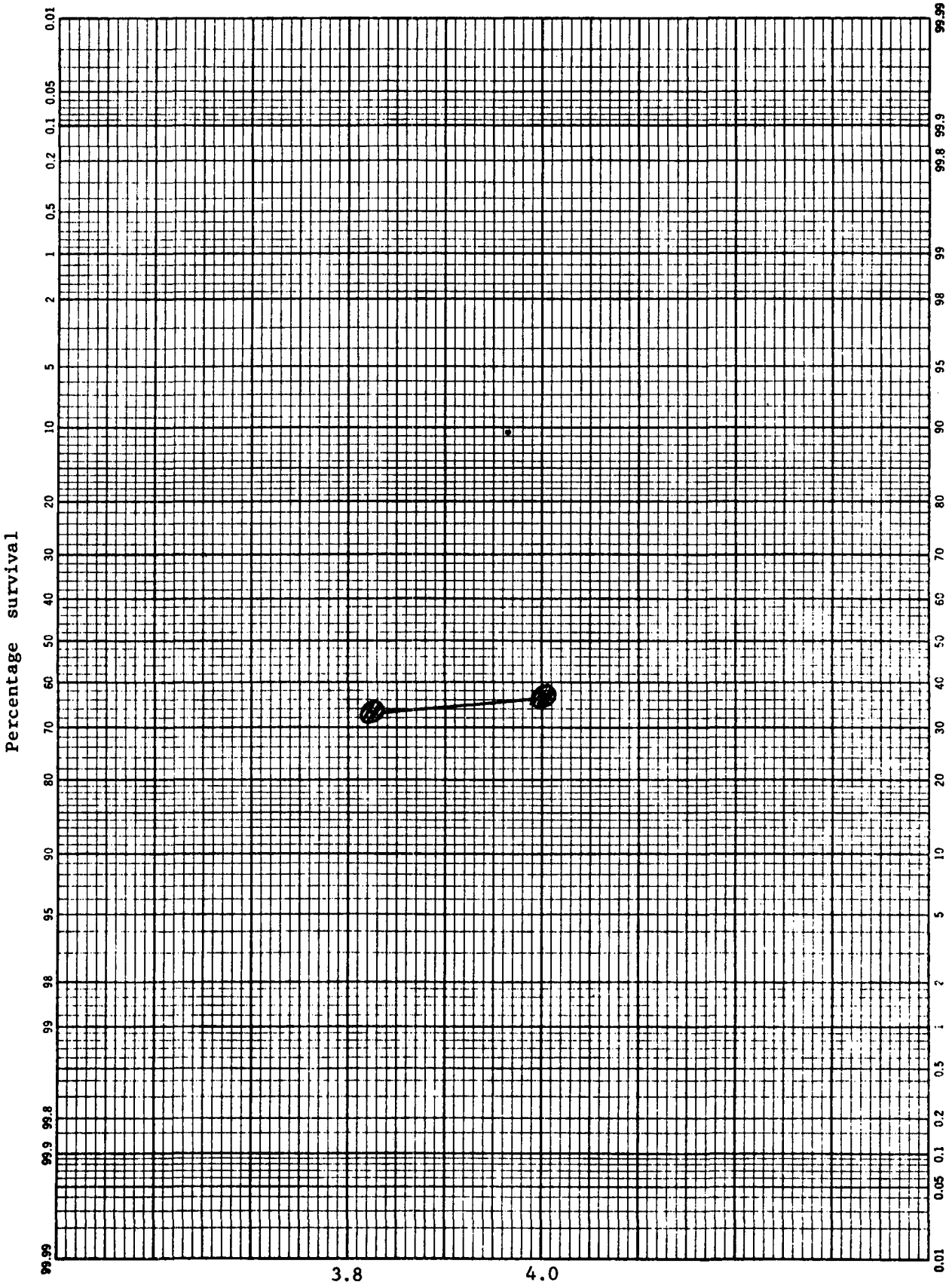
$PbCl_2$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	51	2/2	NA 100.0	NA	NA	NA	NA	NA	NA
9645	51	9/10	90.0 90.0	305 ±97 ±31 (10)	NA	118 ±12 ±4 (10)	44 ±13 ±4 (9)	107 ±12 ±4 (9)	63 ±5 ±2 (9)
5787	51	9/10	90.0 90.0	325 ±35 ±11 (10)	NA	116 ±10 ±3 (10)	54 ±27 ±9 (10)	115 ±31 ±10 (9)	55 ±14 ±5 (9)

PbO / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	33,39	6/6	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (6)	NA	105 ±6 ±2 (6)	64 ±15 ±6 (6)	106 ±33 ±13 (6)	43 ±18 ±7 (6)
10,000	33,39	7/11	63.6 63.6 ±15.7 ±4.7	217 ±165 ±50 (11)	9 ±4 ±2 (4)	103 ±10 ±3 (11)	32 ±19 ±7 (7) ** 50%	96 ±15 ±6 (7) ns	64 ±8 ±3 (7) * 149%
6,666	39	6/9	66.7 66.7	228 ±161 ±54 (9)	13 ±4 ±2 (3)	97 ±4 ±1 (9)	44 ±12 ±5 (6) * 69%	105 ±14 ±6 (6) ns	61 ±12 ±5 (6) m 142%

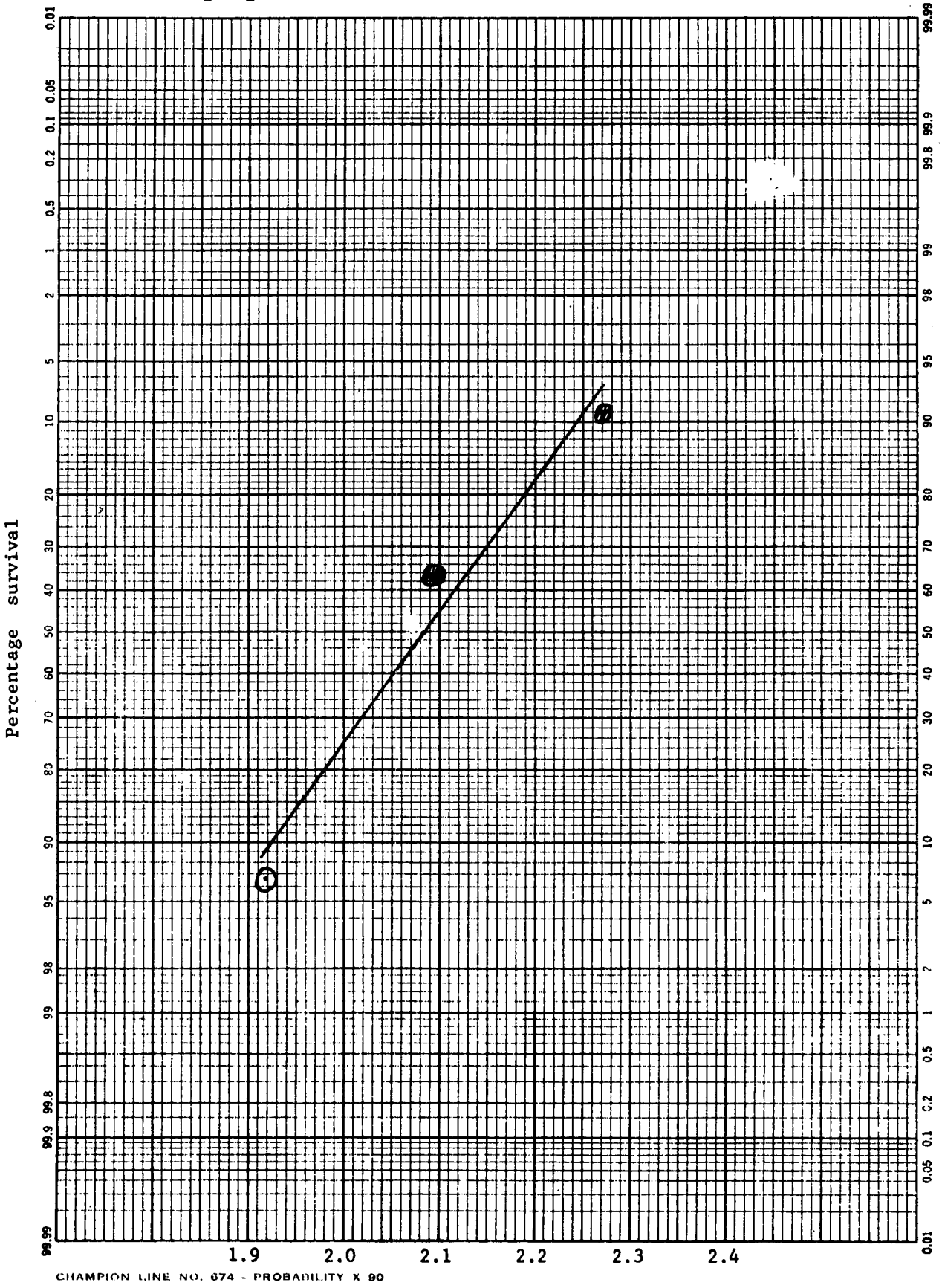
PbO / Oral administration



$PdCl_2 \cdot 2H_2O$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	17,24, 37	8/8	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (8)	NA	97 ±7 ±2 (8)	60 ±8 ±3 (8)	118 ±15 ±5 (8)	57 ±9 ±3 (8)
279	17,24	0/5	NA 0.0 ±0.0 ±0.0	17 ±6 ±3 (5)	17 ±6 ±3 (5)	95 ±6 ±2 (5)	NA	NA	NA
186	17,24, 37	1/11	9.1 9.1 ±8.7 ±2.6	59 ±94 ±28 (11)	32 ±22 ±7 (10)	96 ±5 ±2 (11)	NA	NA	NA
124	17,24, 37	4/11	36.4 36.4 ±19.5 ±5.9	172 ±138 ±42 (11)	78 ±58 ±22 (7)	101 ±4 ±1 (11)	33 ±19 ±9 (5) * 55%	86 ±8 ±4 (4) *** 73%	46 ±6 ±3 (4) * 81%
82.7	24,37	11/11	92.6[a] 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	336 ±0 ±0 (11)	98 ±8 ±2 (11)	59 ±8 ±2 (11) ns	119 ±11 ±3 (11) ns	60 ±8 ±2 (11) ns
55.1	24,37	11/11	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	336 ±0 ±0 (11)	97 ±5 ±2 (11)	57 ±11 ±3 (11) ns	116 ±21 ±6 (11) ns	59 ±13 ±4 (11) ns

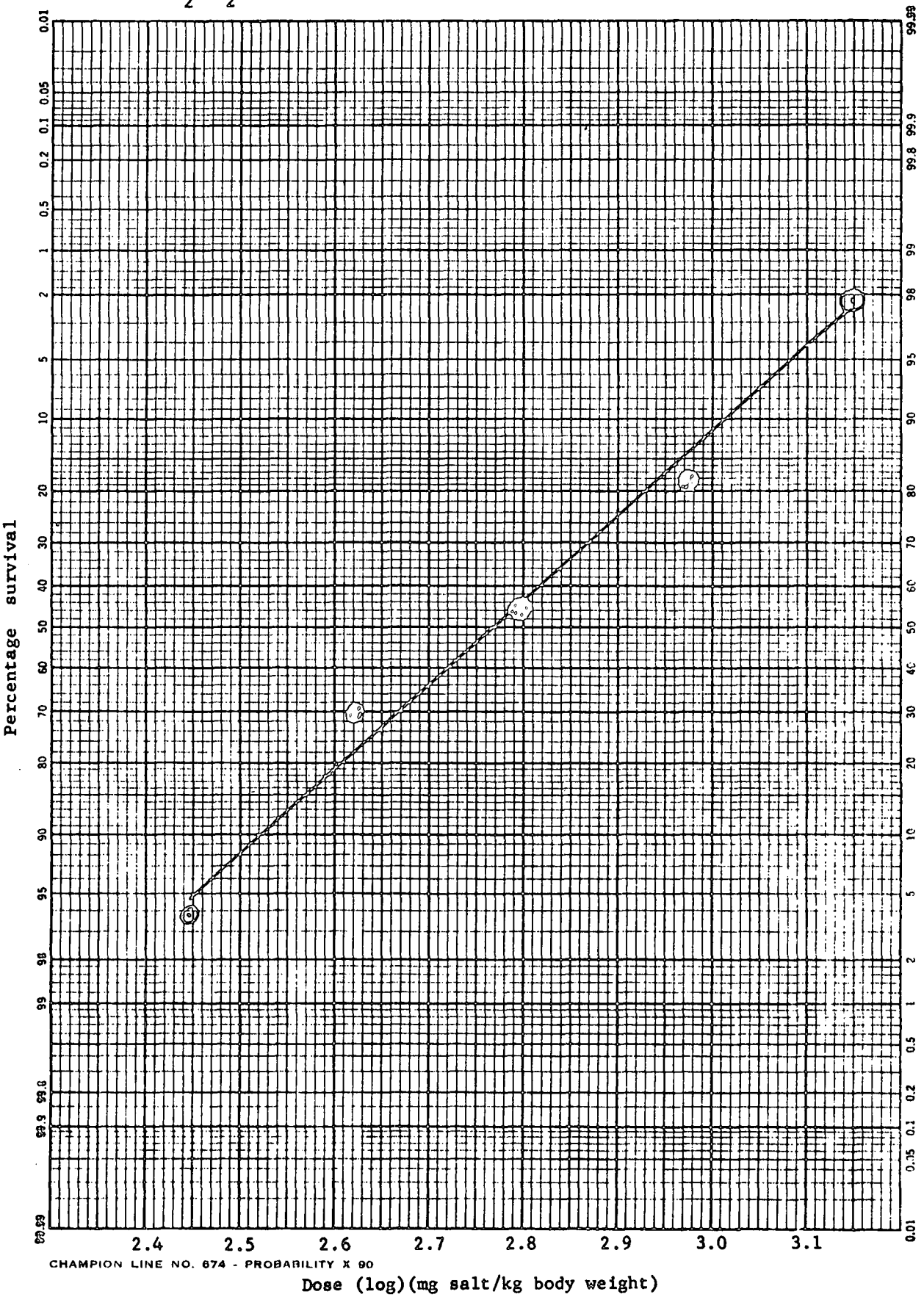
$PdCl_2.2H_2O$ / Intraperitoneal injection



$PdCl_2 \cdot 2H_2O$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr) all animals	Duration of survival (hr) non-survivors	Body weight (g) on day 0	Weight gain (g) days 0-7	Weight gain (g) days 0-14	Weight gain (g) days 7-14
Controls	15,16, 18,25	9/9	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (9)	NA	108 ±13 ±4 (9)	57 ±8 ±3 (9)	115 ±13 ±4 (9)	59 ±9 ±3 (9)
1410	16,18, 25	0/11	2.2[a] 0.0 ±0.0 ±0.0	58 ±57 ±17 (11)	58 ±57 ±17 (11)	109 ±11 ±3 (11)	NA	NA	NA
940	15,16, 18,25	2/11	18.2 18.2 ±31.2 ±9.4	125 ±108 ±32 (11)	79 ±31 ±10 (9)	102 ±11 ±3 (11)	NA	NA	NA
627	15,16, 18,25	5/11	45.5 45.5 ±32.6 ±9.8	213 ±130 ±39 (11)	110 ±76 ±31 (6)	106 ±8 ±3 (11)	14 ±23 ±9 (6) *** 25%	69 ±31 ±14 (5) ** 60%	51 ±13 ±6 (5) ns 86%
418	15,16, 18,25	7/10	70.0 70.0 ±29.2 ±9.2	276 ±100 ±32 (10)	136 ±52 ±30 (3)	103 ±8 ±3 (10)	11 ±20 ±7 (8) *** 35%	57 ±40 ±15 (7) ** 50%	42 ±23 ±9 (7) m 71%
279	15,16 18,25	11/11	96.2[a] 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	NA	107 ±11 ±3 (11)	37 ±28 ±8 (11) * 65%	89 ±30 ±9 (11) * 77%	52 ±9 ±3 (11) ns 88%
186	15	3/3	NA 100.0	336 ±0 ±0 (3)	NA	98 ±5 ±3 (3)	49 ±10 ±6 (3) ns 86%	106 ±14 ±8 (3) ns	57 ±4 ±3 (3) ns

$PdCl_2.2H_2O$ / Oral administration



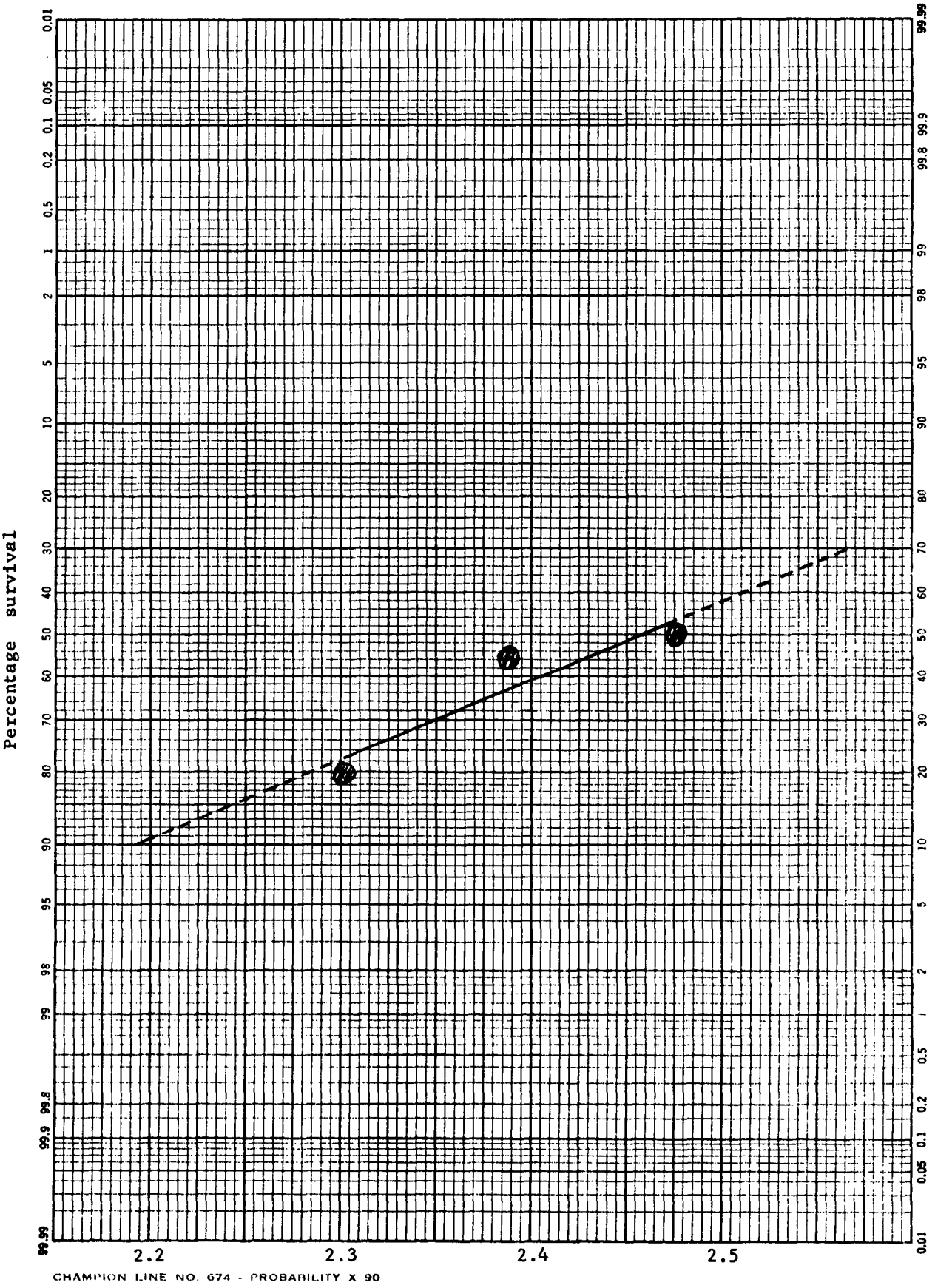
PdO / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	33,54	5/5	NA	336	NA	108	69	130	62
			100.0	±0		±7	±12	±10	±7
			±0.0	±0		±3	±5	±4	±3
			±0.0	(5)		(5)	(5)	(5)	(5)
10,000	33,54	6/6	100.0	336	NA	103	50	98	48
			100.0	±0		±13	±18	±35	±17
			±0.0	±0		±5	±7	±14	±7
			±0.0	(5)		(6)	(6)	(6)	(6)
						ns	m	m	ns
							72%	75%	77%

$PdSO_4$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	22,40, 47	9/9	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (9)	NA	107 ±4 ±2 (9)	62 ±10 ±3 (9)	126 ±19 ±6 (9)	64 ±12 ±4 (9)
450	40	0/4	NA 0.0	52 ±52 ±26 (4)	52 ±52 ±26 (4)	101 ±6 ±3 (4)	NA	NA	NA
300	40,47	5/10	50.0 50.0 ±21.5 ±6.8	219 ±127 ±40 (10)	101 ±40 ±18 (5)	103 ±7 ±2 (10)	44 ±10 ±4 (5) ** 71%	89 ±39 ±17 (5) m 71%	45 ±30 ±13 (5) ns 70%
245	47	5/9	55.6 55.6	245 ±114 ±38 (9)	132 ±60 ±30 (4)	110 ±10 ±3 (9)	38 ±16 ±7 (6) ** 61%	86 ±33 ±15 (5) * 68%	46 ±18 ±8 (5) m 72%
200	22,40, 47	8/10	80.0 80.0 ±17.2 ±5.4	294 ±88 ±28 (10)	NA	105 ±6 ±2 (10)	31 ±16 ±6 (8) *** 50%	75 ±34 ±12 (8) ** 60%	44 ±25 ±9 (8) m 69%
133.3	22,40	4/4	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (4)	NA	104 ±13 ±6 (4)	34 ±21 ±10 (4) * 55%	90 ±33 ±17 (4) m 71%	56 ±14 ±7 (4) ns 88%

$PdSO_4$ / Intraperitoneal injection



$PtCl_2$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	18,52 53	6/6	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (6)	NA	104 ±24 ±10 (6)	66 ±18 ±7 (6)	124 ±17 ±7 (6)	57 ±4 ±2 (6)
1111	52,53	1/4	NA 25.0 ±16.6 ±8.3	89 ±165 ±82 (4)	7 ±6 ±3 (3)	108 ±21 ±11 (4)	NA	NA	NA
741	52,53	3/9	33.3 33.3 ±18.9 ±6.3	151 ±167 ±56 (9)	58 ±118 ±48 (6)	112 ±12 ±4 (9)	6 ±23 ±12 (4) ** 9%	53 ±52 ±30 (3) m 43%	46 ±24 ±14 (3) ns 81%
578	52,53	3/6	50.0 50.0 ±38.7 ±15.8	175 ±177 ±72 (6)	13 ±21 ±12 (3)	107 ±8 ±3 (6)	16 ±12 ±7 (3) ** 24%	58 ±27 ±15 (3) ** 47%	43 ±15 ±9 (3) ns 75%
450	18,53	9/10	90.0 90.0 ±5.3 ±1.7	307 ±91 ±29 (10)	NA	117 ±12 ±4 (10)	39 ±16 ±5 (9) ** 59%	100 ±25 ±8 (9) * 81%	61 ±12 ±4 (9) ns
250	18,53	8/10	80.0 80.0 ±10.5 ±3.3	329 ±18 ±6 (10)	NA	117 ±16 ±5 (10)	53 ±11 ±3 (10) ns 80%	107 ±21 ±7 (8) ns 86%	56 ±12 ±4 (8) ns

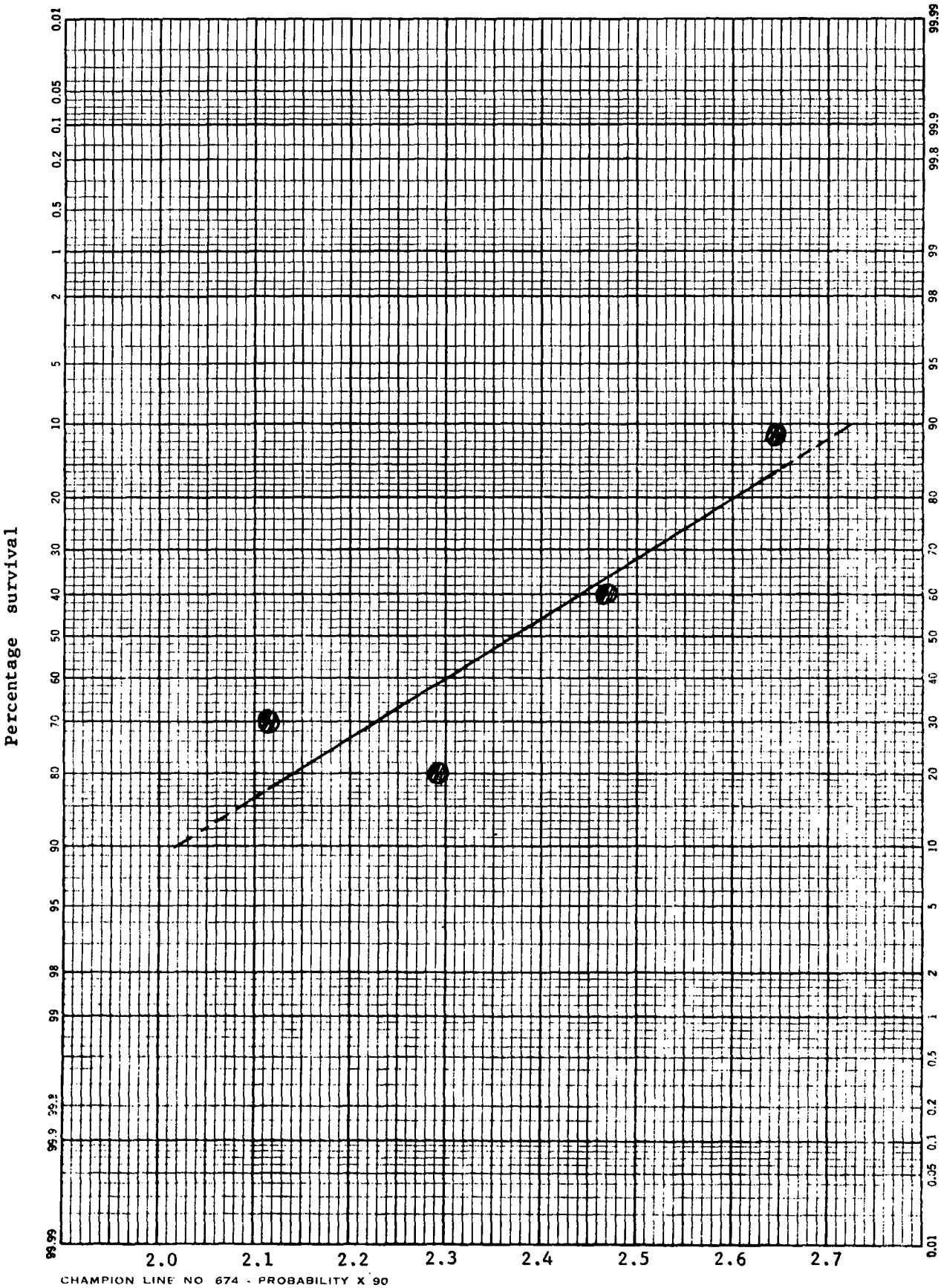
$PtCl_4$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	42,45, 50	6/6	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (6)	NA	95 ±12 ±5 (6)	61 ±6 ±3 (6)	120 ±11 ±5 (6)	59 ±7 ±3 (6)
108	42,45	0/6	NA 0.0 ±0.0 ±0.0	0 ±0 ±0 (6)	0 ±0 ±0 (6)	105 ±7 ±3 (6)	NA	NA	NA
64.8	42,45, 50	1/10	10.00 10.0 ±12.2 ±4.1	34 ±106 ±34 (10)	1 ±0 ±0 (9)	101 ±9 ±3 (10)	NA	NA	NA
38.9	45,50	2/10	20.00 20.0 ±0.0 ±0.0	107 ±123 ±39 (10)	50 ±29 ±10 (8)	100 ±6 ±2 (10)	NA	NA	NA
23.3	45,50	10/10	97.5[d] 100.0 ±0.0 ±0.0	336 ±0 ±0 (10)	NA	100 ±9 ±3 (10)	22 ±22 ±7 (10) ***	87 ±26 ±8 (10) ** 73%	62 ±14 ±4 (10) ns
14.0	45	5/5	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (10)	NA	102 ±8 ±3 (5)	57 ±11 ±5 (5) ns	96 ±6 ±3 (5) ** 80%	38 ±11 ±5 (5) ** 64%

$PtCl_4$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	31,43, 46	7/8	NA 87.5 ±23.1 ±8.2	310 ±72 ±26 (8)	NA	100 ±15 ±5 (8)	65 ±18 ±7 (7)	108 ±35 ±13 (7)	44 ±29 ±11 (7)
660	29	0/6	NA 0.0	0 ±0 ±0 (6)	0 ±0 ±0 (6)	98 ±9 ±4 (6)	NA	NA	NA
440	29,46	1/9	11.11 11.1 ±10.5 ±3.5	38 ±112 ±37 (9)	1 ±3 ±1 (8)	100 ±6 ±2 (9)	NA	NA	NA
293.3	31,43, 46	4/10	40.0 40.0 ±22.0 ±6.9	140 ±170 ±53 (10)	9 ±10 ±4 (6)	109 ±10 ±3 (10)	16 ±38 ±19 (4) * 25%	81 ±34 ±17 (4) ns 75%	65 ±14 ±7 (4) ns 148%
195.6	31,43,	8/10	80.0 80.0 ±18.3 ±5.8	270 ±138 ±44 (10)	NA	103 ±12 ±4 (10)	NA	NA	NA
130.4	31,43, 46	7/10	70.0 70.0 ±31.6 ±10.0	236 ±162 ±51 (10)	1 ±0 ±0 (3)	102 ±9 ±3 (10)	57 ±13 ±5 (7)	115 ±22 ±8 (7)	58 ±22 ±8 (7)
86.9	31,43	2/3	NA 66.7	240 ±166 ±96 (3)	NA	106 ±15 ±9 (3)	NA	NA	NA

$PtCl_4$ / Oral administration



PtO_2 / Oral administration

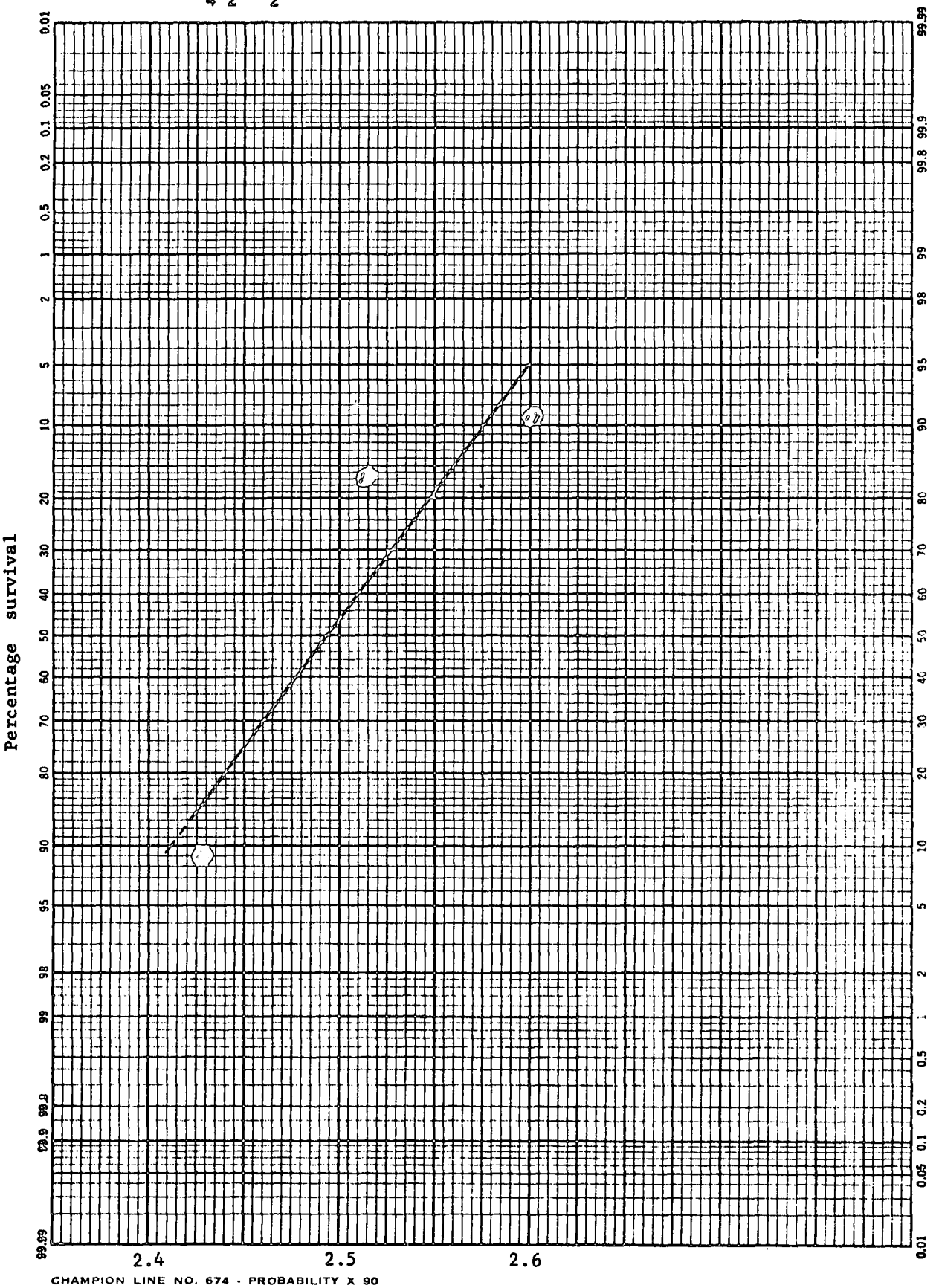
Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	9,20, 33,51	9/9	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (9)	NA	106 ±16 ±5 (9)	66 ±20 ±7 (9)	117 ±35 ±12 (9)	51 ±18 ±6 (9)
8000	9,33, 51,52	5/7	71.4 71.4 ±48.8 ±18.4	253 ±143 ±54 (7)	NA	118 ±11 ±4 (7)	68 ±15 ±7 (5) ns	114 ±17 ±8 (5) ns	47 ±10 ±4 (5) ns
4444	20,52	5/6	83.3 83.3 ±12.9 ±5.3	326 ±25 ±10 (6)	NA	115 ±21 ±9	62 ±18 ±7 (6) ns	106 ±36 ±16 (5) ns 90%	37 ±28 ±13 (5) ns 73%

$Pt(SO_4)_2 \cdot 4H_2O$ / Intraperitoneal injection

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	14,22, 24,32,	16/16	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (16)	NA	106 ±10 ±3 (16)	59 ±7 ±2 (16)	114 ±14 ±4 (16)	55 ±13 ±3 (16)
600	14,22, 24	0/10	NA[b] 0.0 ±0.0 ±0.0	28 ±16 ±5 (10)	28 ±16 ±5 (10)	100 ±4 ±1 (10)	NA	NA	NA
400	14,22, 24	1/11	9.1 9.1 ±20.2 ±6.1	67 ±92 ±28 (11)	41 ±23 ±7 (10)	102 ±11 ±3 (11)	NA	NA	NA
326.6	32,34	2/12	16.7 16.7 ±0.0 ±0.0	117 ±106 ±31 (12)	73 ±31 ±10 (10)	108 ±11 ±3 (12)	NA	NA	NA
267	14,22, 24	10/11	90.9 90.9 ±8.7 ±2.6	308 ±93 ±28 (11)	NA	101 ±8 ±2 (11)	39 ±17 ±5 (10) ** 66%	96 ±26 ±8 (10) m 84%	56 ±11 ±3 (10) ns
178	14,22, 24	11/11	NA[b] 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	NA	104 ±11 ±3 (11)	36 ±14 ±4 (11) *** 61%	94 ±32 ±10 (11) m 82%	58 ±21 ±6 (11) ns

Pt^{4+} salt obtained from ICN/K and K Laboratories

$Pt(SO_4)_2.4H_2O$ / Intraperitoneal injection



$Pt(SO_4)_2 \cdot 4H_2O$ (Goldsmith) / Intraperitoneal injection

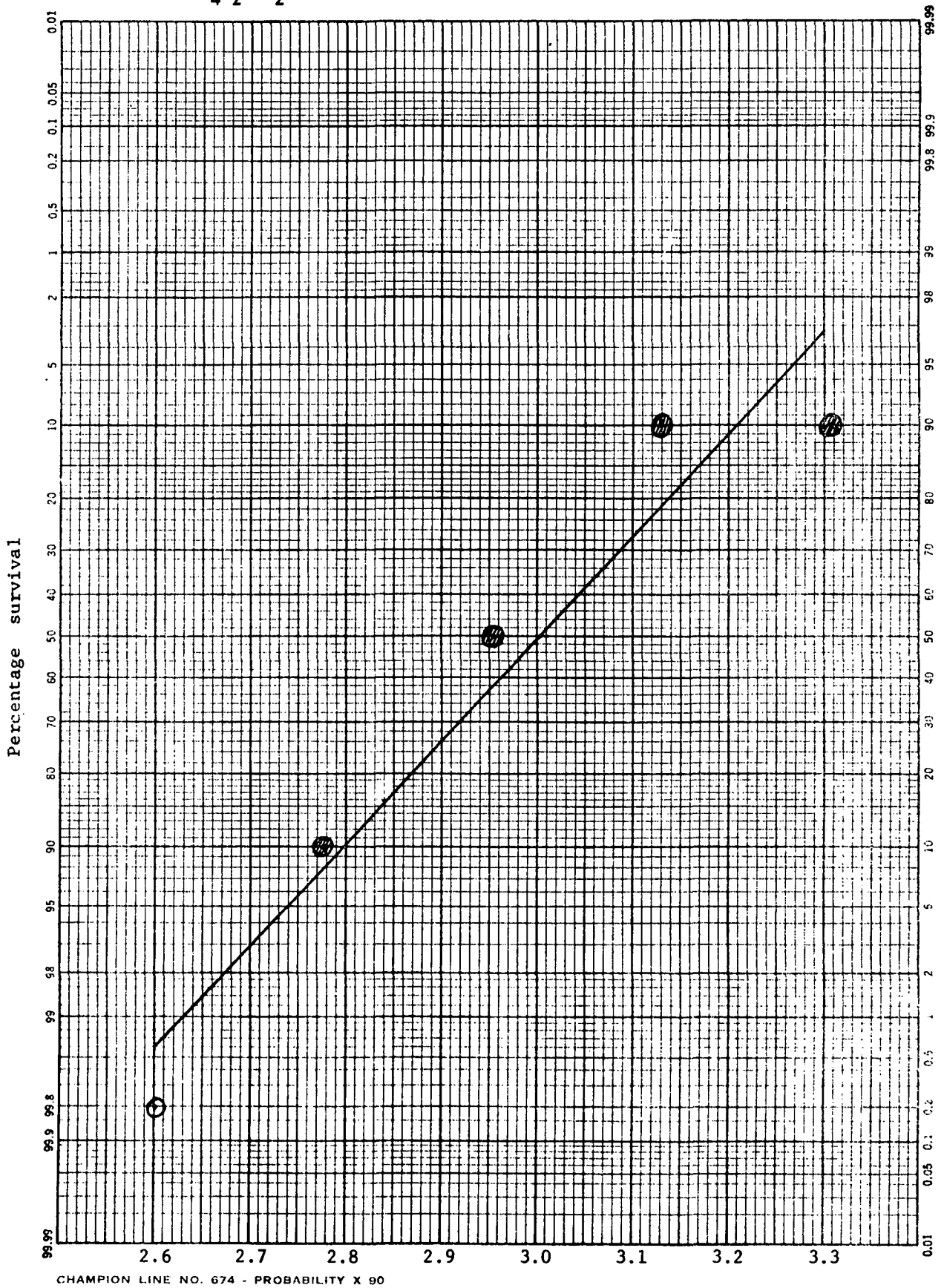
Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	56,57	4/4	NA 100.0 ±0.0 ±0.0	336 ±0 ±0	NA	105 ±18 ±9 (4)	52 ±12 ±6 (4)	106 ±15 ±8 (4)	54 ±5 ±2 (4)
400	55	1/3	NA 33.3	114 ±167 ±96 (3)	NA	121 ±16 ±9 (3)	NA	NA	NA
267	55,56 57	0/7	3.6[d] 0.0 ±0.0 ±0.0	76 ±13 ±5 (7)	76 ±13 ±5 (7)	112 ±13 ±5 (7)	NA	NA	NA
119	56,57	6/7	85.7 85.7 ±17.8 ±6.7	307 ±78 ±29 (7)	NA	114 ±7 ±3 (6)	8 ±31 ±13 (6) * 16%	56 ±34 ±14 (6) * 52%	47 ±19 ±8 (6) ns 88%
52.7	56	4/4	NA 100.0	336 ±0 ±0 (4)	NA	120 ±18 ±9 (4)	56 ±16 ±8 (4) ns	117 ±24 ±12 (4) ns	61 ±9 ±4 (4) ns 113%

Pt^{4+} salt obtained from D. F. Goldsmith Chemical and Metal Corp.

$Pt(SO_4)_2 \cdot 4H_2O$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	14,16, 23,25	11/11	NA 100.0 ±0.0 ±0.0	336 ±0 ±0 (11)	NA	106 ±12 ±3 (11)	50 ±8 ±2 (11)	108 ±16 ±5 (11)	58 ±11 ±3 (11)
2025	14,16, 23,25	1/10	10.0 10.0 ±31.6 ±10.0	46 ±104 ±33 (10)	13 ±24 ±8 (9)	109 ±13 ±4 (10)	NA	NA	NA
1350	14,16, 23,25	1/10	10.0 10.0 ±31.6 ±10.0	43 ±103 ±33 (10)	11 ±10 ±3 (9)	109 ±11 ±3 (10)	NA	NA	NA
900	14,16, 23,25	5/10	50.0 50.0 ±45.1 ±14.3	172 ±173 ±55 (10)	8 ±4 ±2 (5)	109 ±10 ±3 (10)	27 ±33 ±15 (5) ns 54%	78 ±48 ±21 (5) ns 72%	51 ±19 ±8 (5) ns 88%
600	14,16, 23,25	9/10	90.0 90.0 ±16.1 ±5.1	307 ±92 ±29 (10)	NA	108 ±11 ±3 (10)	37 ±25 ±8 (9) ns 74%	106 ±17 ±6 (9) ns	70 ±29 ±10 (9) ns
400	14,16, 23	7/7	99.8[a] 100.0 ±0.0 ±0.0	336 ±0 ±0 (7)	NA	109 ±7 ±3 (7)	52 ±15 ±6 (7) ns	116 ±20 ±8 (7) ns	64 ±6 ±2 (7) ns 110%

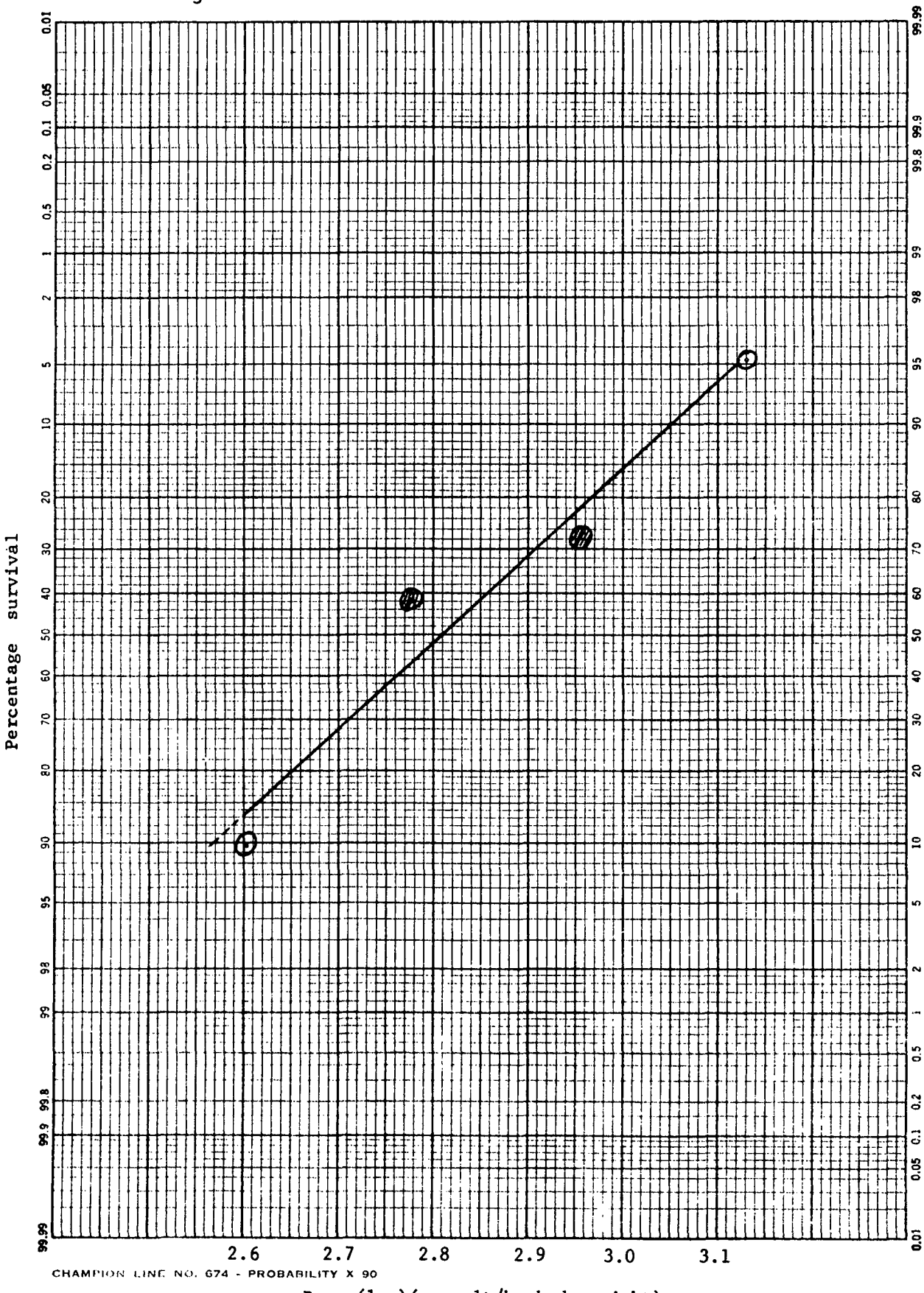
$Pt(SO_4)_2.4H_2O$ / Oral administration



$RuCl_3$ / Oral administration

Dose (mg salt /kg body weight)	Expt.	Number survivors /number tested	Percentage survival	Duration of survival (hr)		Body weight (g) on day 0	Weight gain (g)		
				all animals	non-survivors		days 0-7	days 0-14	days 7-14
Controls	4,7, 35,36	8/9	NA 88.9 ±22.0 ±7.3	335 ±4 ±1 (9)	NA	114 ±16 ±5 (9)	57 ±11 ±4 (8)	113 ±16 ±6 (8)	52 ±17 ±6 (7)
1350	35,36	0/11	4.8[a] 0.0 ±0.0 ±0.0	36 ±16 ±5 (11)	36 ±16 ±5 (11)	110 ±11 ±3 (11)	NA	NA	NA
900	35,36	3/11	27.3 27.3 ±12.2 ±3.7	139 ±132 ±40 (11)	65 ±45 ±16 (8)	109 ±7 ±2 (11)	NA	10 ±31 ±18 (3) *** 9%	NA
600	7,35, 36	5/12	41.7 41.7 ±28.9 ±8.3	150 ±164 ±47 (12)	18 ±17 ±7 (7)	102 ±10 ±3 (12)	44 ±7 ±4 (3) * 77%	102 ±19 ±8 (5) ns 90%	71 ±4 ±2 (3) * 137%
400	4,7, 35,36	12/12	90.1[a] 100.0 ±0.0 ±0.0	336 ±0 ±0 (12)	NA	114 ±13 ±4 (12)	45 ±12 ±5 (6) m 79%	101 ±26 ±7 (12) ns 89%	56 ±11 ±5 (6) ns
200	4	2/2	NA 100.0	336	NA	132	NA	NA	NA

$RuCl_3$ / Oral administration



TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-76-010a		

4. TITLE AND SUBTITLE	5. REPORT DATE
ASSESSMENT OF TOXICITY OF AUTOMOTIVE METALLIC EMISSIONS Volume I; Assessment of Fuel Additives Emission Toxicity via Selected Assays of Nucleic Acid and Protein Synthesis	January 1976
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
David J. Holbrook, Jr.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Department of Biochemistry School of Medicine University of North Carolina Chapel Hill, N.C. 27514	1AA601
	11. CONTRACT/GRANT NO.
	68-02-1205
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, N.C. 27711	Final
	14. SPONSORING AGENCY CODE
	EPA-ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Various parameters of toxicity have been studied for salts of manganese, lead, palladium, and platinum. Acute toxicities (LD-50 doses) are reported for both intraperitoneal injection and oral administration for the following salts: $PtCl_4$, $Pt(SO_4)_2$, $PdCl_2$, $MnCl_2$, $PdSO_4$, $PtCl_2$, $RuCl_3$, PtO_2, PbO, PdO, and MnO_2. Concentrations of metallic ions following dietary administration are reported, as are effects on weights of five organs (liver, kidney, spleen, heart, testes). Also following dietary administration, hepatic microsomes were isolated and the following parameters related to in vitro drug metabolism were measured: yield of microsomal protein/g liver; in vitro activities of aniline hydroxylase and aminopyrine demethylase; content of cytochromes P-450 and b5/mg microsomal protein.

Development of a rapid and convenient method for the analysis of ribosomal RNA in studies of RNA synthesis is reported.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicity Metabolism Manganese Lead (metal) palladium platinum Exhaust emissions Ribonucleic acids Ribonucleic acids		06 F, T 21 D

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	67
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

INSTRUCTIONS

1. **REPORT NUMBER**
Insert the EPA report number as it appears on the cover of the publication.

2. **LEAVE BLANK**

3. **RECIPIENTS ACCESSION NUMBER**
Reserved for use by each report recipient.

4. **TITLE AND SUBTITLE**
Title should indicate clearly and briefly the subject coverage of the report, and be displayed prominently. Set subtitle, if used, in smaller type or otherwise subordinate it to main title. When a report is prepared in more than one volume, repeat the primary title, add volume number and include subtitle for the specific title.

5. **REPORT DATE**
Each report shall carry a date indicating at least month and year. Indicate the basis on which it was selected *(e.g., date of issue, date of approval, date of preparation, etc.)*.

6. **PERFORMING ORGANIZATION CODE**
Leave blank.

7. **AUTHOR(S)**
Give name(s) in conventional order *(John R. Doe, J. Robert Doe, etc.)*. List author's affiliation if it differs from the performing organization.

8. **PERFORMING ORGANIZATION REPORT NUMBER**
Insert if performing organization wishes to assign this number.

9. **PERFORMING ORGANIZATION NAME AND ADDRESS**
Give name, street, city, state, and ZIP code. List no more than two levels of an organizational hirearchy.

10. **PROGRAM ELEMENT NUMBER**
Use the program element number under which the report was prepared. Subordinate numbers may be included in parentheses.

11. **CONTRACT/GRANT NUMBER**
Insert contract or grant number under which report was prepared.

12. **SPONSORING AGENCY NAME AND ADDRESS**
Include ZIP code.

13. **TYPE OF REPORT AND PERIOD COVERED**
Indicate interim final, etc., and if applicable, dates covered.

14. **SPONSORING AGENCY CODE**
Leave blank.

15. **SUPPLEMENTARY NOTES**
Enter information not included elsewhere but useful, such as: Prepared in cooperation with, Translation of, Presented at conference of, To be published in, Supersedes, Supplements, etc.

16. **ABSTRACT**
Include a brief *(200 words or less)* factual summary of the most significant information contained in the report. If the report contains a significant bibliography or literature survey, mention it here.

17. **KEY WORDS AND DOCUMENT ANALYSIS**
(a) DESCRIPTORS - Select from the Thesaurus of Engineering and Scientific Terms the proper authorized terms that identify the major concept of the research and are sufficiently specific and precise to be used as index entries for cataloging.

(b) IDENTIFIERS AND OPEN-ENDED TERMS - Use identifiers for project names, code names, equipment designators, etc. Use open-ended terms written in descriptor form for those subjects for which no descriptor exists.

(c) COSATI FIELD GROUP - Field and group assignments are to be taken from the 1965 COSATI Subject Category List. Since the majority of documents are multidisciplinary in nature, the Primary Field/Group assignment(s) will be specific discipline, area of human endeavor, or type of physical object. The application(s) will be cross-referenced with secondary Field/Group assignments that will follow the primary posting(s).

18. **DISTRIBUTION STATEMENT**
Denote releasability to the public or limitation for reasons other than security for example "Release Unlimited." Cite any availability to the public, with address and price.

19. & 20. **SECURITY CLASSIFICATION**
DO NOT submit classified reports to the National Technical Information service.

21. **NUMBER OF PAGES**
Insert the total number of pages, including this one and unnumbered pages, but exclude distribution list, if any.

22. **PRICE**
Insert the price set by the National Technical Information Service or the Government Printing Office, if known.