

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-120

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

NDA 21120

Novantrone, Mitoxantrone hydrochloride

KEY WORDS:

Reviewer Name Paul Roney
Division Name DNDP
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Information to sponsor: Yes () No ()

Sponsor (or agent): Immunex Corporation
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Manufacturer for drug substance Wyeth-Ayerst
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P.O. Box AC, Pueblo Station
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Drug:

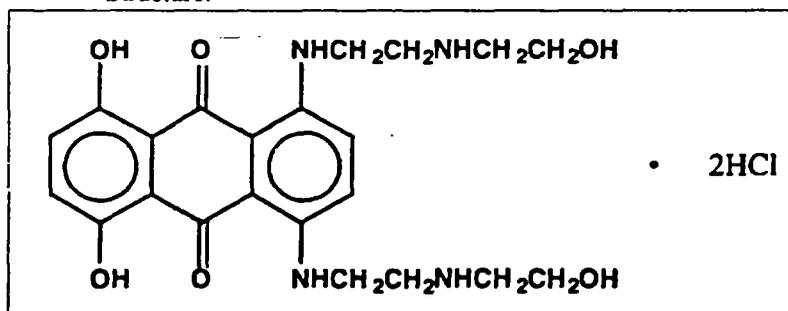
Code Name:
Generic Name: Mitoxantrone Hydrochloride
Trade Name: Novantrone
Chemical Name: 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione

dihydrochloride

CAS Registry Number:

Molecular Formula/ Molecular Weight: 517.41 / C₂₂H₂₈N₄O₆.2HCl

Structure:



Relevant INDs/NDAs/DMFs:

NDA 19297, IND

Drug Class: Cytotoxic synthetic anthracenedione

Indication: Treatment of secondary-progressive multiple sclerosis

Clinical formulation:

Route of administration: Intravenous injection

Proposed clinical protocol or Use: 12 mg/m², every 3 months

Previous clinical experience:

Disclaimer -- use of sponsor's material

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Introduction and Drug History

Mitoxantrone is a cytotoxic synthetic anthracenedione. It has previously been approved by the FDA in 1987 for use in treatment (in combination with other approved drugs) of acute non-lymphocytic leukemia. The recommended dose was 12 mg/m² for 3 consecutive days, or a total of 36 mg/m² per cycle. The cycles could be repeated two to four times. In 1996, mitoxantrone was approved for treatment of pain (in combination with corticosteroids) associated with hormone-refractory prostate cancer. The recommended dose was 12-14 mg/m² for every 3 weeks.

Studies reviewed within this submission:

All of the pivotal studies have been reviewed under NDA 19297 by J Sun

Studies not reviewed within this submission:

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PHARMACOLOGY:

Mechanism of Action:

Mitoxantrone exerts its cellular effects by interfering with DNA structure and function. It intercalates into DNA causing DNA crosslinks and DNA double- and single-strand breaks. Mitoxantrone also interferes with RNA molecules in the cell nucleus. In addition, mitoxantrone is a potent inhibitor of topoisomerase II, an enzyme responsible for uncoiling and repairing damaged DNA. Due to the sensitivity of the immune system to agents which interfere with DNA structure and function, mitoxantrone is an immunosuppressive agent.

The etiology of multiple sclerosis is not completely understood. Environmental factors (possibly a viral infection before the age of 15) appear to predispose individuals to contracting the disease later in life. It is thought that an autoimmune reaction causes the initiation of the disease and its exacerbations later in life. Patients with multiple sclerosis have increased levels of serum antibodies to myelin proteins, such as myelin basic protein. The drugs currently approved agents for the treatment of multiple sclerosis reflect this theory of etiology. These drugs include immunosuppressive corticosteroids and other agents which suppress immune function¹. The sponsor proposes that since mitoxantrone is an immunosuppressive drug, it would be an effective agent in the treatment of multiple sclerosis.

Drug Activity Related to Proposed Indication:

An animal model for multiple sclerosis is experimental allergic encephalomyelitis (EAE). EAE attempts to mimic the suspected autoimmune etiology of multiple sclerosis by raising autoantibodies against myelin antigens such as myelin basic protein (MBP). The resulting pathology is characterized by perivascular mononuclear infiltrates and disruption of the blood brain barrier, which resembles the lesions observed in multiple sclerosis. Mitoxantrone has been tested for its ability to treat EAE in various species. For example, in Lewis rats, EAE was induced by injection of guinea pig spinal cord homogenate. This resulted in paralysis starting on day 8 and resolving by day 10-14. On day 14, rats were treated with 1 mg/kg (6 mg/m²) mitoxantrone either IP or SC. Mitoxantrone delayed the relapse of paralysis observed in all control rats starting on days 17 to 22. In contrast, only 2/6 rats treated with IP mitoxantrone developed paralysis and the onset of symptoms in these rats were delayed and milder than in untreated rats. No rats treated with mitoxantrone SC developed the paralysis. In another study, AB/H mice with EAE were treated with 2.5 mg/kg (7.5 mg/m²) IP twice weekly. By Day 50 of the study, 12/12 control mice had relapses of EAE, but only 1/13 mitoxantrone treated mice had a relapse, and the symptoms in this mouse was milder than the symptoms in the control mice. A summary of studies (taken from NDA submission) is provided below. These data suggest that mitoxantrone is effective in the EAE model of multiple sclerosis.

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¹ Adams, RD, M Victor, and AH Ropper. 1997. Multiple Sclerosis and Allied Demyelinative Diseases. In *Principles of Neurology*. 6th Edition. McGraw-Hill, New York. Pages 902-927.

Table A.1.1.7: Summary of EAE Study Results

Therapeutic Protection in Active EAE Models			
Study	Type of EAE model	Mitox Treatment Regimen	Results
Levine, 1986	Relapsing disease Lewis rats	1 mg/kg IP or SC (lcg) Single dose Day 14	All 6 control rats relapsed. Mitox prevented relapse in 12 of 14 rats.
Watson, 1991	Relapsing disease Biozzi AB/H mice	2.5 mg/kg IP, twice weekly Days 27-40	Mitox prevented relapse in 12/13 mice ($p < 0.002$).
Effector-Level Inhibition in Active EAE Models			
Study	Type of EAE model	Mitox Treatment Regimen	Results
Ridge, 1985	Acute disease Lewis rats	0.25 - 0.5 mg/kg/day IP Days 7-16 Compared to 1.25 - 5.0 mg/kg CP	Mitox at 0.25 - 0.5 mg/kg suppressed clinical and histological lesions ($p < 0.05$).
Watson, 1991	Acute disease Biozzi AB/H mice	1 - 2.5 mg/kg/day IP Days 12-19	Mitox at 2.5 mg/kg prevented development of acute disease.
Baker, 1992	Acute disease Biozzi AB/H mice	0.5 - 5.0 mg/kg IP Single dose Day 9	A single dose of 5 mg/kg Mitox completely inhibited the development of EAE.
Prophylactic Effects in Active EAE Models			
Study	Type of EAE model	Mitox Treatment Regimen	Results
Ridge, 1985	Acute disease Lewis rats	0.125 - 0.5 mg/kg/day IP for 16 days starting on Day 0. Compared to 1.25 - 5.0 mg/kg CP	Mitox at 0.25 - 0.5 mg/kg suppressed clinical and histological lesions ($p < 0.05$).
Lublin, 1987	Acute disease (SJL/J x BALB/c) F1 mice	0.25 or 0.5 mg/kg/day IP 10 days starting on Day 0	Mitox prevented the development of EAE.
Lublin, 1987	Delayed onset disease SJL/J mice	0.05 mg/kg IP 3X/week for 12 weeks.	Mitox significantly delayed the onset of disease.
Mustafa, 1993	Acute disease Lewis rats	0.5 mg/kg IP, alternate days (Days 0-15). Compared to CsA at 3.0 or 20 mg/kg, alternate days (Days 0-22)	Mitox completely protected rats from EAE.
Treatment of Passive EAE			
Study	Type of EAE model	Mitox Treatment Regimen	Results
Ridge, 1985	Lewis rats immunized with sensitized, syngeneic immune cells	•Donors: 0.5 mg/kg/day x 14 days •Cells in vitro: 0.01-0.001 mg/mL •Recipient pretreatment: 0.5 mg/kg/day IP x 5 days	Mitox prevented the development of passive EAE in all three experimental designs.

Mitox = mitoxantrone; CP = cyclophosphamide; CsA = cyclosporin A.

Ancillary Pharmacology Studies:

Ancillary pharmacology studies have explored the immunosuppressive effects of mitoxantrone. Mitoxantrone prevented the development of adjuvant arthritis in rats at doses as low as 0.063 mg/kg/day (0.378 mg/m²). Mitoxantrone (0.25 mg/kg (1.5 mg/m²) every other day) also delayed the rejection of allogeneic heart transplants in rats.

More specific studies examined the effects of mitoxantrone on the components of the immune system. Further studies in rats and mice suggest that mitoxantrone inhibits the activity of T-cells, B-cells and macrophages. The effects on T-cells appear complex. Inhibition of T helper function appeared to be mediated by macrophages,

but T suppressor and cytotoxic activity was mediated directly on the T cells. In summary, mitoxantrone inhibits a variety of immune mediated activities in *in vivo* and *in vitro* models of immune system activity.

Summary of pharmacology:

Mitoxantrone is active in an experimental model of multiple sclerosis (experimental allergic encephalopathy). It also immunosuppressive actions on B-cell, T-cell and macrophage activity. Since multiple sclerosis is thought to have an autoimmune component, the pharmacology studies support the potential therapeutic utility of mitoxantrone in this disease.

SAFETY PHARMACOLOGY:

Neurological effects:

Mitoxantrone (25 to 50 mg/kg IP) had no effect on pentylentetrazole induced seizures, tetrabenazine induced depression, or amphetamine lethality. In addition, mitoxantrone did not induce analgesia at 25 mg/kg IP.

Cardiovascular effects:

In rats, mitoxantrone (3 mg/kg IP, given 20 hours apart) had no effect on heart rate or blood pressure. It decreased the response of rats to tyramine, epinephrine, and angiotensin amide, suggesting an inhibitory effect on vasoconstrictive mechanisms. Mitoxantrone (20 mg/kg IP) had no effect on heart rate or blood pressure in spontaneously hypertensive rats. In cats, 20 mg/kg IP caused a transient increase in blood pressure 15 to 22% during the 30 minutes after injection; no effects were observed on heart rate or ECG monitored for five hours following injection.

Pulmonary effects: No data

Renal effects: No data

Gastrointestinal effects: No data

Abuse liability: No data

Other:

Conclusions:

Summary:

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PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

The available pharmacokinetic data generally measure radiolabelled mitoxantrone and do not discriminate between parent compound and metabolites. Data for single dose pharmacokinetic data in rats are presented below (Report 33). Data were not available on the pharmacokinetic parameters of mitoxantrone in other species.

Parameters	Dose						Mean	C.V. (%)
	0.25 mg/kg		0.50 mg/kg		0.75 mg/kg			
	M	F	M	F	M	F		
Terminal rate constant; λ_z (day ⁻¹)	0.066	0.062	0.053	0.059	0.037	0.054	0.059	7.6
Biological half-life; $t_{1/2}$, β ; (days)	10.50	11.20	12.60	11.70	12.20	12.80	11.83	7.4
AUC (0- ∞) (ng·day/mL)	9.76	*	23.30	21.80	32.40	35.90	0.044 [†]	7.7
Volume of distribution; V_d	388.10	*	390.10	388.10	406.70	392.9	392.00	2.0
Total plasma Clearance, Cl_p (mL/min/kg)	17.80	*	14.90	15.90	16.10	14.70	15.84	8.1
Renal clearance; Cl_r ; (mL/min/kg)	1.60	*	1.90	1.70	2.10	1.65	1.71	14.0
Non-renal clearance; Cl_{nr} ; (mL/min/kg)	16.20	*	13.00	14.20	14.00	13.00	14.10	8.9

* Not sufficient data for estimation

[†] Normalized with respect to the dose (AUC/dose; day·g/mL)

Absorption:

Mitoxantrone is administered as an intravenous injection, so that absorption is not an issue. The sponsor claims that mitoxantrone is poorly absorbed orally, although no data were presented to support this statement.

Distribution:

The distribution of mitoxantrone was examined using radiolabelled mitoxantrone. This method does not distinguish between parent mitoxantrone and its metabolites in these tissues. A summary of the tissue distribution studies is presented below.

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Mitoxantrone Tissue Distribution in the Rat, Dog, and Monkey:
A Chart of Tissues with High Concentrations in at Least One of the Species at Intervals of
1 or 2, 4 or 10, 20 or 35, or 58 Days After Dosing Compared with Plasma
(ng/g)

(Reports 28 through 30, 33 and 43)

Species: (Days after dose):	Rat (2)	Dog (1)	Monkey (1)	Rat (10)	Dog (10)	Monkey (1)	Rat (30)	Dog (30)	Monkey (35)	Rat (58)	Dog (58)	Rat (120)
Dose (mg/kg):	0.3	0.37	1.0	0.3	0.37	1.0	0.3	0.37	1.0	0.3	0.37	0.3
M/sex:	2 M	1 M	1 F	2 M	2M/1F	1 F	2 M	1 F	1 F	2 M	1 M	3 M
Reports:	28,29	29	30	29,45	29	30	29	29	30	29	29	33
Tissue												
Parotid gland	ND	1,160	5,970	ND	432	12,000	ND	192	3,670	ND	135	ND
Lymph nodes ^a	ND	397	1,442	ND	210	2,338	ND	142	508	ND	325	ND
Gallbladder	ND	2,330	6,060	ND	479	1,930	ND	346	455	ND	298	ND
Bile	ND	28,100	28,500	ND	400	2,060	ND	206	262	ND	ND	ND
Pancreas	103	1,500	5,290	592	937	4,570	117	866	2,040	43	537	20
Liver	672	2,350	4,230	456	1,295	1,735	115	1,170	331	70	1,095	29
Spleen	1,752	1,055	4,000	1,306	946	2,260	589	608	313	477	516	70
Lung	1,265	1,085	695	1,433	302	415	1,010	149	155	226	191	48
Kidney	2,091	2,550	11,400	1,839	1,900	6,000	534	659	744	213	404	22
Heart	1,038	593	2,905	825	432	1,081	229	390	1,310	167	136	11
Adrenal gland	999	484	2,060	1,036	107	1,030	527	70	622	238	53	154
Thyroid gland	ND	228	2,398	ND	ND	1,530	ND	935	1,110	ND	741	ND
Tonsil	ND	603	1,130	ND	ND	957	ND	ND	229	ND	43	ND
Salivary gland(s) ^b	ND	753	2,605	ND	ND	2,678	ND	645	645	ND	748	ND
Stomach	288	1,060	1,560	239	179	1,210	37	129	252	49	55	13
Small intestine	403	839	1,910	381	100	518	60	57	147	102	41	10
Large intestine	337	505	1,880	137	64	464	39	65	111	24	15	5
Fallopian tube	ND	ND	1,200	ND	ND	1,260	ND	ND	202	ND	ND	ND
Bone marrow	96	479	229	504 ^c	283	1,010	166	88	314	78	59	ND
Adipose fat	189	66	373	162 ^c	41	361	77	24	614	20	25	3
Plasma ^d	3	0	22 ^e	4 ^f	3	10	4 ^f	5	4	4 ^f	1	0

^a Mean values; ^b Submaxillary gland; ^c N = 2; ^d Values are given in mg/ml; ^e N = 4; ^f N = 3; ND = not determined

Metabolism:

The binding of ¹⁴C-mitoxantrone to human plasma, human serum albumin and al-acid glycoprotein was investigated in vitro (Report 58). At concentrations between 26 and 455 ng/ml, 78% of the drug was bound to protein. Mitoxantrone (50 and 200 ng/ml) did not affect the protein binding of seven other drugs (diphenylhydantoin, doxorubicin, methotrexate, prednisone, prednisolone, heparin, acetylsalicylic acid) in vitro (Report 59).

Mitoxantrone is metabolized to a mono- and dicarboxylic acid derivatives and their glucuronide conjugates. No data were reported which distinguished between plasma and tissue levels of mitoxantrone and its metabolites. In rats administered 1 mg/kg intravenously, approximately 75% of the radiolabel in the bile were metabolites of mitoxantrone; 60% of the mitoxantrone in the urine was unconjugated (Report 53).

Elimination:

Studies were conducted on the excretion of radioactive label following intravenous injections of ¹⁴C-mitoxantrone in rats, dogs, and monkeys. These studies suggest that mitoxantrone (and its metabolites) are eliminated primarily via the bile (57 to 66% of label was in the feces) and urine (6 to 17% of label was in the urine). Mitoxantrone and its metabolites are excreted slowly; only 54, 40, and 23 percent of administered label was recovered by 48 hours in rats, dogs and monkeys, respectively.

Other studies:

Mitoxantrone did not induce hepatic mixed function oxidases in rats at an intravenous dose of 0.75 mg/kg for three consecutive days.

Comments:

Summary:

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TOXICOLOGY:

Acute Toxicity

Mice

Ten males and 10 females were used for each dose level. The LD10 and LD50 values are shown in the table:

Route	Study	Vehicle	Dose Range (mg/kg)	Sex	LD10 (mg/kg)	LD50 (mg/kg)
IV	63, 64	Saline	1.9-17.1	Male	7.8	11.3
			1.9-17.1	Female	7.1	9.7
	68	0.8 %NaCl/ 0.2% Na metabisulfite	7-14.4	Male/ Female	-	12.2
	69	0.8 %NaCl/ 0.01% Na metabisulfite/ 0.005% Na Acetate/ 0.046% Acetic Acid	2.5-20	Male	-	10.4
			2.5-20	Female	-	10.6
			2.5-20	Male	-	11.1
	IP	65, 66	Saline	4.5-21.5	Male	7.1
4.5-21.5				Female	2.3	19.7

In the mouse IV study 63, 64, deaths occurred at doses of 7.0 mg/kg or above, and only on days 9 through 15. In the mouse IP study, deaths occurred at doses of 2.9 mg/kg or above, and only on days 9 through 20. In both studies signs of toxicity were: salivation, paleness, rough fur, hair loss, decreased body weight gain, and body weight loss. Additional signs of toxicity from the IP study 65, 66 were abdominal distention, external abdominal staining, diarrhea, peritonitis and ascites. The LD50 was similar for all vehicles tested.

Rats

Acute lethality in rats

Route	Study	Vehicle	Dose Range (mg/kg)	Sex	LD10 (mg/kg)	LD50 (mg/kg)
IV	70, 71	Saline	1.7-7.5	Male	3.5	4.8
			3.0-8.9	Female	3.6	5.2
	76	Saline	1.25-15	Male	-	5.3
			1.25-15	Female	-	7.1
	77	0.8 %NaCl/ 0.01% Na metabisulfite/ 0.005% Na Acetate/ 0.046% Acetic Acid	1.25-15	Male	-	5.2
			1.25-15	Female	-	7.1
IP	72,73	Saline	4.7-10.8	Male	6.2	8.0
			6.2-14.3	Female	9.9	11.7
PO	74	Saline	200-1000	Male	422	682
			200-1000	Female	474	721

In the IV and IP-dosed rats, signs of toxicity were: epistaxis, chromodacryorrhea, rough fur, swelling of the nasal region, salivation, abdominal distention, diarrhea, paleness, external abdominal staining, lacrimation, hematuria, decreased body-weight gain, and body-weight loss. Peritonitis was seen in IP-dosed rats. In PO-dosed rats, signs of

toxicity were: rapid or shallow breathing, sedation, loose and/or bloody feces, nasal discharge, dehydration of skin, chromodacryorrhea, and areas of hair loss. Additional acute toxicity studies showed that there was no difference in toxicity of mitoxantrone when administered in either of the two vehicles (physiological saline and acetate-buffered formulation).

Single-dose IV toxicity, Rat (Report 78, 79)

Volume 9, Pages 1-119

Conducting Laboratory: Lederle Laboratories, Tokyo, Japan

Date of Study Initiation: 12/07/1978

GLP Compliance: No

Species: Rats, CRJ: CD(SD)

Design: Single IV injection with recovery period

No of animals: 15/sex/dose level

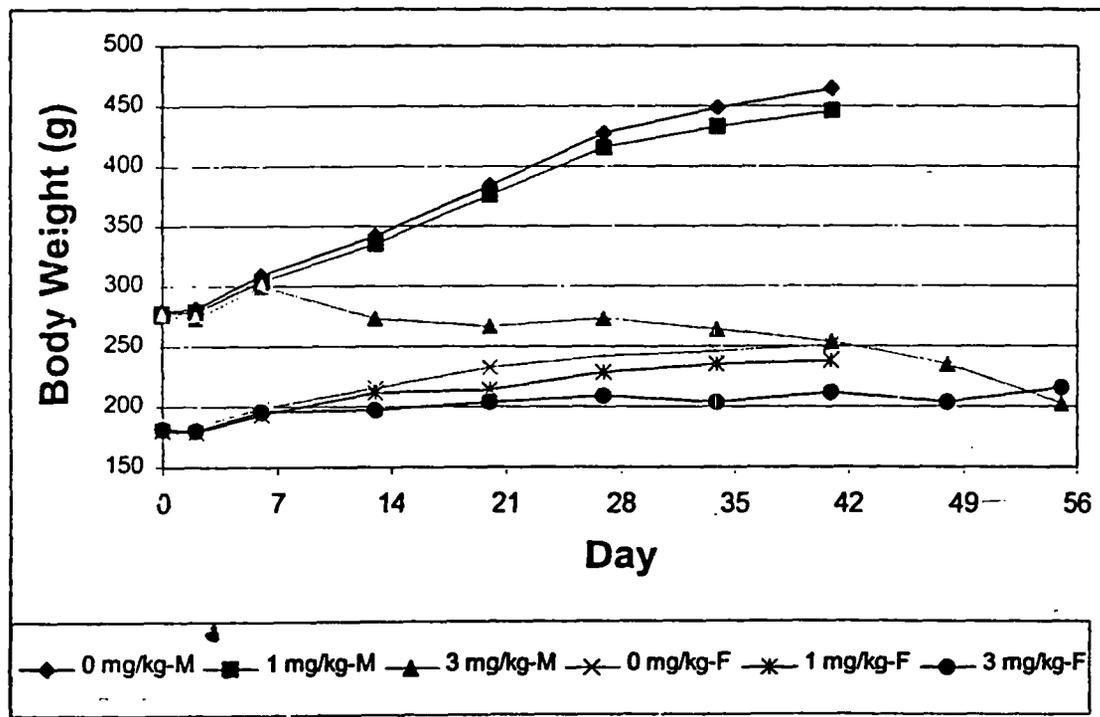
Dose levels: 0, 0.03, 0.1, 0.3, 1.0, 3.0 mg/kg (0, 0.18, 0.6, 1.8, 6, 18 mg/m²)

Design: single IV injection, 3 rats/sex/timepoint sacrificed at days 3, 7, 14, 21, and 42 with the exception of rats receiving 3 mg/kg which were sacrificed at days 3, 7, 14, 21, and 56

Mortality-3 rats died at 3 mg/kg (deaths occurred on Days 18, 34, and 54)

Clinical Signs- 3 mg/kg only, Days 13-55-body weight loss, decreased weight gain, Days 5-56- epistaxis, rough fur, paleness, hematuria, diarrhea, hypothermia, and hair loss.

Body Weight



Decreased body weight in males at 3 mg/kg; slight decrease (9-15%) in body weight gain at 3 mg/kg in females

Blood Chemistry- No effects at 3 and 7 days.

Clinical pathology results (expressed as per cent of controls)

Sign	Day	0.3 mg/kg		1 mg/kg		3 mg/kg	
		Males	Females	Males	Females	Males	Female
BUN	14	101	90	101	84	370	142
	21	93	93	85	91	253	247
	42	109	106	107	92	2723	216
Cholesterol	14	102	71	120	92	685	329
	21	100	87	165	137	805	511
	42	110	110	237	148	289	577

Triglycerides	14	99	61	117	73	575	154
	21	91	94	156	78	971	578
	42	121	117	205	136	213	675
Alkaline Phosphatase	14	73	140	64	105	22	44
	21	75	144	82	92	22	45
	42	93	92	78	119	86	61

In addition, less dramatic (<20%) increases in alpha globulin fraction and decreases in total protein, albumin and gamma globulin fractions were observed at 3 mg/kg

Clinical pathology results are consistent with severe kidney toxicity at 3 mg/kg

Hematology

Hematology results (expressed as per cent of controls); Values in Bold are <90% of control

Sign	Day	0.03 mg/kg		0.1 mg/kg		0.3 mg/kg		1 mg/kg		3 mg/kg	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Female
WBC	3	108	84	78	64	60	77	39	34	25	28
	7	87	84	73	91	58	59	52	44	17	21
	14	99	100	72	117	93	100	57	61	36	42
	21	141	71	111	101	96	71	68	58	54	33
	42	146	91	147	93	129	86	146	83	29	66
Lymph (Absol count)	3	115	82	85	64	61	76	38	34	22	27
	7	85	83	69	91	54	59	49	46	17	23
	14	100	109	70	124	90	101	51	57	22	37
	21	144	75	110	103	91	70	59	58	31	29
	42	155	95	149	86	128	86	138	78	14	61
RBC	3	91	98	95	93	94	94	90	95	89	95
	7	98	100	95	100	95	100	92	91	82	85
	14	99	105	103	108	96	106	98	104	64	73
	21	100	94	95	89	95	100	93	96	62	59
	42	100	100	102	98	99	99	87	98	56	61
Hg	3	96	97	100	94	98	93	96	93	95	91
	7	98	105	94	100	98	105	92	94	82	89
	14	101	101	104	103	98	100	100	99	61	72
	21	96	96	100	97	97	100	94	98	58	55
	42	103	101	104	80	102	99	93	99	53	64

Persistent decreases in RBC and hemoglobin at 3 mg/kg consistent with kidney failure

Persistent leukopenia at 0.3 mg/kg in females and 1 mg/kg in males

Transient leukopenia at 0.03 mg/kg

Organ Weights

Sign	Day	0.03 mg/kg		0.1 mg/kg		0.3 mg/kg		1 mg/kg		3 mg/kg	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Female
Thymus	3	96	108	109	88	80	92	44	47	35	45
	7	89	109	88	111	76	79	30	33	15	15
	14	153	84	106	103	128	74	71	50	25	14
	21	108	107	113	108	105	116	108	96	18	17
	42	116	92	104	122	117	104	118	118	29	99
Spleen	3	101	95	100	81	86	75	68	58	58	57
	7	103	92	83	86	76	72	65	64	45	47
	14	110	84	90	94	96	84	77	69	65	64
	21	106	112	92	97	102	94	93	104	99	95
	42	138	113	124	117	131	104	150	119	96	131
Seminal Vesicles	3	98	-	100	-	103	-	97	-	103	-
	7	99	-	114	-	100	-	105	-	86	-
	14	80	-	113	-	85	-	107	-	56	-
	21	118	-	120	-	128	-	127	-	61	-
	42	96	-	88	-	97	-	97	-	31	-

Kidney	3	102	116	101	104	100	107	97	112	100	111
	7	102	100	111	103	109	103	105	103	117	105
	14	97	107	102	119	99	119	105	110	159	165
	21	96	110	93	101	109	118	104	116	176	191
	42	106	99	99	107	99	104	146	140	179	201
Heart	3	95	99	97	99	100	91	103	97	100	101
	7	94	95	102	98	102	104	102	102	101	99
	14	91	107	104	103	99	111	102	108	95	121
	21	99	101	97	89	104	98	96	121	111	112
	42	105	91	105	96	106	95	106	110	182	132
Liver	3	97	104	103	104	99	102	103	104	102	104
	7	99	99	99	104	105	101	97	105	117	110
	14	102	103	100	102	102	101	102	107	147	163
	21	106	99	99	96	110	109	111	122	184	209
	42	104	98	103	106	101	103	125	129	196	217

Gross Necropsy

Early Deaths- Fluid in thoracic cavity, kidneys enlarged (1/3) or discolored (2/3) lungs congested (2/3); small prostate and seminal vesicles.

Scheduled Sacrifices-

3 mg/kg- Small thymus and spleen were observed starting on Day 3 through Day 21 and 14, respectively; kidney enlargement and discoloration starting on Day 14 through 21 and 56, respectively

1 mg/kg Small thymus and spleen were observed starting on Day 3 through Day 14 and 7, respectively

Histopathology

Early deaths- Kidneys- slight to severe fibrotic glomeruli (3/3), hydropic degeneration of proximal tubular epithelium (2/3), proliferation of interstitial tissue with round cell infiltration.

Severe lymphocytic depletion of thymus and spleen.

Liver- slight to moderate increase in basophilic granules in hepatocytes (3/3) and slight to moderate fatty degeneration of hepatocytes (2/3)

Scheduled Deaths-

	0 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Males						
Thymus, Cortical lymphocytic depletion	NP	NP	NP	Min (Day 3-7)	Mod-Sev (Day 3-14)	Mod-Sev (Day 3-56)
Spleen, Follicular lymphocytic depletion	NP	NP	Min (Day 3)	Min (Day 3-7)	Min-Sli (Day 3-21)	Mod (Day 3-56)
Lymph Nodes, Lymphocytic depletion	NP	NP	NP	NP	Slight (Day 3-14)	Mod-Sev (Day 3-56)
Bone Marrow, Cellularity decrease	NP	NP	NP	Min (Day 3)	Severe (Day 3-7)	Severe (Day 3-14)
Bone Marrow, increase in fat tissue	NP	NP	NP	NP	Sli-Mod (Day 7)	Severe (Day 7-21)
Kidney, Hydropic degeneration	NP	NP	NP	NP	Sli-Mod (Day 21-42)	Sli-Mod (Day 7-21)
Kidney, Interstitial tissue proliferation	NP	NP	NP	NP	NP	Sli-Sev (Day 14-56)
Kidney, Fibrosis	NP	NP	NP	NP	NP	Severe Day 21-56
Liver, Basophilic granules	NP	NP	NP	NP	Slight Day 56	Sli-Sev Day 14-56
Females						
Thymus, Cortical lymphocytic depletion	NP	NP	NP	NP	Mod (Day 3-7)	Severe (Day 3-56)
Spleen, Follicular lymphocytic depletion	NP	NP	NP	Slight (Day 3-7)	Sli-Mod (Day 3-21)	Mod-Sev (Day 3-56)

	0 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Lymph Nodes, Lymphocytic depletion	NP	NP	NP	Slight (Day 3-7)	Sli-Mod (Day 3-21)	Sli-Sev (Day 3-21)
Bone Marrow, Cellularity decrease	NP	NP	NP	Slight (Day 3)	Severe (Day 3-7)	Severe (Day 3-14)
Bone Marrow, increase in fat tissue	NP	NP	NP	NP	Slight (Day 7)	NP-Mod (Day 21)
Kidney, Hydropic degeneration	NP	NP	NP	NP	Slight (Day 21-42)	Sli-Sev (Day 14-56)
Kidney, Interstitial tissue proliferation	NP	NP	NP	NP	NP	Slight (Day 14-56)
Kidney, Fibrosis	NP	NP	NP	NP	NP	Slight (Day 21-56)
Liver, Basophilic granules	NP	NP	NP	NP	NP	Sli-Mod (Day 14-56)

Key findings

- 3 mg/kg caused deaths, which occurred up to 55 days post dose. Deaths were associated with kidney failure, as indicated by kidney fibrosis, hydropic degeneration of proximal tubular epithelium and interstitial tissue proliferation, and severe lymphocytic depletion of thymus and spleen and leukopenia.
- Decreased body weight was observed at 3 mg/kg in males; decreased body weight gain was observed at 3 mg/kg in females.
- Leukopenia, characterized by decreased lymphocyte counts, were observed at 0.03 mg/kg and above; the severity of the leukopenia increased with the dose. There was minimal recovery of leukocyte counts at 1 mg/kg at time points out to last observation day (Day 42 for 1 mg/kg, Day 56 for 3 mg/kg).
- Decreased red blood cell counts, with associated decreased hemoglobin levels, were observed at 3 mg/kg from Day 3 to 56. From Day 14 to 56, red blood cell counts were 56 to 73% of controls. The anemia is potentially related to effects on the bone marrow and the kidneys (see below)
- Associated with the leukopenia and anemia was decreased cellularity and increased fat tissue in the bone marrow starting at 0.3 mg/kg. The severity and duration of the lesions increased with dose. Bone marrow alterations resolved after day 21 post dosing. The prolonged anemia could also be related to kidney toxicity (see below).
- In addition to changes in bone marrow, alterations to other lymphoid system organs were observed. These alterations were lymphocytic depletions of the thymus (0.1 mg/kg and higher), spleen (0.3 mg/kg and higher) and lymph nodes (0.3 mg/kg and higher). The severity and duration of the alterations increased with dose; no recovery was noted in males at Day 56 and females at Day 21. Decreased spleen and thymus weights (80% of control or less) were observed at 0.3 mg/kg (Days 3-14, 74-80% of control), 1 mg/kg (Days 3-14, 58-77% of control), and 3 mg/kg (Days 3-42, 14 to 65% of control).
- The kidney is a target organ at 1 and 3 mg/kg. Slight hydropic degeneration was observed at 1 mg/kg from Days 21 to 42). Slight to severe hydropic degeneration, interstitial tissue proliferation and fibrosis were observed at 3 mg/kg from days 15 to 56. Related changes included increased BUN at 3 mg/kg from Day 14 to 56, decreased red blood cells from day 3 to 56 (this finding could also be related to effects on the bone marrow, see above), and hematuria (between Day 5 to 56). Finally, kidney weight was increased at 1 (Day 42, increased 42 to 46%) and 3 mg/kg (Days 14-56, increased 59 to 101%).
- Basophilic granules were also observed in the livers of rats treated with 1 mg/kg (Day 42 only, slight) and 3 mg/kg (days 14 to 56, slight to severe).
- Decreased relative seminal vesicle weights were observed at 3 mg/kg from days 14 to 56 (31 to 61% of control).
- Increased serum cholesterol at 1 (Day 14 to 42, 120 to 237 per cent of control) and 3 mg/kg (Day 14 to 56, 289 to 805% of control); increased triglycerides at 3 mg/kg from day 14 to 56 (154 to 971% of controls). These findings are common in progressive kidney failure.
- The Lowest Effect Level was 0.03 mg/kg for leukopenia. A No Effect Level could not be determined from this study.

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Dogs

Dog, Single-Dose IV Toxicity (Report 84, 85)

Volume 9, Pages 222-260

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 6/12/1978

GLP Compliance: No

Species: Beagle Dog

Design: Single IV injection with recovery period

No. of animals: 1/dose

Doses: 0.25, 0.5, 1.0, 4.0, 40 mg/kg (5, 10, 20, 80, 800 mg/m²)

Design: single IV injection, 1 dog/dose, 3 dogs received 0.5, 1, 40 mg/kg, 2 E dogs received

0.25, 4 mg/kg; dogs sacrificed on Day 8 of study

Mortality: 40 mg/kg- died 5 hours post dosing on Day 0; 4 mg/kg- sacrificed moribund on Day 3

Clinical Signs:

40 mg/kg- depression, edema

4 mg/kg and above- diarrhea, bloody diarrhea, hypothermia

1 mg/kg and above- emesis, salivation, weakness

0.5 mg/kg and below- no significant signs.

Body weight- 4 mg/kg-18% decrease on Day 3; 1 mg/kg- 9% decrease on Day 8; 0.5 mg/kg- no effect

Food Consumption; 1 mg/kg anorexia starting on Day 5

Hematology- 4 mg/kg dog considered dehydrated, resulting in higher than expected hematology values.

Dose (mg/kg)	White Blood Cells (% of Predose)			Red Blood Cells (% of Predose)		
	Day 2	Day 4	Day 7	Day 2	Day 4	Day 7
4	103	34*	--	87	106*	--
1	59	23	29	97	85	74
0.5	48	42	26	96	85	85
0.25	70	63	57	94	92	88

*Day 3 value,

Clinical Chemistry

1 mg/kg and above- Increased BUN and cholesterol; lack of predosing values make comparisons difficult.

Gross Pathology

40 mg/kg- Liver and kidney congestion, pulmonary edema

4 mg/kg- emaciation, duodenum hemorrhage, blood in large intestine and anus, hyperemic colon and kidney, congested cerebrum and pituitary

1 mg/kg- lymph nodes edema and hemorrhage, kidney hyperemic, hematoma in cervical subcutaneous tissue, thyroid gland petechial hemorrhages

0.5 mg/kg- enlarged prostate gland, tonsils, and spleen, congested spleen

0.25 mg/kg- enlarged adrenal gland

Histopathology

40 mg/kg- liver congestion, pneumonia, hepatitis, spleen extramedullary hematopoiesis

4 mg/kg- colitis, renal congestion, spleen depletion of lymphoid tissue

1 mg/kg- pulmonary congestion with lymphocyte accumulation, marked lymph node hemorrhage, bone marrow hypocellularity, skeletal muscle degeneration

0.5 mg/kg- congested spleen, liver, and lymph nodes, granulomatous pneumonia, lymph node hyperplasia

0.25 mg/kg- congested spleen, liver, and lung, granulomatous pneumonia,

Key study findings

1. Results are consistent with a cytotoxic drug. Primary target organs include the blood, gastro-intestinal tract, lung and kidney.
2. Dose dependent decreases in WBC counts were observed starting at 0.25 mg/kg (the lowest level tested) starting on Day 2 (43% decrease in WBC count at 0.25 mg/kg on Day 7).

3. Dose dependent decreases in RBC counts were observed starting at 0.25 mg/kg (the lowest level tested), the magnitude of the decrease was less than that of the WBC decrease (12% decrease at 0.25 mg/kg on Day 7).
4. Increased BUN at 1 mg/kg and above with pathological evidence of kidney toxicity (renal congestion at 4 mg/kg and hyperemic kidneys at 1 mg/kg) suggest that the kidney is also an important target organ.
5. Lung toxicity (lung congestion and pneumonia) was observed at 0.25 mg/kg and above.
6. Gastro-intestinal toxicity (colitis, hemorrhage) was observed at 4 mg/kg and above.
7. A No Effect Level could not be determined from this study.

A single intravenous toxicity study of CL 232,315 with 60-67 days of recovery in beagle dogs (Reports 86, 87)

Volume 10, Pages 1-243

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 12/07/1978

GLP Compliance: No

Design: Single IV injection with recovery period

Dosing Information

Species: Beagle Dog

Age: 12-20 months

Weight: 8.4 to 10.7 kg (males), 6.6 to 9.8 kg (females)

No. of animals: 2/sex/dose experimental, 4/sex controls

Doses: 0, 0.1875, 0.25, 0.375, 0.5, 1 mg/kg (3.75, 5, 7.5, 10, 20 mg/m²)

Mortality: All deaths occurred on days 9-10, one death at 0.5 mg/kg was a moribund sacrifice

Dose	0	0.1875	0.25	0.375	0.5	1
Mortality	0/8	0/4	0/4	0/4	3/4	4/4

Clinical Signs

Reporting Incomplete

Dose	0	0.1875	0.25	0.375	0.5	1
Salivation	0/8	3/4	0/4	3/4 (Days ?-19)	4/4 (Days 6-10)	4/4 (Days 6-10)
Inactivity	0/8	0/4	1/4 (Day 8)	0/4	4/4 (Days 6-20)	4/4 (Days 6-10)
Emesis	0/8	0/4	0/4	3/4 (Days ?-19)	4/4 (Days 6-10)	4/4 (Days 6-10)
Edema	0/8	0/4	0/4	3/4 (Days ?-19)	4/4 (Days 6-41)	4/4 (Days 6-10)
Diarrhea	0/8	0/4	0/4	0/4	4/4 (Days 6-10)	4/4 (Days 6-10)
Conjunctivitis	0/8	0/4	0/4	0/4	2/4	1/4
Paw Soreness	0/8	0/4	0/4	3/4 (Days ?-19)	1/4	0/4

Body Weight- 6-9% decrease in body weight at Day 7 at 1 mg/kg;

Food Consumption- Slight decreases in food consumption at 0.375 mg/kg and above

Ophthalmoscopy- 2 dogs at 0.5 mg/kg and 1 dog at 1 mg/kg developed conjunctivitis, which was attributed to infectious agent- effect may be due to leukopenia; No other effects noted.

EKG- No effects according to sponsor (no data provided)

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Hematology- Values expressed as percent of predose values. Values in bold are outside normal range.

Sign	Day	0.1875 mg/kg		0.25 mg/kg		0.375 mg/kg		0.5 mg/kg		1 mg/kg	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
WBC	1	81	78	81	82	81	64	73	89	70	80
	4	67	75	59	52	58	45	45	40	26	27
	6	67	58	49	43	47	32	16	28	7	5
	13	85	78	67	76	66	71		33		
	21	83	78	98	86	109	119		79		
Neutrophils	1	88	94	98	101	95	68	100	121	111	104
	4	67	99	76	58	59	50	52	32	24	29
	6	72	71	44	42	44	19	9	23	3	2
	13	97	102	69	92	64	84		49		
	21	81	92	141	111	117	163		145		
RBC	1	99	96	96	95	99	95	94	105	99	98
	4	100	94	98	97	95	93	89	105	94	93
	6	99	95	95	89	92	86	81	94	79	74
	13	99	94	94	97	89	79		96		
	21	100	94	99	99	89	78		97		

Clinical Chemistry- No significant effects

Urinalysis--According to sponsor no significant effects (no data provided)

Gross Pathology

Sign	0 mg/kg	0.1875 mg/kg	0.25 mg/kg	0.375 mg/kg	0.5 mg/kg	1 mg/kg
Heart, hemorrhage	0/8	0/4	0/4	0/4	3 / 4	4/4
Stomach, red discoloration	0/8	0/4	0/4	0/4	1 / 4	4/4
Large Intestine, Hemorrhage	0/8	0/4	0/4	0/4	1 / 4	1 / 4
Large Intestine, Ulceration	0/8	0/4	0/4	0/4	2/4	1 / 4
Tonsil, red discoloration	0/8	0/4	1 / 4	0/4	3 / 4	4/4
Tonsil, irregular or ulcerated	0/8	0/4	0/4	1 / 4	3 / 4	3 / 4
Thymus, atrophy	0/8	0/4	0/4	0/4	2/ 4	4/4
Lymph node, red discoloration	0/8	2 / 4	0/4	0/4	2/4	4/4
Bone Marrow Discoloration	1/8	0/4	0/4	1 / 4	1 / 4	2/4

Organ Weights- Expressed as percent of control absolute weight; numbers in bold statistically significantly different from control values; organ weights not taken from prematurely deceased dogs.

Organ	Dose (mg/kg)					
	0.1875		0.25		0.375	
	Males	Females	Males	Females	Males	Females
Thymus	139	164	98	119	193	308
Testes	82	--	48	--	66	--

Histopathology

0.5 mg/kg and above- depletion of lymphoid tissues (reduced numbers of lymphocytes and smaller germinal centers in the lymph nodes and the white pulp of the spleen; in addition, depletion of Peyer's patches in the intestine were noted); necrosis of the tonsils; lung hemorrhages in males only

No significant histopathology in dogs which survived to terminal sacrifice (sponsor conclusion, difficult to interpret the study due to poor presentation of the data.)

Key Study Findings

1. Lethal dose was 0.5 mg/kg
2. Fatal doses were associated with depletion of lymphoid tissue and lung hemorrhages (males only)
3. Leukopenia (with neutropenia) observed at doses as low as 0.1875 in females and 0.25 in males; Recovery between days 13 and 21
4. Decreased red blood cell counts at 1 mg/kg
5. Necrosis of tonsils at 0.5 mg/kg.
6. Clinical signs observed at 0.5 mg/kg and above included inactivity and diarrhea.
7. Clinical signs observed at 0.375 mg/kg and above included salivation, emesis, edema, and paw soreness.
8. The no effect level in males was 0.1875 mg/kg; a no effect level could not be determined for females.

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Monkeys

1 and 5 day preliminary intravenous toxicity studies in male and female Cynomolgus monkeys with anticancer compound CK 232,315 (Reports 89, 90)

Volume 11, Pages 12-54

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 12/07/1978

GLP Compliance: No

Design: Single IV injection with 8 day recovery

Dosing Information

Species: Cynomolgus monkeys (*Macaca fascicularis*)

Age: ?

Weight: 2.90 to 4.45 kg (males), 2.50 to 3.80 kg (females)

No. of animals: 1/dose

Doses: 1.5, 3, 6, 12, 60 mg/kg (18, 36, 72, 144, 720 mg/m²)

Mortality: 60 mg/kg (4 hours post dosing), 12 mg/kg (Day 8)

Clinical Signs:

60 mg/kg- inactive 2.5 to 3 hours post dosing, no apparent reflexes in arms or legs, clinic convulsions 3 hours post dosing, red frothy fluid in mouth and nose just prior to death

12 mg/kg- emesis and watery stools on Day 7

No other clinical signs

Body weight- 12 mg/kg- 8% decreased in body weight

Food consumption- Food consumption decreased at 1.5 mg/kg and above

Ophthalmoscopy- Conducted, but not reported

Hematology

	Day	1.5 mg/kg	3.0 mg/kg	6.0 mg/kg	12 mg/kg
White Blood Cells	2	63	35	45	39
	4	53	24	33	33
	7	41	22	25	17
Neutrophils	2	109	34	44	55
	4	64	21	35	50
	7	18	2	0	0
Lymphocytes	2	39	33	44	37
	4	54	27	29	24
	7	54	44	44	25
Red Blood Cells	2	96	84	97	81
	4	91	84	91	87
	7	85	78	77	109
Hemoglobin	2	94	83	96	83
	4	89	81	90	84
	7	85	78	78	108
Hematocrit	2	95	81	96	82
	4	91	81	90	86
	7	84	76	76	109

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Clinical Chemistry- Values expressed as percent of predose values

	Day	1.5 mg/kg	3.0 mg/kg	6.0 mg/kg	12 mg/kg
BUN	2	112	89	110	123
	4	76	58	60	85
	7	60	67	83	323
Glucose	2	101	97	85	85
	4	115	70	80	83
	7	103	120	125	234
Chloride	2	99	109	97	109
	4	99	110	99	116
	7	105	108	100	114
Creatinine	2	111	90	100	90
	4	111	80	111	100
	7	133	100	144	550
Total Protein	2	--	78	--	88
	4	99	88	104	94
	7	104	90	96	133
Alkaline Phosphatase	2	104	95	118	113
	4	112	91	139	119
	7	122	113	126	155
CPK	2	1082	134	441	118
	4	6631	153	2839	121
	7	414	71	139	70

Gross Pathology
Histopathology

	1.5 mg/kg	3.0 mg/kg	6.0 mg/kg	12 mg/kg	60 mg/kg
Pulmonary Edema	+	+	+	+	-
Enteritis	-	+	+	+	+
Bone Marrow Hypocellularity	-	+	-	+	-
Bone Marrow Aplasia	N/A	+	N/A	N/A	N/A
Thymus Atrophy	N/A	N/A	N/A	+	N/A
Myocarditis, Focal subacute	-	-	-	-	+
Lymph Node Hyperplasia	+	+	+	+	+
Spleen, Congestion	+	-	-	-	-
Hepatocyte Degeneration	+	+	+	-	-
Hepatic Congestion	-	+	-	-	-
Visceral Congestion and Hemorrhage	+	+	+	+	+
Bright Blue coloration of GI mucosa	-	-	-	-	+

Key findings

1. The lethal dose was 12 mg/kg after 8 days; 60 mg/kg was lethal 4 hours post dosing. The highest dose not causing death after 8 days was 3 mg/kg.
2. Decreased body weight was observed at 12 mg/kg; decreased food consumption at 1.5 mg/kg and above.
3. Leukopenia, characterized by decreased neutrophil and lymphocyte counts, was observed at 1.5 mg/kg and above. The severity of the leukopenia increased with dose and was more severe 7 days post dosing than at 2 days post dosing.
4. Accompanying the leukopenia was effects on the bone marrow, lymph nodes, and thymus. Bone marrow changes included hypocellularity and aplasia. Lymph node changes included hyperplasia observed at all dose levels. Thymus changes included atrophy at 12 mg/kg (the only dose at which the thymus was examined histopathologically). Lymph node hyperplasia and thymus atrophy are characteristics of immunosuppressive agents in dogs and rodents.
5. Decreased red blood cell counts, accompanied by decreased hematocrit and hemoglobin levels, were observed at 1.5 mg/kg. The effect was less at some of the higher doses due to the dehydration of the monkeys.

6. Diarrhea and emesis were observed at 12 mg/kg. Clinical pathology signs attributable to this include increased blood glucose, BUN, and total protein.
7. Enteritis was observed at 3 mg/kg and above. Enteritis is a characteristic toxicity of cytotoxic drugs.
8. Pulmonary edema was observed at 1.5 mg/kg and above.
9. The liver was another target organ. Hepatocyte degeneration was observed between 1.5 and 6 mg/kg.
10. The lowest effect level in this study was 1.5 mg/kg for leukopenia, pulmonary edema, visceral congestion and hemorrhage. A No Effect Level could not be determined from this study.

A single dose intravenous toxicity study of CL 232,315 in male and female Cynomolgus monkeys with a 2-month observation/recovery period (Reports 91, 92)

Volume 11, Pages 55-273

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 05/21/1979

GLP Compliance: No

Design: Single IV injection with 64-5 day recovery

Dosing Information

Species: Cynomolgus monkeys (*Macaca fascicularis*)

Age: ?

Weight: 2.8 to 5.1 kg (males), 1.9 to 3.5 kg (females)

No. of animals: 2/sex/dose

Doses: 0, 0.25, 0.5 and 1 mg/kg (0, 3, 6, 12 mg/m²)

Mortality

1 mg/kg- 1 female died on Day 13; animal was inactive beginning on day 11 and was anorexic from day 10

Clinical Signs- No effect on body temperature

1 mg/kg- 1 male was inactive for most of study; had 1 day of diarrhea

0.5 mg/kg- 1 male was inactive from day 28 through 63

Body Weight

1 mg/kg- body weight was decreased 8% in males and 5 % in females at 7days; slow recovery to baseline weight at 42 days in females and 49 days in males

Food consumption

1 mg/kg- 2/4 monkeys had below average food consumption

Ophthalmology- Sponsor states there were no significant effects, but data were not provided

EKG- Sponsor states significant effects on EKG or blood pressure, but data were not provided

Hematology- Expressed as percent of control

Sign	Day	0.25 mg/kg		0.5 mg/kg		1 mg/kg	
		Male	Female	Male	Female	Male	Female
WBC	4	51	75	45	106	31	90
	8	57	82	31	59	24	42
	11	56	81	27	65	12	17
	14	30	57	25	51	26	42
	18	57	116	74	130	65	168
	25	94	106	119	140	98	121
Neutrophils	4	46	71	41	95	25	116
	8	41	79	14	30	11	10
	11	35	73	1	39	1	4
	14	12	42	4	11	20	22
	18	33	127	58	171	55	254
	25	104	115	137	120	133	147
Lymphocytes	4	63	85	51	120	40	63
	8	89	93	61	99	48	86
	11	96	95	69	88	32	48
	14	64	73	66	98	36	69
	18	104	117	108	107	88	112
	25	68	106	83	150	68	103

Sign	Day	0.25 mg/kg		0.5 mg/kg		1 mg/kg	
		Male	Female	Male	Female	Male	Female
RBC	4	101	96	96	90	97	86
	8	97	93	78	83	88	74
	11	93	88	82	79	76	55
	14	96	93	86	80	73	53
	18	101	97	92	81	77	54
	25	101	99	99	87	80	70
Hematocrit	4	101	94	94	95	88	90
	8	97	92	76	86	79	75
	11	93	87	80	83	66	53
	14	98	92	86	83	66	55
	18	104	97	95	86	71	61
	25	106	99	103	94	77	83
Hemoglobin	4	98	92	91	94	88	90
	8	94	88	76	86	81	75
	11	90	84	80	82	68	56
	14	94	89	85	81	65	56
	18	99	93	94	85	69	59
	25	99	95	100	90	74	81

Clinical Chemistry- No significant effects

Urinalysis- Sponsor states there were no significant effects, but data were not provided

Organ Weights-

No significant effects observed

Gross Pathology

1 mg/kg- In the deceased animal, the following gross pathology signs were observed: froth on cut lung surface, fibrin in the pleural cavity, hydrothorax, hydropericardium, contaneous congestion and hemorrhages and a 3 mm rectal ulcer.

No significant gross pathology in animals at scheduled sacrifice.

Histopathology

1 mg/kg- In the deceased animal, the following histopathological lesions were observed: pulmonary edema, sub acute perivascular inflammation of the kidneys, and regenerating bone marrow.

No significant histopathology in animals at scheduled sacrifice

Key findings

1. The lethal dose in this study was 1 mg/kg; the death occurred on Day 13 post dosing. Relevant histological findings included pulmonary edema, perivascular inflammation of the kidney, and regenerating bone marrow.
2. Leukopenia was observed at 0.25 mg/kg and was dose dependent. WBC counts were depressed on Day 4 of the study and reached a nadir Days 11 to 14. The leukopenia was characterized by neutropenia and, to a lesser extent, lymphocytopenia. The regenerating bone marrow in the deceased monkey is consistent with the severe leukopenia observed in treated animals.
3. Anemia was also observed, primarily at 1 mg/kg; There were related decreases in hemoglobin and hematocrit. The lack of an increase in reticulocytes suggest that the bone marrow was affected.
4. The Lowest Effect Level was 0.25 mg/kg for leukopenia and neutropenia. A No Effect Level could not be determined.

Acute Toxicity Summary

A listing of the lowest lethal intravenous doses and the highest nonlethal dose in various species is presented in the following table. The lowest lethal doses are consistent across species and range from 0.5 mg/kg in the dog to 7 mg/kg in mice, a factor of 14; when doses are expressed as mg/m², the range is from 10 to 21, a factor of 2. The signs of toxicity were consistent across species and doses. At the lower range of lethality, the time between exposure and death was often more than seven days. The primary toxicity observed during these studies was effects on the bone marrow resulting in leukopenia and anemia. Other target organs included the intestinal mucosa, kidney, lungs (primarily at higher doses). The long recovery periods given to the experimental animals makes it more difficult to assess the acute histopathological effects of mitoxantrone.

Species	Lethal Dose (mg/kg)	Lethal Dose (mg/m ²)	Nonlethal Dose (mg/kg)	Nonlethal Dose (mg/m ²)
Mouse	7	21	5.5	16.5
Rat	3.0	18	1	6
Dog	0.5	10	0.375	7.5
Monkey	1	18	0.5	9

	Rat		Dog		Monkey	
	Mg/kg	Mg/m ²	Mg/kg	Mg/m ²	Mg/kg	Mg/m ²
Leukopenia	0.03 (N/A)	0.18 (N/A)	0.1875 (N/A)	3.75 (N/A)	0.25 (N/A)	4.5 (N/A)
Anemia	3 (1)	18 (6)	1 (0.5)	20 (10)	1 (0.5)	18 (9)

Subchronic Toxicology

Anticancer agent CL 232,315: A one month intravenous toxicity study in rats. (Reports 93, 94)

Volume 12, Pages 1-130

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 08/11/1980

GLP Compliance: No

Design: Daily IV injections for 30 days

Dosing Information

Species: Rat CRJ:CD(SD)

Age: 6 weeks

Weight: 205.2 to 238.3 g (males), 156.5 to 182.2 g (females)

No. of animals 10 rats/sex/dose

Doses: 0, 0.003, 0.01, 0.03, 0.1, 0.3 mg/kg
(0, 0.018, 0.06, 0.18, 0.6, 1.8 mg/m²)

Route: IV into tail vein over 10 seconds

Clinical signs-

	0		0.003		0.01		0.03		0.1		0.3	
	M	F	M	F	M	F	M	F	M	F	M	F
Number of Rats	10	10	10	10	10	10	10	10	10	10	10	10
Mortality	0	0	0	0	0	0	0	0	0	0	10	10
Epistaxis	0	0	0	0	0	0	0	0	0	0	9	6
Perinasal Swelling	0	0	0	0	0	0	0	0	0	0	10	8
Lacrimation	0	0	0	0	0	0	0	0	0	0	5	4
Chromodacryorrhea	0	0	0	0	0	0	0	0	0	0	5	4
Rough fur	0	0	0	0	0	0	0	0	0	0	7	10
Paleness	0	0	0	0	0	0	0	0	0	5	7	9
Inactivity	0	0	0	0	0	0	0	0	0	0	7	7

Males died between days 11 and 15; females died between days 11-25

Body Weight

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Anticancer Agent CL 232.315 : A One Month Intravenous Toxicity Study in Rats
 — Mean Body Weight (grams) —

Sex	Dose mg/kg/day ^a	Predose	Day of Test ^b				
			7	14	21	29	
Male	0.0	Mean	220.4	261.3	295.5	323.9	344.9
		S.D. (N)	8.97 10	9.61 10	13.31 10	16.97 10	19.26 10
	0.003	Mean	222.8	272.6	312.6	343.3	367.4
		S.D. (N)	11.47 10	15.01 10	22.39 10	30.61 10	35.10 10
	0.010	Mean	219.9	268.8	305.8	336.6	361.0
		S.D. (N)	6.84 10	11.42 10	16.30 10	19.71 10	23.91 10
0.030	Mean	219.9	265.7	300.5	331.2	354.6	
	S.D. (N)	10.18 10	15.01 10	23.15 10	32.78 10	38.65 10	
0.100	Mean	223.5	264.9	299.9	323.3	344.8	
	S.D. (N)	6.70 10	11.55 10	13.79 10	15.35 10	17.31 10	
0.300	Mean	218.3	252.3	233.6 ^c	--	--	
	S.D. (N)	8.20 10	15.41 10	0.07 2			
Female	0.0	Mean	165.7	183.4	209.5	228.3	244.2
		S.D. (N)	7.06 10	7.02 10	8.58 10	8.65 10	10.53 10
	0.003	Mean	170.3	189.9	213.9	229.8	245.2
		S.D. (N)	7.19 10	9.98 10	11.74 10	14.13 10	16.11 10
	0.010	Mean	170.1	191.2	212.5	230.7	243.1
		S.D. (N)	4.92 10	8.20 10	6.74 10	8.27 10	11.81 10
0.030	Mean	169.7	190.4	214.1	228.8	243.9	
	S.D. (N)	5.97 10	9.23 10	11.45 10	11.97 10	13.09 10	
0.100	Mean	166.5	185.5	201.9	214.0	223.2	
	S.D. (N)	8.65 10	7.26 10	8.85 10	11.10 10	12.86 10	
0.300	Mean	166.9	185.6	183.0 ^c	197.2 ^c	--	
	S.D. (N)	5.04 10	6.16 10	15.75 8	0.0 1		

The statistical comparison in each case is with the control group (0 mg/kg/day).

^{cc} P < 0.01

S.D.: Standard deviation (N): Number of animals
 a: As the free base b: Day first dose given designated as day 0.
 c: Not evaluated statistically due to insufficient number of animals.
 --: Not determined because all animals had died.

Food consumption-

0.3 mg/kg- decreased food consumption in males (days 7-13) and females (days 14-20)

Urinalysis- No effects

EKG- Sponsor says no effects (no data presented)

Hematology- 0.3 mg/kg not examined because all the rats had died; Expresses as % of controls; values in bold statistically significant difference

	0.003		0.01		0.03		0.1	
	Male	Female	Male	Female	Male	Female	Male	Female
WBC	104	111	104	84	65	64	32	34
Neutrophils	86	122	110	83	77	68	35	41
Lymphocytes	109	111	105	84	64	62	32	33
RBC	98	97	99	97	96	94	64	58
Hb	96	96	97	98	96	94	67	59
PCV	95	97	97	97	96	94	63	56

Clinical Chemistry- 0.3 mg/kg not examined because all the rats had died; no effects

Organ Weights- no data on 0.3 mg/kg rats due to early deaths

	0.003		0.01		0.03		0.1	
	Male	Female	Male	Female	Male	Female	Male	Female
Thymus	94	104	102	103	80	80	14	13
Spleen	95	96	92	90	73	79	55	64
Heart	103	98	100	97	107	99	110	109
Lungs	99	105	100	103	106	103	108	113
Uterus	-	86	-	105	-	94	-	77

Gross Necropsy

	Sex	0 m/kg	0.003 mg/kg	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Thymus: Small	Male	0/10	0/10	0/10	1/10	10/10	10/10
	Female	0/10	0/10	0/10	0/10	10/10	10/10
Thymus: Congestion	Male	0/10	0/10	0/10	0/10	0/10	6/10
	Female	0/10	0/10	0/10	1/10	0/10	1/10
Lymph Nodes: Small	Male	0/10	0/10	0/10	0/10	0/10	10/10
	Female	0/10	0/10	0/10	0/10	0/10	10/10
Lymph Nodes: Hemorrhage	Male	0/10	0/10	0/10	0/10	0/10	10/10
	Female	0/10	0/10	0/10	0/10	0/10	10/10
Lungs: Congestion and Hemorrhage	Male	0/10	0/10	0/10	0/10	0/10	6/10
	Female	0/10	0/10	0/10	0/10	0/10	1/10
Stomach: Petechia	Male	0/10	0/10	0/10	0/10	0/10	8/10
	Female	0/10	0/10	0/10	0/10	0/10	8/10

Histopathology

0.1 mg/kg- bone marrow hypocellularity characterized by decreased erythroblasts, neutrophils, eosinophils, lymphocytes,

0.03 mg/kg and above- decrease lymphocytes in bone marrow

Histopathological Changes- Males

	0 mg/kg	0.003 mg/kg	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Thymus: Atrophy	1/10	0/10	0/10	6/10	10/10	9/9
Very Slight	1			5		
Slight						1
Moderate				1	4	3
Severe					6	5
Thymus: Cortical lymphocytic depletion	1/10	0/10	0/10	8/10	10/10	9/9
Very Slight	1			7		
Slight				1		
Moderate						1
Severe					10	8
Spleen: Atrophy	0/10	0/10	1/10	4/10	8/10	10/10
Very Slight			1	3		2
Slight				1	8	8
Spleen: Follicular lymphocytic depletion	0/10	0/10	10/10	10/10	10/10	10/10
Very Slight			8	5		
Slight			2	5	1	
Moderate					9	7
Severe						3
Lymph Nodes: Hemorrhage	0/10	1/10	0/10	0/9	1/10	6/8
Slight		1			1	3
Moderate						1
Severe						2
Lymph Nodes: lymphocytic depletion	0/10	0/10	0/10	1/9	10/10	8/8

	0 mg/kg	0.003 mg/kg	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Very Slight					2	
Slight				1	3	
Moderate					5	8
Small Intestine: lymphocytic depletion of Peyer's Patches	0/10	0/9	0/10	0/10	9/9	4/4
Very Slight					1	
Slight					5	1
Moderate					3	2
Severe						1
Bone Marrow: Hypocellularity	0/10	0/10	0/10	6/10	10/10	10/10
Very Slight				6		
Moderate					3	1
Severe					7	9
Bone Marrow: Increased Fat Tissue	0/10	0/10	0/10	6/10	10/10	9/10
Slight				6		1
Moderate					3	1
Severe					7	7
Heart: Atrophy	0/10	0/10	0/10	0/10	8/10	10/10
Very Slight					1	2
Slight					7	5
Moderate						3
Heart: Hydropic and necrotic changes of muscular fibers	0/10	0/10	0/10	0/10	3/10	7/10
Very Slight					3	2
Slight						5
Heart: Proliferation of mesenchymal cells	0/10	0/10	0/10	0/10	9/10	10/10
Very Slight					6	1
Slight					3	9
Kidney: Cloudy swelling of proximal tubular epithelium	0/10	0/10	0/10	0/10	4/10	2/2
Very Slight					2	
Slight					2	
Moderate						2
Lungs: Pulmonary edema	0/10	0/10	0/10	0/10	0/10	8/10
Moderate						3
Severe						5
Lung: Congestion and hemorrhage	0/10	0/10	0/10	0/10	0/10	8/10
Slight						1
Moderate						4
Severe						3
Various organs: Bacterial Clumps	0/10	0/10	0/10	0/10	0/10	1/10

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Histopathological Changes- Females

	0 mg/kg	0.003 mg/kg	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Thymus: Atrophy	0/10	0/10	0/10	3/10	10/10	10/10
Very Slight				3		
Severe					10	10
Thymus: Cortical lymphocytic depletion	0/10	0/10	0/10	6/10	10/10	10/10
Very Slight				6		
Severe					10	10
Spleen: Atrophy	0/10	0/10	0/10	6/10	10/10	9/9
Very Slight				5	1	
Slight				1	4	2
Moderate					5	2
Severe						5
Spleen: Follicular lymphocytic depletion	0/10	0/10	10/10	10/10	10/10	9/9
Very Slight			10	1		
Slight				8	1	
Moderate				1	8	3
Severe					1	6
Lymph Nodes: Hemorrhage	0/10	0/10	0/10	0/10	1/10	8/10
Slight					1	1
Moderate						1
Severe						6
Lymph Nodes: lymphocytic depletion	0/10	0/10	0/10	0/10	10/10	10/10
Very Slight					1	
Slight					2	
Moderate					7	3
Severe						7
Small Intestine: lymphocytic depletion of Peyer's Patches	0/10	0/10	0/10	0/10	9/9	2/2
Slight					7	
Moderate					2	1
Severe						1
Bone Marrow: Hypocellularity	0/10	0/10	0/10	3/10	10/10	10/10
Very Slight				3		
Slight					3	
Moderate					2	
Severe					5	10
Bone Marrow: Increased Fat Tissue	0/10	0/10	0/10	6/10	10/10	9/10
Slight				6		1
Moderate					3	7
Severe					7	1
Heart: Atrophy	0/10	0/10	0/10	0/10	8/10	10/10
Very Slight					3	
Slight					5	7
Moderate						2
Severe						1
Heart: Hydropic and necrotic changes of muscular fibers	0/10	0/10	0/10	0/10	6/10	8/10
Very Slight					5	1
Slight					1	5
Moderate						2
Heart: Proliferation of mesenchymal cells	0/10	0/10	0/10	0/10	10/10	10/10
Very Slight					8	2
Slight					2	7

	0 mg/kg	0.003 mg/kg	0.01 mg/kg	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg
Severe						1
Kidney: Cloudy swelling of proximal tubular epithelium	0/10	0/10	0/10	2/10	9/10	0/0
Very Slight				2	9	
Lungs: Pulmonary edema	0/10	0/10	0/10	0/10	0/10	9/10
Very Slight						1
Slight						1
Moderate						3
Severe						4
Lung: Congestion and hemorrhage	0/10	0/10	0/10	0/10	0/10	6/10
Moderate						4
Severe						2
Various organs: Bacterial Clumps	0/10	0/10	0/10	0/10	0/10	8/10

Key Findings

1. The lowest lethal dose was 0.3 mg/kg/day, which caused 100% mortality; rats died between Days 11 and 25 of the study. The highest non-lethal dose was 0.1 mg/kg/day.
2. Clinical signs observed in rats given 0.3 mg/kg included epistaxis and chromodacryorrhea, which is consistent with internal hemorrhages (see below). In addition, lacrimation, inactivity, and perinasal swelling were observed.
3. Decreased body weight gain was observed at 0.3 mg/kg in males (body weight 79% of control at Day 14) and 0.1 mg/kg in females (91% of control at Day 29). Decreased food consumption was also observed at 0.3 mg/kg in males (days 7-13) and females (days 14-20).
4. Statistically significant leukopenia, characterized by decreased neutrophil and lymphocyte counts was observed at 0.03 mg/kg and above. The decrease was dose dependent (62-77% of control at 0.03 mg/kg and 32 to 41% of control at 0.1 mg/kg). Leukopenia was also observed in females at 0.01 mg/kg (83-84% of control); this decrease was not statistically significant, however it is consistent with decreases at higher doses; in addition, is consistent with the lymphocyte depletion in the spleen of rats treated at 0.01 mg/kg (see below).
5. A smaller decrease in red blood cell, hemoglobin, and hematocrit were observed at 0.03 mg/kg and above. The decrease was dose dependent (95% of control at 0.03 mg/kg and 58-67% of control at 0.1 mg/kg).
6. No effects on clinical chemistry, urinalysis, or EKG parameters were observed at 0.1 mg/kg and below. Due to premature mortality, these tests were not done on the rats given 0.3 mg/kg.
7. The bone marrow is a significant target organ. Effects on the bone marrow include hypocellularity with increased fat tissue beginning at 0.03 mg/kg; these effects were considered severe at 0.1 mg/kg.
8. The lymphoid organs were also significant target organs. Atrophy with lymphocyte depletion was observed in the thymus, spleen, and lymph nodes (lymphocyte depletion only) at 0.03, 0.01, and 0.03 mg/kg, respectively. The severity of the lesions increased with dose.
9. Adverse effects on the heart (atrophy, necrosis, and mesenchymal cell proliferation) were observed at 0.3 mg/kg.
10. Cloudy swelling of proximal tubular epithelium of the kidney was observed at 0.1 mg/kg.
11. Hemorrhages were observed in the lymph nodes, stomach, small intestine, adrenal glands, brain and lungs at 0.3 mg/kg; petichiae in the stomach were also observed at this dose. Possibly related to these findings, pulmonary edema was observed at 0.3 mg/kg.
12. Bacterial clumps suggestive of a decrement in the immune system was observed in the heart, lungs, tongue, stomach, kidneys, ovaries, lymph nodes and brain at 0.3 mg/kg.
13. Evaluation of target organs at 0.3 mg/kg is difficult due to extensive autolysis in treated animals and lack of blood data. As a result, little or no data was available on effects on the liver, kidney, and intestines. Based on results of acute toxicity studies, it is reasonable to expect that the intestinal mucosa would be affected by Novantrone.
14. The highest no effect level in this study was 0.003 mg/kg.

Chronic Studies

Rat, IV Administration Once Every 21 Days for 12 months (Reports 104-112)

Volume 16, page 1 to Volume 20, page 107

Conducting Laboratory: Lederle (Japan) Laboratories, American Cyanamid Co, Tokyo, Japan

Date of Study Initiation: 03/30/1981

GLP Compliance: No

Design: See Table

Dosing Information

Species: Rat CRJ:CD(SD)

Age: 6 weeks

Weight: 238.0 to 322.7 g (males), 175.0 to 225.3 g (females)

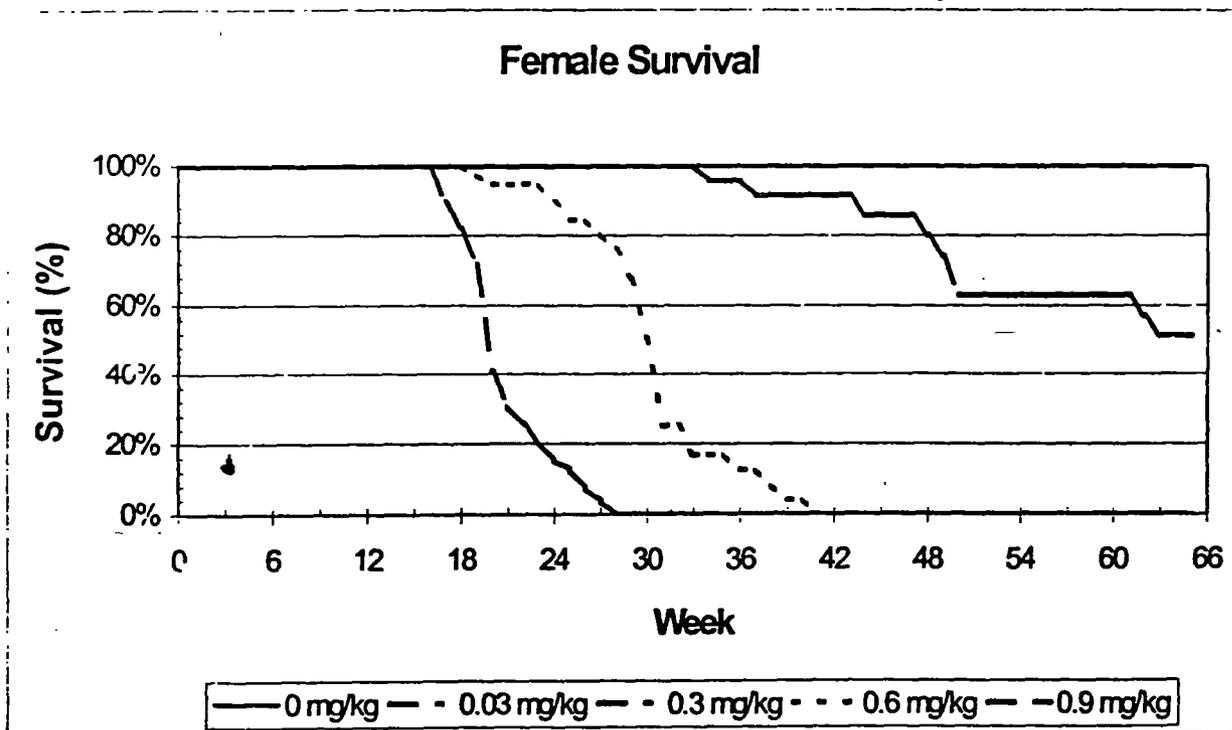
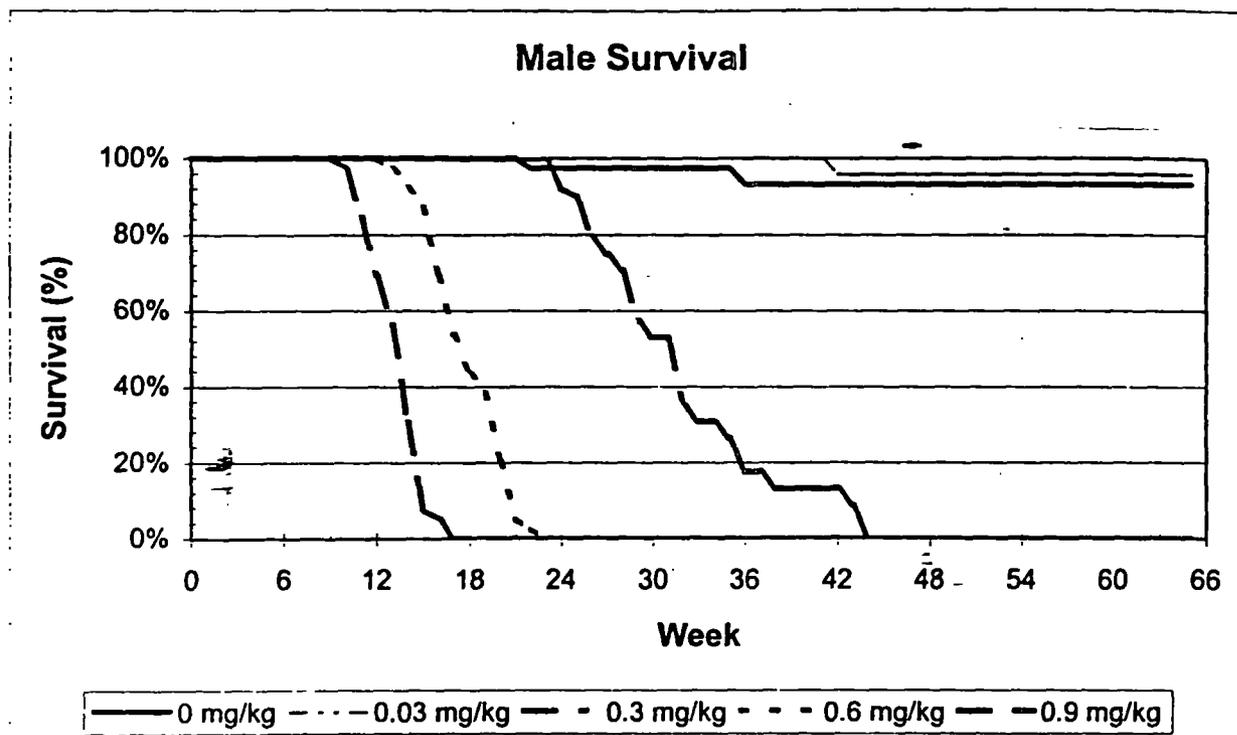
Route: IV into tail vein over 10 seconds

- Experimental Design -								
Sex	Dose		No. of Rats	Type of Segment	Number Doses Given	Cumulative Dose per Animal		Day of ^c Sacrifice
	mg/kg ^a	mg/m ² b				mg/kg	mg/m ²	
Male	0.0 ^d	0.0	15	6 Month	9	0.0	0.0	Days 189-193
			18	12 Month	18	0.0	0.0	Days 378-382
			6	Recovery	18	0.0	0.0	Days 477-479
	0.03	0.18	15	6 Month	9	0.27	1.62	Days 189-193
			18	12 Month	18	0.54	3.24	Days 378-382
			6	Recovery	18	0.54	3.24	Days 477-479
	0.3	1.8	15	6 Month	9	2.7	16.2	Days 189-193
			18	12 Month	18	5.4	32.4	Days 378-382
			6	Recovery	18	5.4	32.4	Days 477-479
	0.6	3.6	15	6 Month	9	5.4	32.4	Days 189-193
			18	12 Month	18	10.8	64.8	Days 378-382
			6	Recovery	18	10.8	64.8	Days 477-479
	0.9	5.4	15	6 Month	9	8.1	48.6	Days 189-193
			18	12 Month	18	16.2	97.2	Days 378-382
			6	Recovery	18	16.2	97.2	Days 477-479
Female	0.0 ^d	0.0	15	6 Month	9	0.0	0.0	Days 189-193
			18	12 Month	18	0.0	0.0	Days 378-382
			6	Recovery	18	0.0	0.0	Days 477-479
	0.03	0.18	15	6 Month	9	0.27	1.62	Days 189-193
			18	12 Month	18	0.54	3.24	Days 378-382
			6	Recovery	18	0.54	3.24	Days 477-479
	0.3	1.8	15	6 Month	9	2.7	16.2	Days 189-193
			18	12 Month	18	5.4	32.4	Days 378-382
			6	Recovery	18	5.4	32.4	Days 477-479
	0.6	3.6	15	6 Month	9	5.4	32.4	Days 189-193
			18	12 Month	18	10.8	64.8	Days 378-382
			6	Recovery	18	10.8	64.8	Days 477-479
	0.9	5.4	15	6 Month	9	8.1	48.6	Days 189-193
			18	12 Month	18	16.2	97.2	Days 378-382
			6	Recovery	18	16.2	97.2	Days 477-479

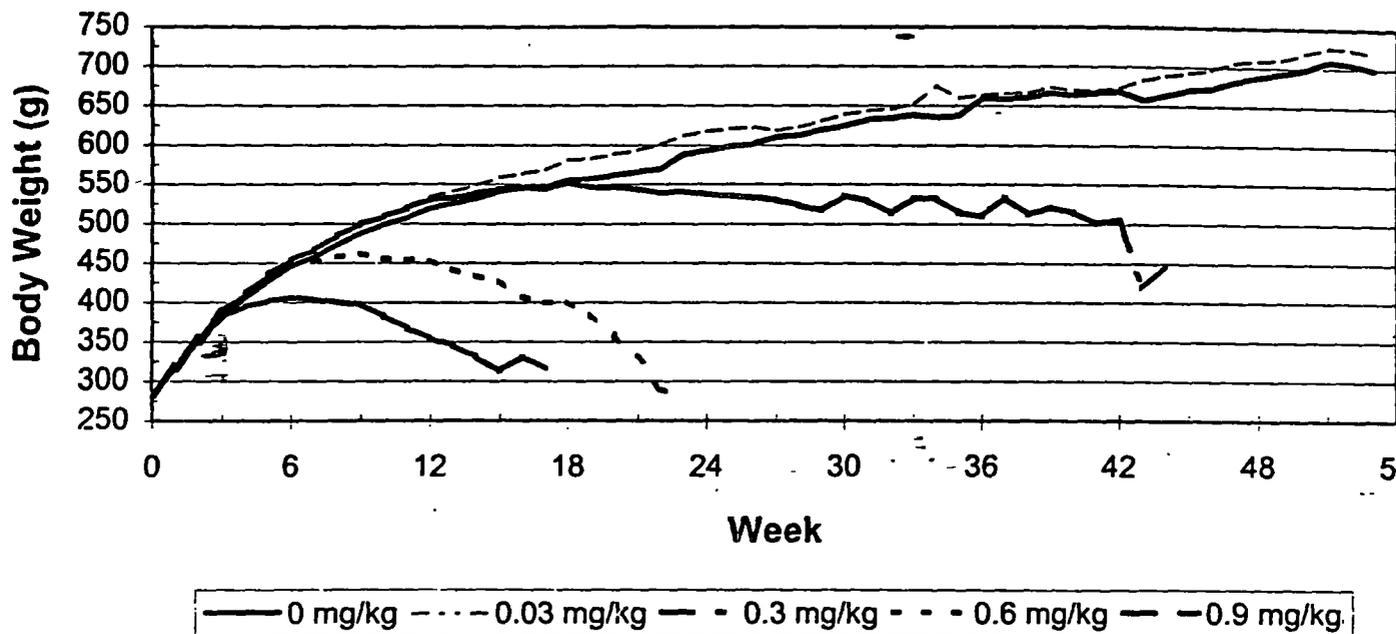
a: As the free base
b: Calculated by the Km factor 6
c: Day first dose given designated as day 0
d: Control animals received the sterile physiological saline.

Due to high mortality, recovery groups were not done generally;

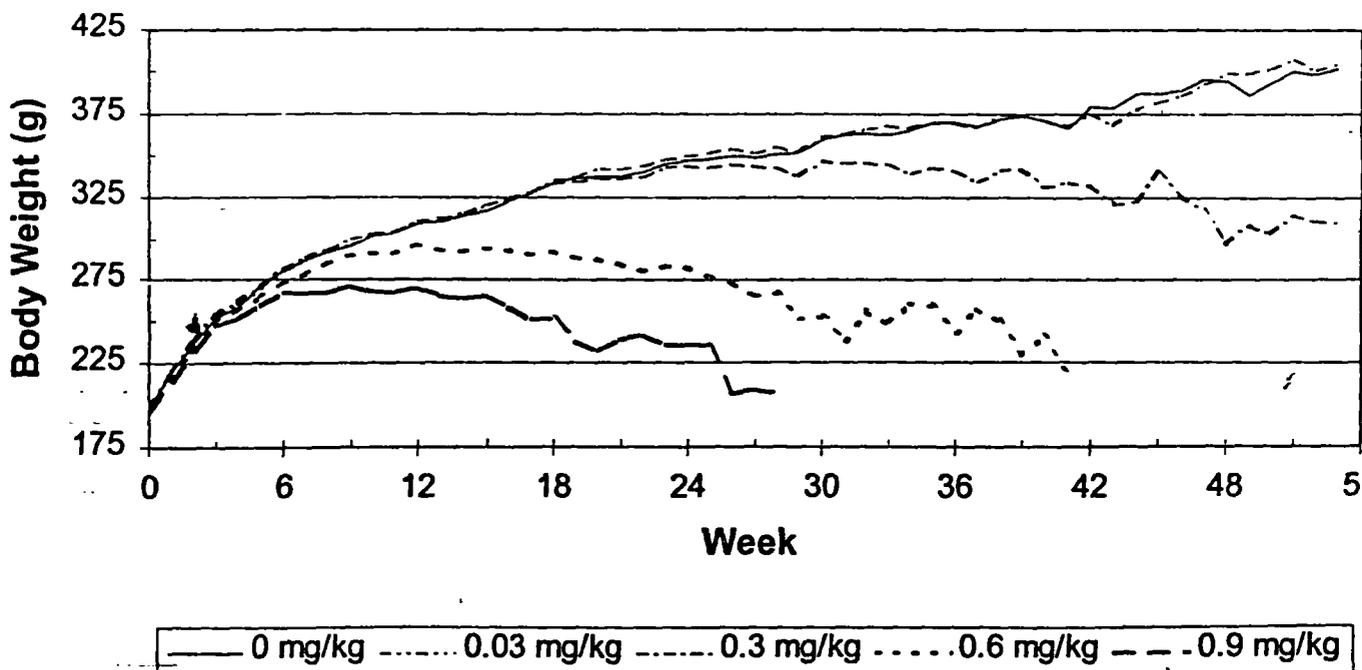
Mortality- Mortality in males occurred when cumulative doses reached about 2.9 mg/kg with most of the rats dying when the cumulative doses reached 4.2 to 4.4 mg/kg. In females, mortality occurred when cumulative doses reached 4.6 mg/kg and most of the females died when the cumulative doses reached 6.5 to 6.7 mg/kg.



Male Body Weight



Female Body Weight



Food Consumption- Decreased food consumption was observed at 0.3 mg/kg and above in both sexes.

Clinical Observations- Values expressed as percent of animals showing symptoms

	Day	0 mg/kg		0.03 mg/kg		0.3 mg/kg		0.6 mg/kg		0.9 mg/kg	
		M	F	M	F	M	F	M	F	M	F
Paleness	0-188	2	0	0	0	31	0	100	90	100	100
	189-290	4	0	0	0	100	8	---	100	---	100
	291-377	0	0	0	0	100	80	---	---	---	---
Thinness	0-188	5	0	0	0	2	0	67	15	46	64
	189-290	4	0	0	0	17	8	---	75	---	100
	291-377	0	0	0	0	33	50	---	---	---	---
Inactivity	0-188	5	0	0	0	5	0	64	10	64	59
	189-290	4	0	0	0	44	4	---	75	---	100
	291-377							---	---	---	---
Epistaxis	0-188	5	0	0	2	13	18	56	13	59	28
	189-290	4	0	0	0	39	0	---	15	---	50
	291-377	0	0	0	0	33	10	---	---	---	---
Chromodacryorrhea	0-188	8	2	2	0	8	8	8	5	15	2
	189-290	4	0	0	0	6	0	---	15	---	0
	291-377	0	8	0	8	33	20	---	---	---	---
Red Colored Fur	0-188	2	0	0	2	5	5	28	8	23	28
	189-290	0	0	0	0	6	0	---	10	---	0
	291-377	0	0	0	0	0	10	---	---	---	---
Hematuria	0-188	2	0	0	0	2	0	0	0	10	0
	189-290	0	0	4	0	0	0	---	0	---	0
	291-377	0	0	8	0	0	0	---	---	---	---

Symptoms generally developed approximately four weeks prior to death

EKG-

0.9 mg/kg- decreased heart rate (-10%), increased PR and QT intervals at days 85-87; decreased heart rate (-22%) at day 169-171 in females only (n=2), all males were deceased at this time point.

0.6 mg/kg was not evaluated at times that symptoms of toxicity were apparent.

0.3 mg/kg- decreased heart rate (-10%) at days 169-171 in males only.

Hematology-Expressed as percent of controls;

	Day	0.03 mg/kg		0.3 mg/kg		0.6 mg/kg	0.9 mg/kg
		M	F	M	F	F	F
WBC	189	105	105	99	85	87	56
	290	81	132		80		
	378	77	85		189		
Neutrophils	189	95	99	137	95	160	63
	290	91	114		104		
	378	111	89		608		
Lymphocytes	189	107	106	85	80	63	55
	290	80	129		71		
	378	69	82		67		
RBC	189	102	103	68	97	72	67
	290	96	104		92		
	378	92	94		81		
Hemoglobin	189	101	103	73	99	74	71
	290	96	104		90		
	378	98	97		83		
Hematocrit	189	101	102	74	99	74	76
	290	97	101		89		
	378	95	93		106		
Platelets	189	106	109	144	124	151	160
	290	98	107		114		
	378	103	104		127		

Values in **Bold** are significantly different from controls; values in *Italics* had too few animals to permit statistical analysis.

Clinical Chemistry- Expressed as percent of controls

	Day	0.03 mg/kg		0.3 mg/kg		0.6 mg/kg	0.9 mg/kg
		M	F	M	F	F	F
Cholesterol	189	116	89	913	233	691	<i>763</i>
	290	82	123		466		
	378	175	95		567		
Triglycerides	189	115	114	812	203	2315	<i>1675</i>
	290	90	76		791		
	378	194	93		1177		
BUN	189	99	99	187	93	410	<i>196</i>
	290	84	95		93		
	378	90	93		135		
Phospholipids	189	118	97	750	200	474	<i>548</i>
	290	86	118		351		
	378	145	100		369		
Glucose	189	102	99	89	93	80	<i>86</i>
	290	97	84		73		
	378	108	107		112		
Calcium	189	104	98	105	102	117	<i>102</i>
	290	96	101		111		
	378	99	103		108		
Phosphate	189	112	92	132	88	205	<i>132</i>
	290	88	98		281		
	378	95	100		110		

Values in **Bold** are significantly different from controls; values in *Italics* had too few animals to permit statistical analysis.

Urinalysis-Expressed as percent of rats

	Day	0		0.03		0.3		0.6		0.9	
		M	F	M	F	M	F	M	F	M	F
Proteinuria*	98	4	8	4	0	96	62	100	100	100	100
	182	0	19	4	0	91	96		100		100
	266	19	25	50	13	100	100		100		
	296	0	17	8	8	100	100				
	371	20	33	64	10		100				
Occult Blood	98	24	12	38	8	20	0	100	4	92	19
	182	35	12	38	4	57	4		20		50
	266	44	19	19	25	75	13		67		
	296	70	0	25	8	67	10				
	371	50	8	36	20		20				
Urine Volume Increase#	98	--	--	N	N	N	Y	Y	Y	Y	Y
	182	--	--	N	N	Y	N		Y		Y
	266	--	--	Y	N	Y	Y		Y		
	296	--	--	N	N	Y	Y				
	371	--	--	N	N		Y				

*>1000 mg/100 ml in males; >300 mg/100 ml in females

#urine volume significantly greater than control (Y/N/)

No significant recovery during recovery periods

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Organ Weights

Organ	Sex	0.003			0.3		0.6
Day		189	290	378	189	290	189
Kidney	M	95	101	107	177		
	F	95	99	107	117	130	139
Spleen	M	93	75	93	124		
	F	95	86	95	91	94	104
Lung	M	96	104	100	119		
	F	94	103	95	100	117	134
Liver	M	101	97	113	154		
	F	95	94	103	118	149	208
Adrenal Gland	M	90	100	89	130		
	F	90	95	129	100	95	114

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Gross Pathology

0.6 mg/kg- pale bone marrow;

0.3 mg/kg- pale brown discoloration of kidneys; hardening of aorta

Histopathology

Non-Neoplastic lesions

	D*	0 mg/kg		0.03 mg/kg		0.3 mg/kg		0.6 mg/kg		0.9 mg/kg	
		M	F	M	F	M	F	M	F	M	F
Bone Marrow Hypocellularity	S	0/25	0/15	0/23	0/14	0/13	0/15		3/13		0/1
	P	0/0		0/2		15/25	5/11	38/39	6/6	36/39	36/36
Thymus: Depletion of Lymphoid Tissue	S	0/25	4/25	4/24	4/25	5/13	10/21		7/12		0/1
	P	0/2		0/3		22/22	9/9	29/29	16/17	34/34	29/29
Lymph Node: Depletion of Lymphoid Tissue	S	0/22	0/24	0/25	0/27	0/12	15/22		9/9		1/1
	P	1/2		0/2		15/16	6/7	26/26	16/16	20/22	24/24
Spleen: Depletion of Lymphoid Tissue	S	0/25	0/27	1/25	0/27	4/13	22/22		13/13		1/1
	P	2/3		1/3		24/24	13/13	37/37	23/23	37/37	35/35
Injection Site: Necrosis	S	0/25	0/24	3/21	1/26	8/13	8/22		6/13		1/1
	P	0/3		0/3		14/26	1/13	24/39	7/26	19/39	14/38
Heart: muscle necrosis	S	1/25	0/26	0/25	0/27	0/13	2/22		12/13		1/1
	P	0/2		1/2		25/25	8/13	38/38	26/26	36/37	36/38
Kidney: Hydropic change/necrosis proximal tubule	S	4/25	4/27	8/25	3/27	13/13	19/22		13/13		1/1
	P	1/1		0/0		18/18	10/10	33/33	21/21	33/33	34/34
Kidney: nephrosclerosis	S	0/25	0/27	4/25	0/27	13/13	4/22		13/13		1/1
	P	0/1		1/1		22/22	8/8	33/33	26/26	33/33	34/35
Small Intestine: Mucosal atrophy	S	0/25	0/27	0/25	0/27	0/14	0/22		13/13		1/1
	P	0/0		0/0		3/4	1/1	13/20	3/5	9/16	15/15
Aorta: Tunica media necrosis and calcification	S	0/25	0/27	0/25	0/25	0/11	0/22		1/13		0/1
	P	0/2		1/2		25/26	6/11	37/39	26/26	37/39	34/35
Lung: Pulmonary Edema	S	0/25	0/27	0/25	0/27	0/13	0/22		0/13		0/1
	P	1/3		3/3		24/26	8/13	26/38	10/25	28/39	7/38

*Deaths; S=scheduled sacrifice, P=premature death

Lesions were not reversed at the end of the three month recovery period.

	0 mg/kg		0.03 mg/kg		0.3 mg/kg		0.6 mg/kg
	M	F	M	F	M	F	F
Total rats with neoplasms	0/11	3/23	0/12	6/24	1/18	8/24	1/17
Benign	0	0	0	1	0	1	0
Malignant	0	3	0	5	0	8	1
Auditory Sebaceous Gland	0/11	0/23	0/12	0/24	1/18	5/24	1/17
Acinas sebaceous carcinoma	0	0	0	0	0	2	0
Squamous cell carcinoma	0	0	0	0	1	3	1
Mammary gland	0/11	3/23	0/12	6/24	0/18	4/24	0/17
Adenoma	0	0	0	1	0	0	0
Preputial Gland Adenoma	0	0	0	0	0	1	0
Papillary Carcinoma	0	0	0	3	0	0	0
Duct Carcinoma	0	1	0	0	0	1	0
Adenocarcinoma	0	1	0	2	0	3	0
Scirrhous carcinoma	0	1	0	0	0	0	0
Fibrosarcoma of lung, kidney and subcutaneous tissue	0/11	0/12	0/12	0/12	0/18	1/11	0/17
Oral cavity: squamous cell carcinoma	0/11	0/23	0/12	0/24	0/18	1/24	0/17

Key Findings

1. Mortality in males occurred when cumulative doses reached about 2.9 mg/kg with most of the rats dying when the cumulative doses reached 4.2 to 4.4 mg/kg. In females, mortality occurred when cumulative doses reached 4.6 mg/kg and most of the females died when the cumulative doses reached 6.5 to 6.7 mg/kg. No mortality was associated with 0.03 mg/kg (cumulative dose 0.54 mg/kg).
2. Clinical signs observed in rats given 0.3 mg/kg or more included epistaxis, red fur, and chromodacryorrhea, which is consistent with internal hemorrhages (see below). Hematuria was observed in males at 0.03 mg/kg and 0.9 mg/kg, this is consistent with urinalysis observations and nephrotoxicity (see below). Additional clinical signs included paleness, thinness, and inactivity. Clinical signs generally appeared about four weeks prior to death.
3. Body-weight decreases were observed at 0.3 mg/kg and above. The decreases in body weight appear dependent on cumulative doses. Male body weight changes became apparent when cumulative doses reached 2.4 to 2.7 mg/kg. Female body weight changes became apparent when cumulative doses reached 3.6 mg/kg. Body weight changes occurred about 6 to 9 weeks prior to death in males and 7 to 13 weeks prior to death in females. No body weight changes were observed at 0.03 mg/kg.
4. Evaluation of hematology and clinical pathology data were complicated by the high early mortality which lead to no data for males at 0.6 and 0.9 mg/kg and little data from these doses in females.
5. Leukopenia was observed at all dose levels. Although not all of the observations were statistically significant, the observation that severe effects on WBC counts were observed in other studies with this compound suggest that decreases of 13 to 23% in WBC count are biologically significant, even if statistical significance was not observed. In other studies, the decrease in WBC count was characterized by a decrease in lymphocytes, which was observed in this study too. These data are also consistent with the bone marrow hypocellularity observed in this study (see below).
6. The RBC counts were decreased in this study too. Parallel decreased in hemoglobin and hematocrit were observed at 0.3 mg/kg and above. Supporting the diagnosis of low RBC counts were clinical observations of paleness (see above).
7. The bone marrow was a significant target organ. The primary effect was hypocellularity beginning at 0.3 mg/kg. This is consistent with the effects on white and red blood cell counts.
8. The lymphoid organs were also significant target organs. Depletion of lymphoid tissue was observed in the thymus, spleen, and lymph nodes at 0.3 mg/kg and above. The severity of the lesions increased with dose.
9. Adverse effects on the heart (necrosis) were observed at 0.3 mg/kg and above. In addition, alterations in ECG parameters were observed (decreased heart rate and increased PR and QT intervals) at 0.9

mg/kg (Days 85-87) and 0.3 mg/kg (Days 169-171). At these times, the clinical signs were apparent in these rats.

10. The kidney is a significant target organ. An increase in kidney histological alterations (proximal tubule necrosis and nephrosclerosis) was observed in males at 0.03 mg/kg and above and in females at 0.3 mg/kg and above. In addition, there were altered urinalysis values (proteinuria, occult blood, and increased urine volume) at these doses. Altered clinical chemistry values were observed (increased cholesterol, triglycerides, phospholipids, phosphate, and BUN) which are associated with kidney failure.
11. Pulmonary edema was commonly observed in animals dying at 0.03 mg/kg and above.
12. Injection site necrosis was observed at 0.03 mg/kg and above.
13. An increased incidence of neoplasms was observed in females at 0.03 mg/kg and 0.3 mg/kg (6/24 and 8/24 tumor bearing animals, respectively vs 3/23 in controls). Higher dose animals generally did not survive long enough to develop neoplasms. The increase was most pronounced in carcinomas of the auditory sebaceous gland at 0.6 mg/kg (0/23, 0/24, 5/24 at 0, 0.03 and 0.3 mg/kg, respectively). An increase was also observed in mammary gland neoplasms (3/23, 6/24, 4/24 at 0, 0.03 and 0.3 mg/kg, respectively).
14. A no effect level could not be determined from this study.

Dog, 30-Week Intravenous Toxicity, Intermittent Dosing (Reports 113-116)

Volume 21, page 1 to Volume 22, page 149

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, -

Date of Study Initiation: 01/18/1979

GLP Compliance: No

Design: See Table

Dosing Information

Species: Dog, Beagle

Age: 12-14 months

Weight: 6.2 to 9.2 kg (males), 7.7 to 9.6 kg (females)

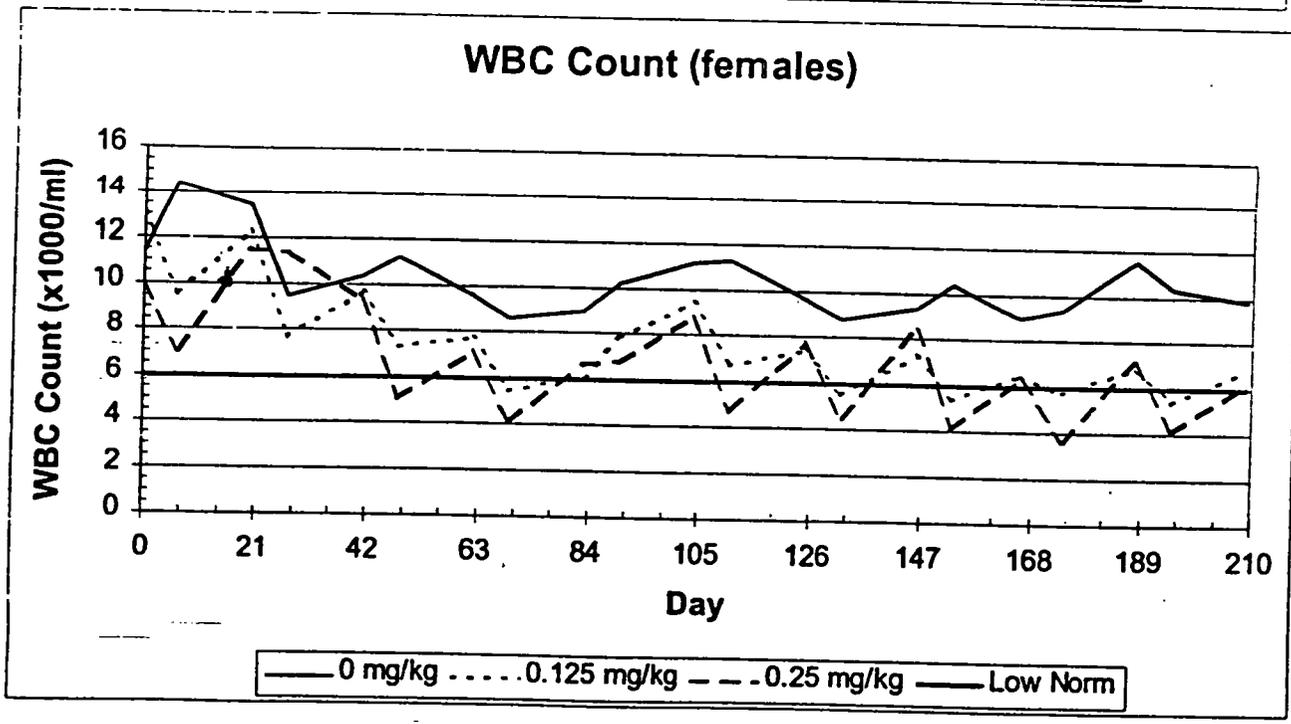
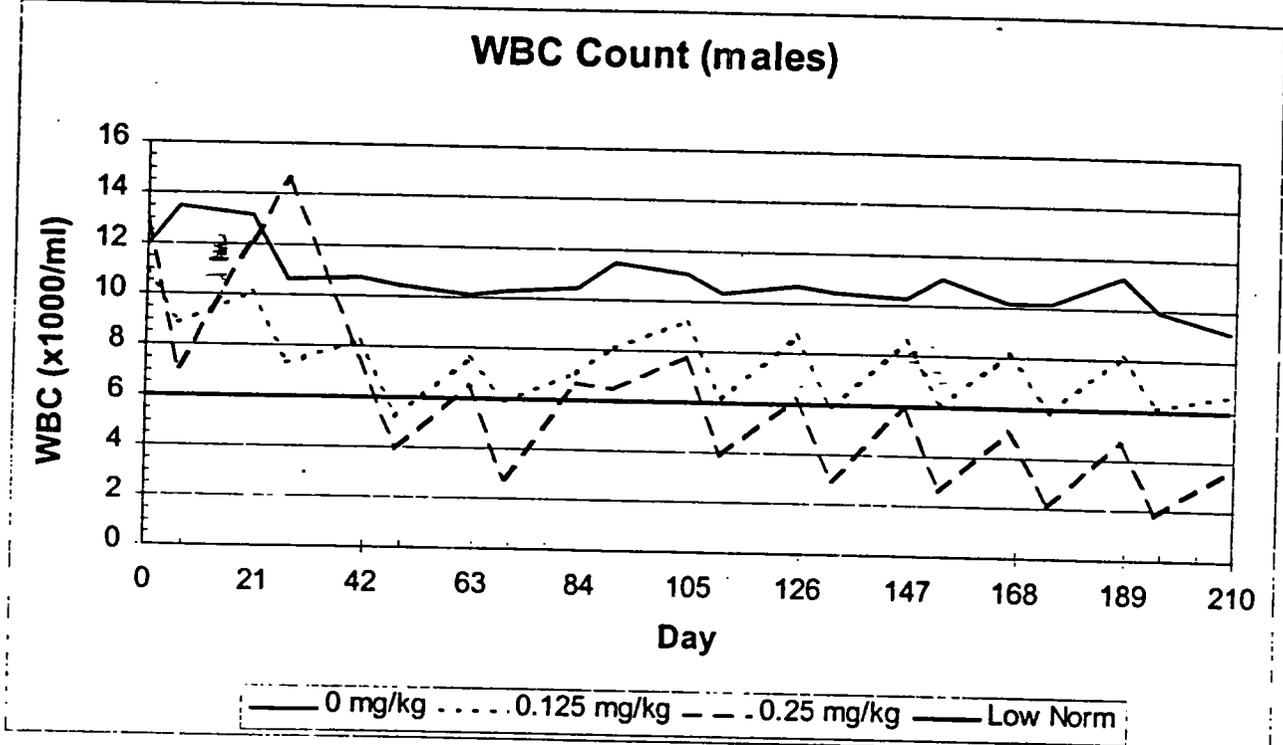
Route: IV

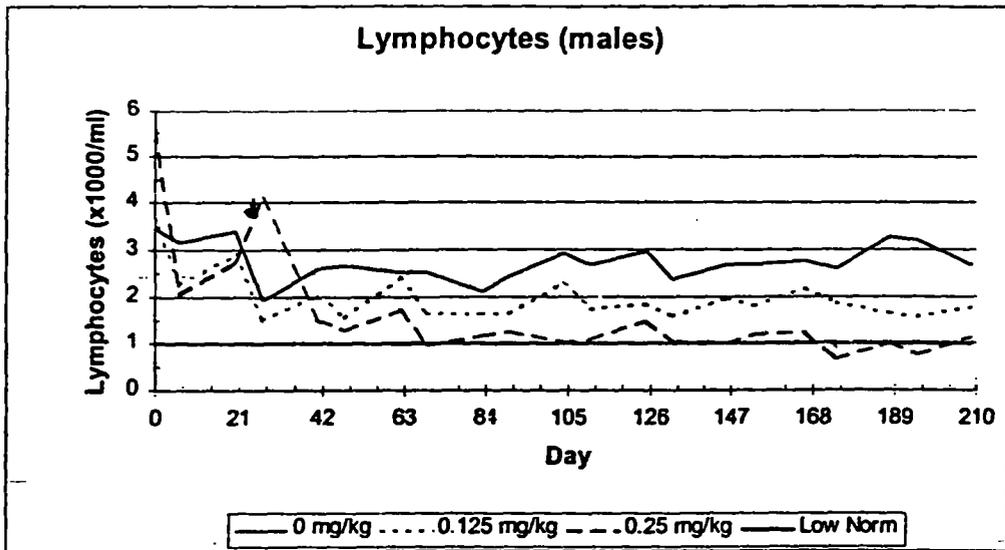
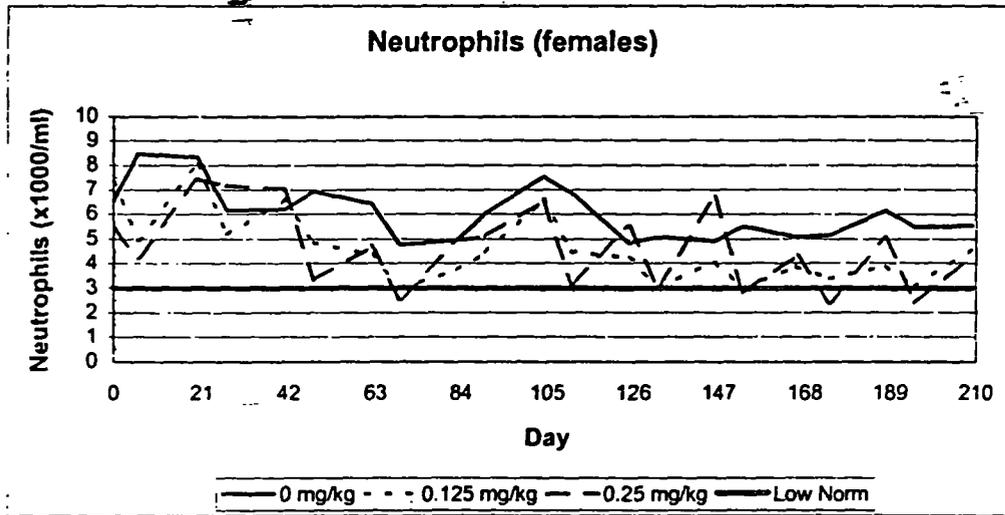
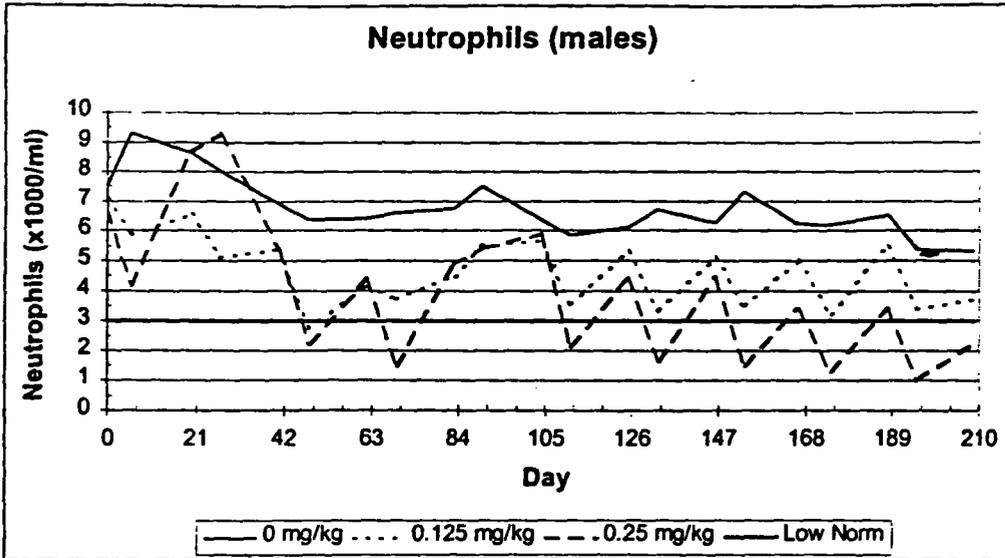
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Group	Computer Number	Unique Animal Number	Sex	Dose mg/kg	Solution Concentration mg/ml	Infusion Time (min)	Rate		Dose ₂ mg/m	Total Dose ₂ mg/m
							mg/kg/min	ml/kg/min		
Control	1	032235	M	Vehicle	0.0	5	0.0	0.4	0.0	0.0
	2	032248	M	0.9% NaCl	0.0	5	0.0	0.4	0.0	0.0
	3	032256	M		0.0	5	0.0	0.4	0.0	0.0
	19	032275	F		0.0	5	0.0	0.4	0.0	0.0
	20	032287	F		0.0	5	0.0	0.4	0.0	0.0
	21	032288	F		0.0	5	0.0	0.4	0.0	0.0
Low (CL 232,315)	4	032234	M	0.125	0.0625	5	0.025	0.4	2.575	25.75
	5	032242	M		0.0625	5	0.025	0.4	2.575	25.75
	6	032251	M		0.0625	5	0.025	0.4	2.575	25.75
	22	032279	F		0.0625	5	0.025	0.4	2.575	25.75
	23	032282	F		0.0625	5	0.025	0.4	2.575	25.75
	24	032292	F		0.0625	5	0.025	0.4	2.575	25.75
High (CL 232,315)	7	032241	M	0.25	0.125	5	0.05	0.4	5.15	30.9
	8	032244	M		0.125	5	0.05	0.4	5.15	30.9
	9	032249	M		0.125	5	0.05	0.4	5.15	30.9
	25	032277	F		0.125	5	0.05	0.4	5.15	30.9
	26	032281	F		0.125	5	0.05	0.4	5.15	30.9
	27	032295	F		0.125	5	0.05	0.4	5.15	30.9
Adriamycin	16	032237	M	1.75	0.87	5	0.35	0.4	36.05	324.4
	17	032247	M		0.87	5	0.35	0.4	36.05	324.4
	18	032254	M		0.87	5	0.35	0.4	36.05	324.4
	34	032278	F		0.87	5	0.35	0.4	36.05	324.4
	35	032285	F		0.87	5	0.35	0.4	36.05	324.4
	36	032291	F		0.87	5	0.35	0.4	36.05	324.4

Mortality- 0.25 mg/kg- 1/6 dogs died on day 118

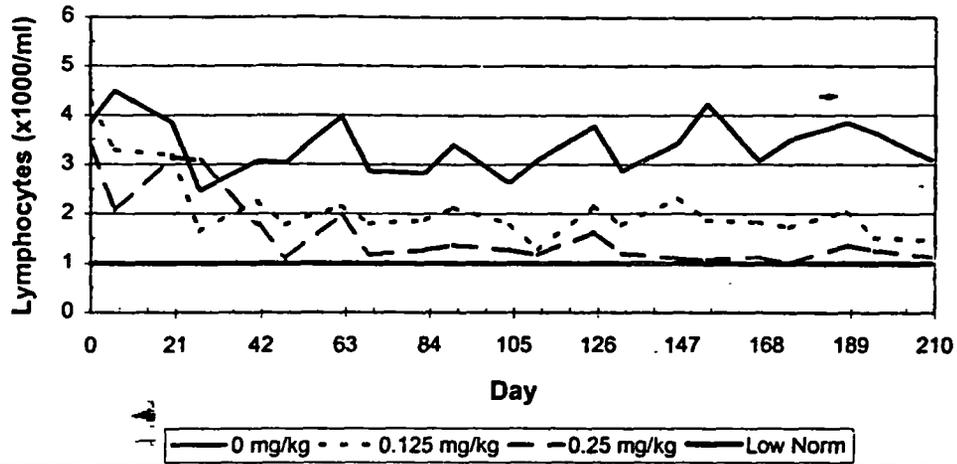
Clinical Observations- No data reported, observations based on sponsor's summary
 0.25 mg/kg- occasional diarrhea and/or bloody diarrhea, cutaneous sores
 0.125 mg/kg and above- soft feces, emesis, salivation, edematous swelling of extremities, isolated diarrhea
 Body Weight- No effects
 Food Consumption- No effects
 Ophthalmoscopy- No adverse effects (data not provided)
 Electrocardiogram- No effects on ECG or blood pressure
 Hematology



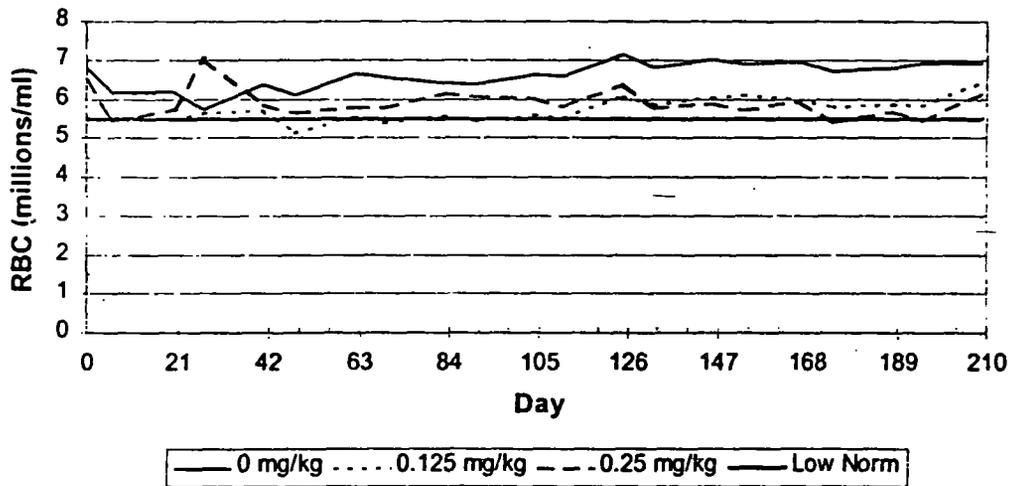


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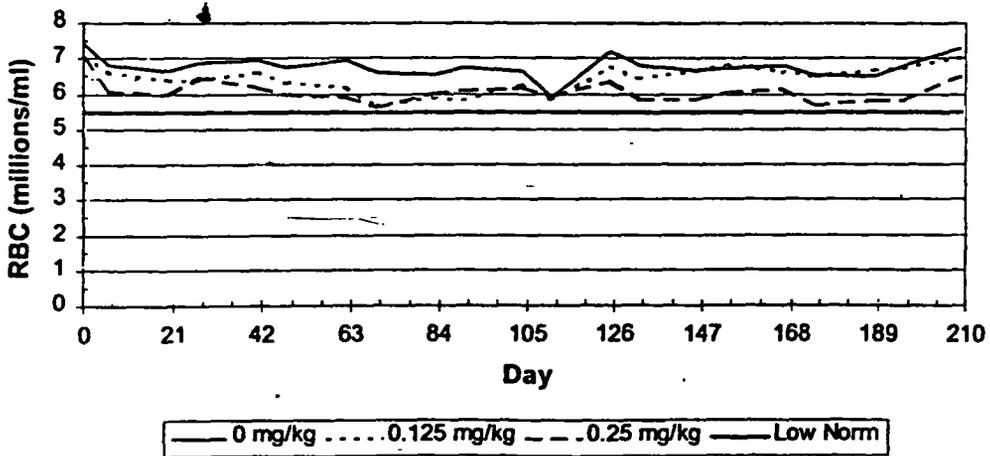
Lymphocytes (females)



RBC (males)



RBC (females)



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Dose dependent decreased in WBC, neutrophil, and lymphocyte counts; decreases more pronounced in males in than in females. RBC, hematocrit, and hemoglobin were reduced, although decrease was not as significant as in WBC parameters.

Clinical Chemistry- No effects

Organ weights- Relative organ weights; values in Bold statistically significantly different from controls.

	0 mg/kg		0.125 mg/kg		0.25 mg/kg	
	M	F	M	F	M	F
Adrenals (mg/kg)	10.4	12.7	12.0	11.1	9.8	17.2
Testes (mg/kg)	96.5	---	54.8	---	47.6	---

Gross Necropsy-

Histopathology

0.125 mg/kg- diffuse tubular atrophy of the testes; aspermia of epididymis

0.25 mg/kg- hypocellular bone marrow, pulmonary infarction with necrotizing pleuritis in deceased dog

Key findings

1. 1 male dog died at 0.25 mg/kg. Necropsy revealed pulmonary infarction with necrotizing pneumonitis and hypocellular bone marrow.
2. A dose dependent decrease in white blood cell counts was observed at 0.125 and 0.25 mg/kg. Males were more affected than females. Both lymphocytes and neutrophils were decreased in treated animals.
3. Red blood cell counts, hemoglobin, and hematocrit were also decreased in treated dogs, but the degree of decrease was not as great as that for white blood cells.
4. The testes are an important target organ. Testicular atrophy with aspermia of the epididymis was observed at 0.125 mg/kg and above. Accompanying the histopathological data was a 43 and 51 percent % decrease in relative testes weight at 0.125 and 0.25 mg/kg, respectively.
5. Increased adrenal gland weight was observed at 0.25 mg/kg in females only. In the absence of histopathological abnormalities in these animals, the significance is uncertain.
6. No adverse effects were observed on the bone marrow or heart of surviving dogs treated with 0.125 or 0.25 mg/kg.
7. A no-effect level could not be determined from this study.

A 44 week comparative toxicity study of mitoxantrone (CL 232,315) and doxorubicin (CL 115,751) given intravenously to Cynomolgus monkeys once every 21 days (Reports 117, 118)

Volume 23, page 1 to Volume 25, page 52

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River

Date of Study Initiation: 03/17/1981

GLP Compliance: No

Design: See Table

Dosing Information

Species: Monkey, Cynomolgus (*Macaca fascicularis*)

Age:

Weight: 3.9 to 5.8 kg (males), 2.3 to 4.2 kg (females)

Route: IV

Group	Compound	Dose/Level		No. of Animals	
		mg/kg	mg/m ²	Males	Females
1	Vehicle ^b	0	0	5	5
2	CL 232,315 ^c	0.125	1.5	5	5
3	CL 232,315 ^c	0.25	3.0	5	5
4	CL 115,751 ^d	1.75	21.0	5	5

^a The first day of dosing, day 0, was March 17, 1981.
^b 0.8% sodium chloride and 0.2% sodium metabisulfite in Sterile Water for Injection.
^c Given on days 0, 21, 42, 63, 84, 105, 126, 147, 167, 189, 231, 259.
^d Given on days 0, 21, 42, 63, 84, 105, 126, 147, 167 and 189.

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Type of Determination	Schedule	Method or Reference
Observation	Daily	Small Animal Weighing System (PDF 1170)
Physical Examination	Produce, Days 41, 83, 183, 223, 238	Manually Recorded
Food Consumption	Daily	Manually Recorded
Body Wt. -	Produce, -0, -1, 5, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 97, 104, 111, 118, 125, 132, 139, 146, 153, 160, 167, 174, 181, 188, 195, 202, 209, 216, 223, 230, 237, 244, 251, 258, 265, 272, 279, 286, 293, 300	Small Animal Weighing System (PDF 1170)
Ophthalmic Examination	Produce, days 49, 119, 153, 196	Indirect and direct ophthalmoscopy, slit lamp biomicroscopy and fundus photography
Electrocardiogram and Blood Pressure	Produce, days 16-17, 36-37, 57-58, 99-100, 121-122, 143-143, 162, 183, 204, 220-239, 263-266, 294	Stewart Packard 1507a Vector Programer 1508 Lead Manipulator with Mobile Chart Drive 10644
Blood Hemogram Hematocrit, Hemoglobin, HGB, WBC, RBC, MCV, MCH, MCHC, MDC, Differential, Reticulocytes Platelet Count	Produce, days 6, 13, 20, 27, 34, 41, 48, 55, 62, 76, 83, 97, 104, 118, 125, 139, 146, 160, 167, 181, 188, 202, 209, 216, 227, 244, 251, 272, 279, 293	Performed by the Clinical Pathology Laboratory, Dept. 973 Method Code 101
Serum Chemistry Profile (Cemtrifichen) Sodium, potassium, chloride, inorganic phosphorus, creatinine, BUN, SCOT, SCPT, albumin phosphorus, glucose, cholesterol, triglycerides, total protein, total bilirubin, calcium, CPK, CKPT	Produce, days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 209, 231, 227, 231, 238, 253, 260, 293	Performed by the Clinical Pathology Laboratory, Dept. 973 Method Code 703/753
Protein Electrophoresis Albumin, globulin alpha-1, alpha-2, beta-1, beta-2, gamma-1	Produce, days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 209, 213, 227, 231, 238, 253, 260, 293	Performed by the Clinical Pathology Laboratory, Dept. 973 Method Code 703/753
CPE Isomerase	Produce, days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 209, 219, 226, 231, 238, 253, 260, 293	Performed by the Clinical Pathology Laboratory, Dept. 973 Method Code 703/753

Mortality

0.25 mg/kg- 3/10 (2 males, 1 female)

0.125 mg/kg- none

0 mg/kg- 1/10 (1 female, unknown cause)

Clinical Observations

0.25 mg/kg- inactivity and decreased food consumption in males who died during the study; heavy menstrual bleeding in female who died during the study.

Body Weight- No effects

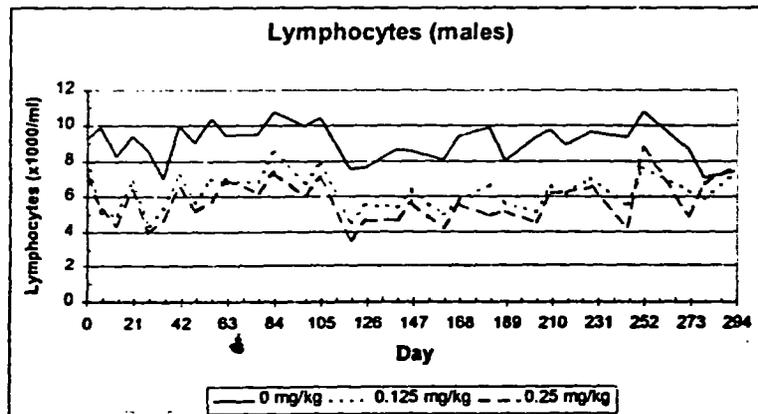
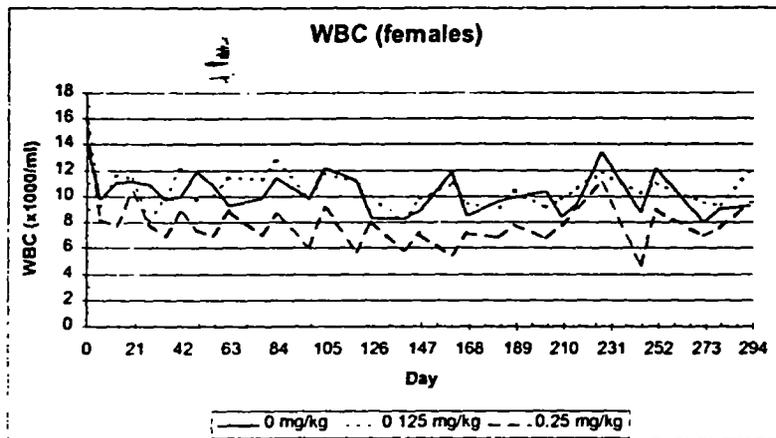
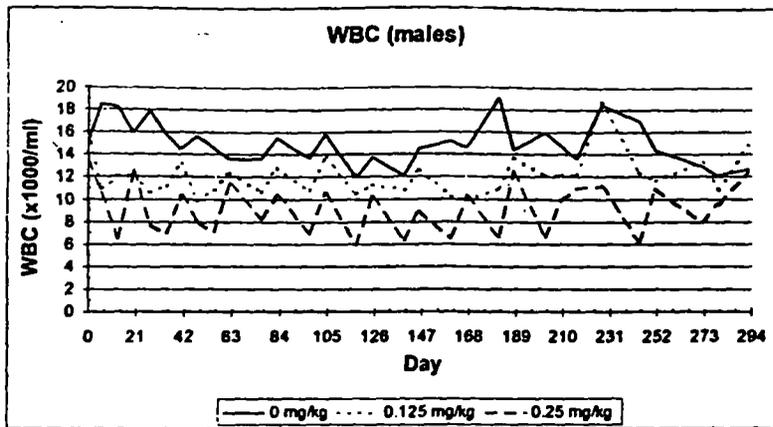
Food consumption- No effects

Ophthalmoscopy- No effects

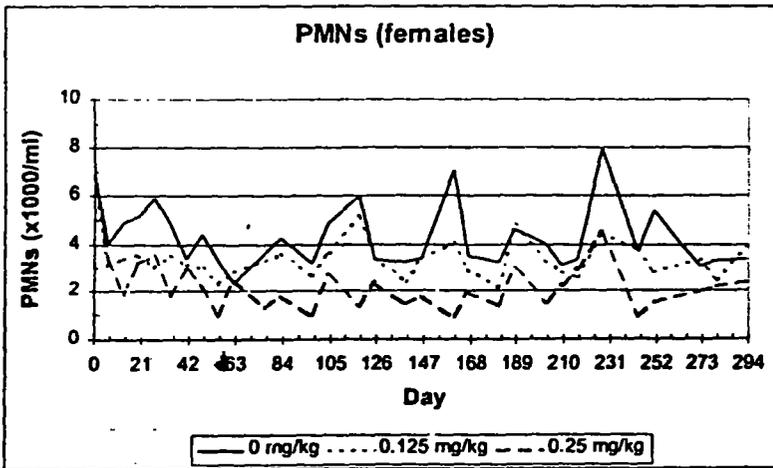
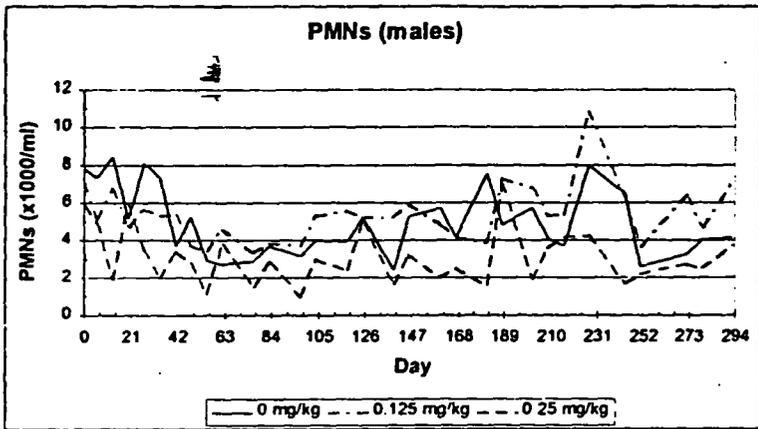
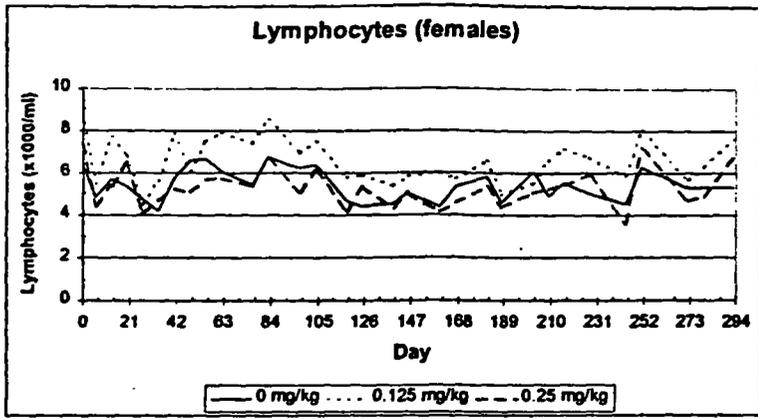
Electrocardiography- No effects on ECG or mean blood pressure

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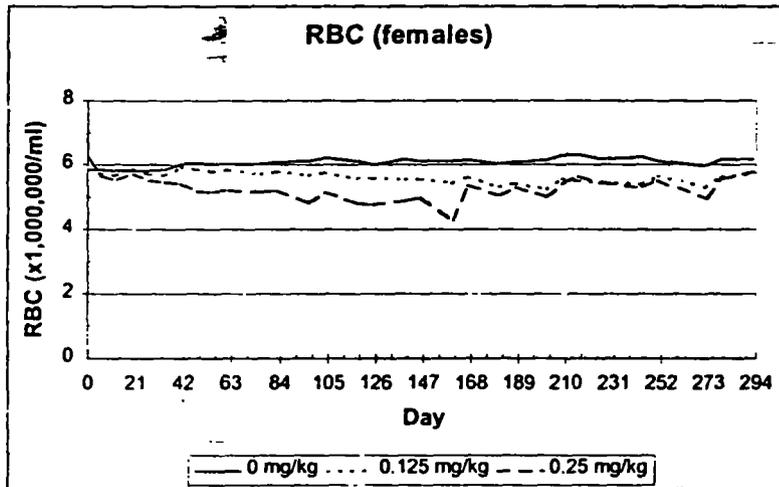
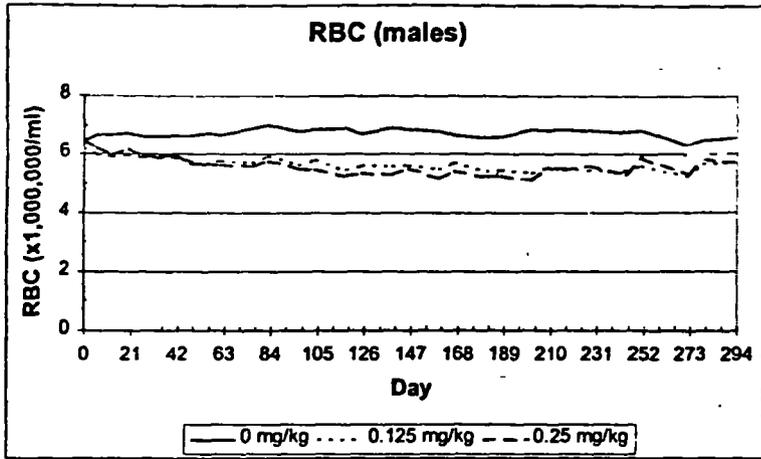
Hematology-



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Clinical Chemistry- No effects

Organ weights- Relative organ weights expressed as percent of controls; values in **Bold** statistically significant from controls

	0.125		0.25 mg/kg	
	Males	Females	Males	Females
Thymus	37	143	54	120
Adrenals	146	99	158	108
Liver	106	84	103	87
Kidney	104	91	107	84
Lung	116	73	115	67

Gross pathology

Early decedents

0.25 mg/kg female- uterine hemorrhage

0.25 mg/kg males- hydrothorax (2/2), thrombus left auricle (1/2), fibrous clots right auricle (1/2), pericardial effusion (1/2)

Incidence of Gross Morphologic Findings

Compound	Vehicle		CL 232,315		CL 232,315	
	0		0.125		0.25	
Dose (mg/kg)						
Sex	M	F	M	F	M	F
Number in group	5	5	5	5	5	5
Cardiac thrombosis	0	0	0	0	2	0
Hydrothorax	0	0	0	0	2	0
Pericardial effusion	0	1	1	1	2	1
Ascites	0	1	0	0	1	0
Renal infarcts	0	0	0	0	0	0
Kidney changes	2	3	2	1	3	1
Pericardial adhesions	0	0	1	0	1	0

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Histopathology

	0 mg/kg		0.125 mg/kg		0.25 mg/kg	
	M	F	M	F	M	F
Heart: Interstitial fibrosis	4/5	2/5	4/5	5/5	5/5	4/5
Mild focal	2	1				
Mild diffuse	1	1	2	3		4
Moderate focal	1		1	2	2	
Moderate diffuse			1		3	
Lung: Fibrosis, pleura, interstitial or alveolar septa	0/5	1/5	2/5	3/5	4/5	2/5
Spleen: Lymphoid tissue depletion	0/5	0/5	2/5	0/5	3/5	0/5
Lymph Nodes: Lymphoid tissue depletion	0/5	0/5	2/5	0/5	1/5	2/5
Thymus: Lymphoid tissue depletion	1/5	1/4	3/5	0/5	4/4	2/5
Epididymis: Oligospermia	0/5	---	0/5	---	2/5	---
Testes: Decreased spermatogenesis	1/5	---	0/5	---	2/5	---

Key findings

- At 0.25 mg/kg, 3/10 monkeys died; inactivity, hydrothorax and cardiac toxicity were the primary observations in the two males who died and heavy menstrual bleeding and uterine hemorrhage was observed in the female who died.
- At 0.125 (males only) and 0.25 mg/kg, decreased white blood cell counts were observed following novantrone injections. The decrease was more severe at 0.25 mg/kg than 0.125 mg/kg. The decrease in white blood cells was characterized by decreases in lymphocytes and PMNs. There was recovery after injection, yielding a characteristic saw toothed time versus white blood cell count, particularly in 0.25 mg/kg males.
- A persistent decrease in red blood cell counts was observed at both 0.125 and 0.25 mg/kg. The effect was less apparent in 0.125 mg/kg females.
- Lymphoid tissue depletion in spleen, lymph nodes and thymus at 0.125 mg/kg in males and 0.25 mg/kg in females.
- Decreased testicular function was apparent at 0.25 mg/kg as indicated by decreased spermatogenesis in the testes and oligospermia in the epididymis.
- Increased severity of heart interstitial fibrosis was observed starting at 0.125 mg/kg. The effects were more pronounced in males than in females.

7. Lung fibrosis was observed in males starting at 0.125 mg/kg; no increase in lung fibrosis was observed in females.
8. The lowest effect level was 0.125 mg/kg; a no effect level could not be determined from this study.

A Comparative intravenous toxicity study in rabbits of Mitoxantrone (CL 232,315) and Doxorubicin Hydrochloride (CL 115,751) (Report 120)

Volume 25, page 156 to Volume 27, page 343

Conducting Laboratory: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Date of Study Initiation: 06/23/1983

GLP Compliance: No

Quality Assurance Report Yes

Design: See Table

Dosing Information

Species: New Zealand White Rabbits

Age:

Weight: 3.9 to 4.5 kg (males), 3.2 to 4.2 kg (females)

Route: IV

Doses 0, 0.125, 0.25 mg/kg (0, 1.5, 3.0 mg/m²)

Experimental Design

Group	Compound	Dose* mg/kg	Number of Animals/Sex	Number of Animals/Sex/Sacrificet			
				1st	2nd	3rd	4th
1	0.9% NaCl (Vehicle)	0	12	3	3	2	4
2	CL 232,315	0.125	12	3	3	2	4
3	CL 232,315	0.25	12	3	3	2	4
4	CL 115,751	1.64	12	3	3	6	-

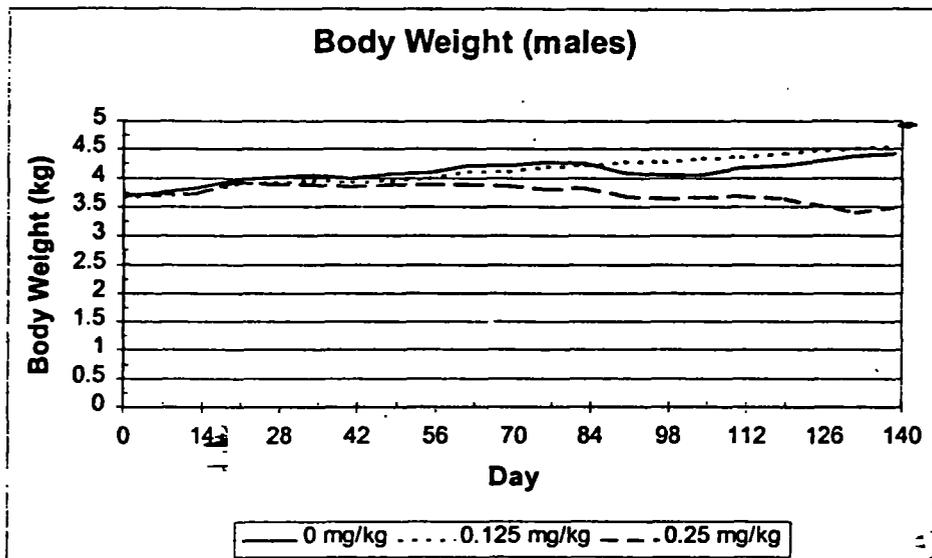
* Each dose was given as a slow bolus infusion at a dosing volume of 1 mL/kg at the frequency of once a week for 15 weeks in the mitoxantrone and control groups, and 12 weeks in the doxorubicin group.

† Interim sacrifices were scheduled after the animal received 5 (1st sacrifice), 10 (2nd sacrifice) or 12 (3rd sacrifice) doses of their respective treatment. All the surviving animals in Group 4 (doxorubicin) were sacrificed after 12 doses because of deteriorating physical condition. The remaining animals in Groups 1, 2 and 3 received 3 more doses and were sacrificed after 15 doses (4th sacrifice) with a recovery period of up to 7 weeks.

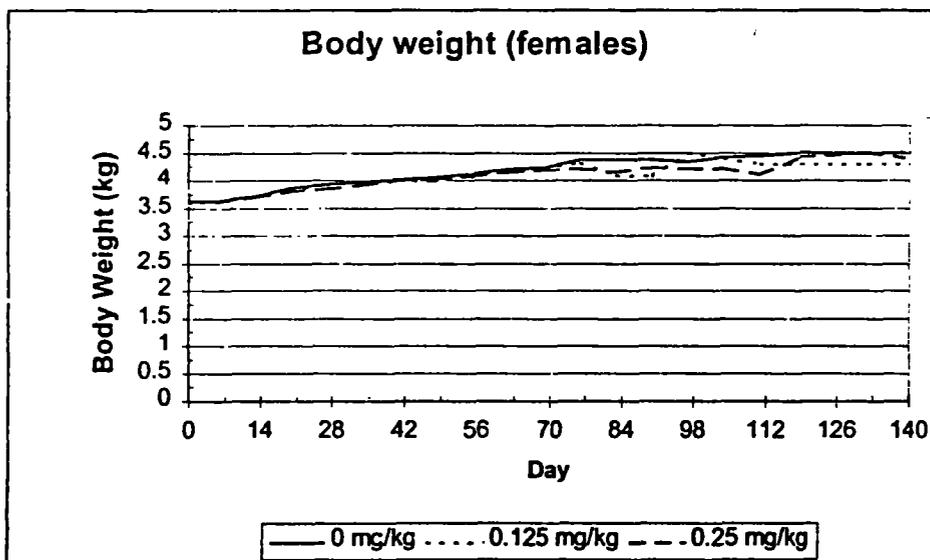
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Deceased animals had lost weight, were hypotensive and had ECG changes indicative of heart damage. No significant clinical observations were made in animals surviving to sacrifice, but minimal clinical exams were made

Body Weight- Decreased body weight in males at 0.25 mg/kg



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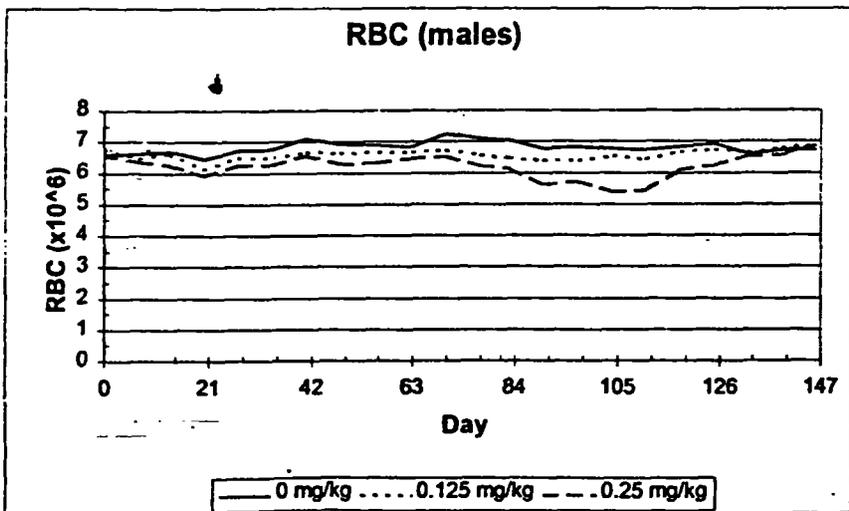
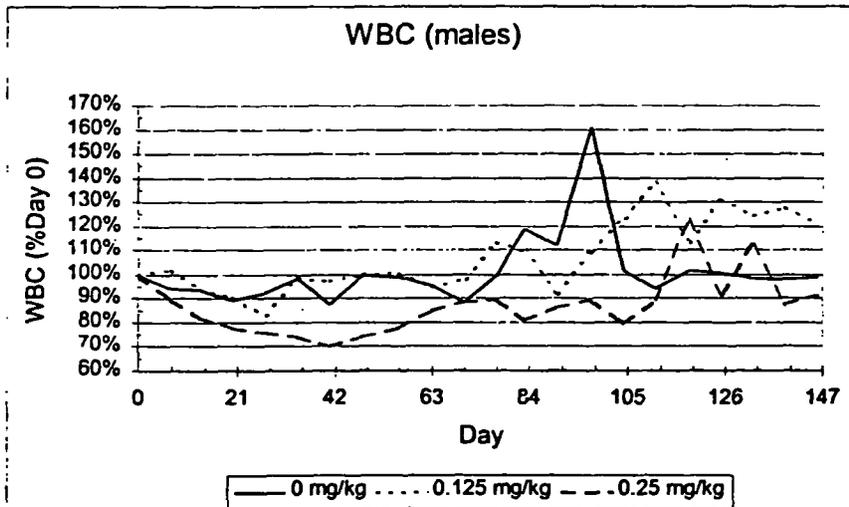
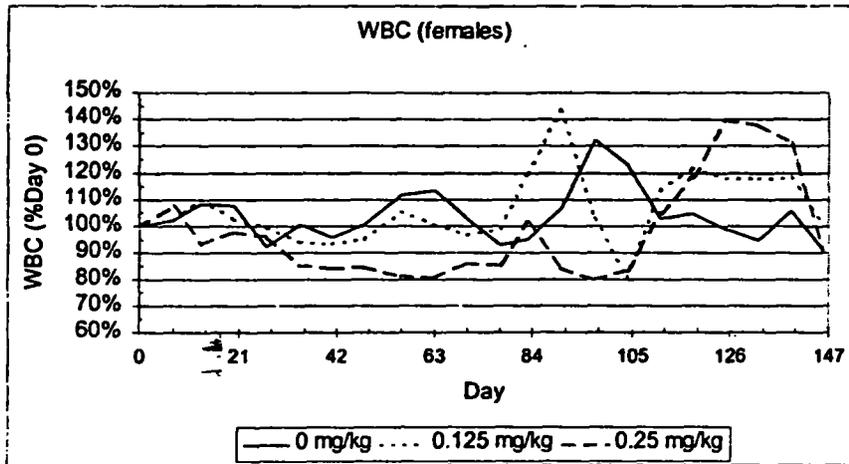
Food Consumption- No effects

Ophthalmoscopy- not done

Electrocardiography- No effects; sponsor did not provide raw data; no obvious effects on blood pressure

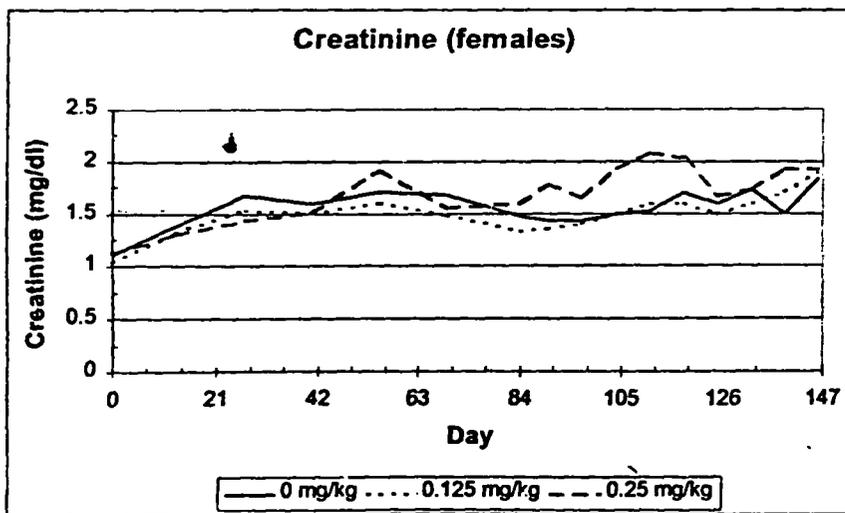
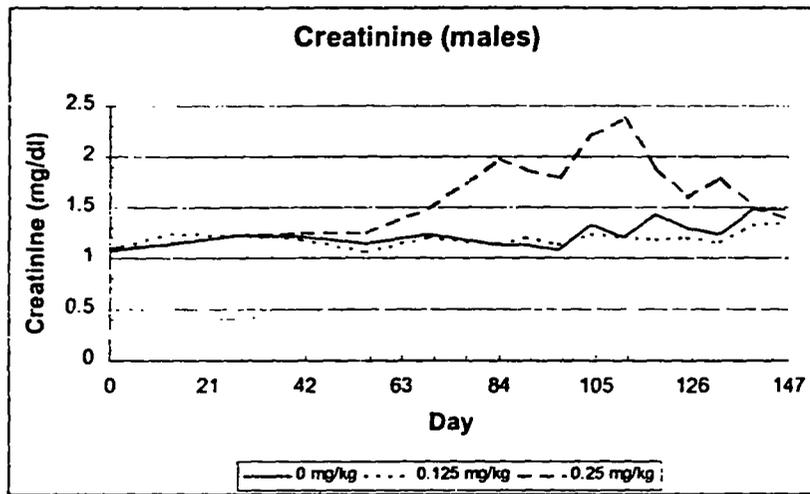
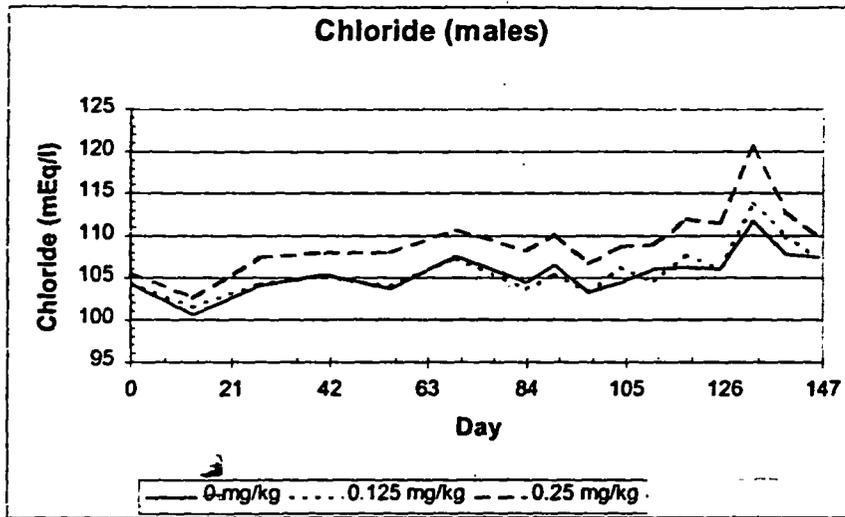
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Hematology- 0.25 mg/kg caused decreased WBC counts in males and females; RBC counts were decreased in males at 0.25 mg/kg starting on Day 90. There was recovery of cell counts starting about seven days after the last dose of mitoxantrone on Day 98 (see graphs). Due to differences in baseline WBC counts, these data are presented as percent of predose values.

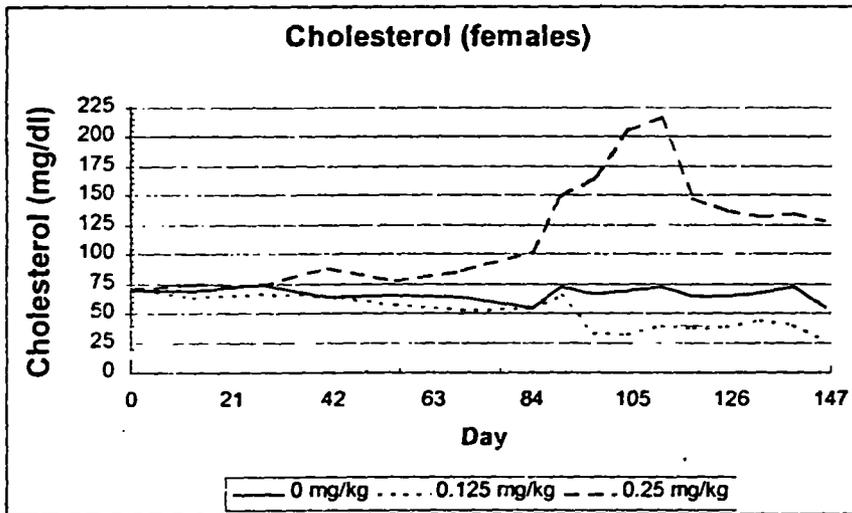
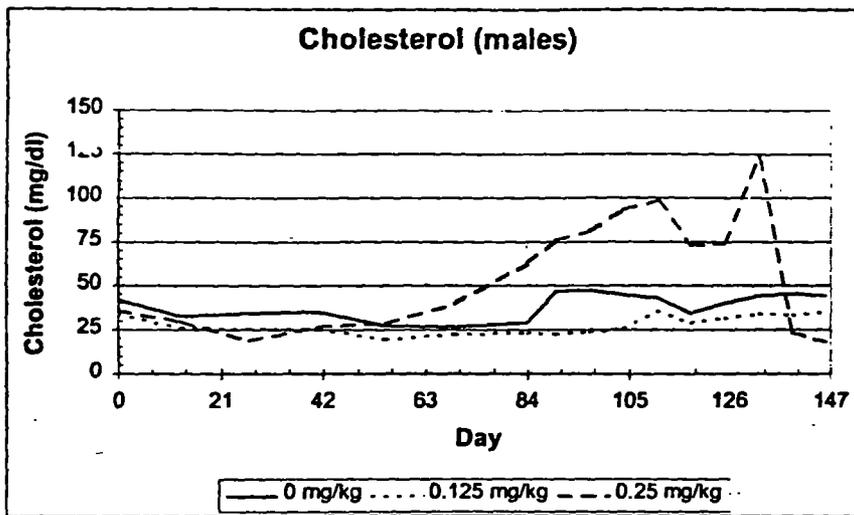


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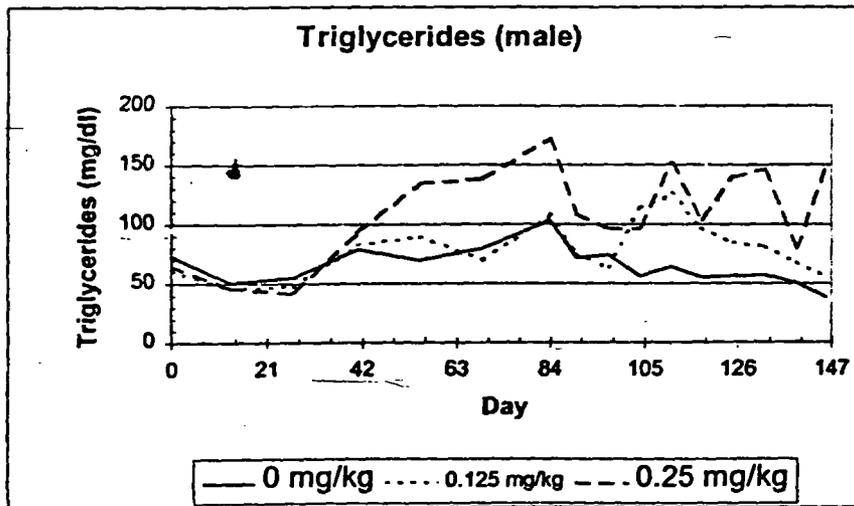
Clinical Chemistry-
 0.25 mg/kg- Increased chloride (males only), creatinine, cholesterol, triglycerides

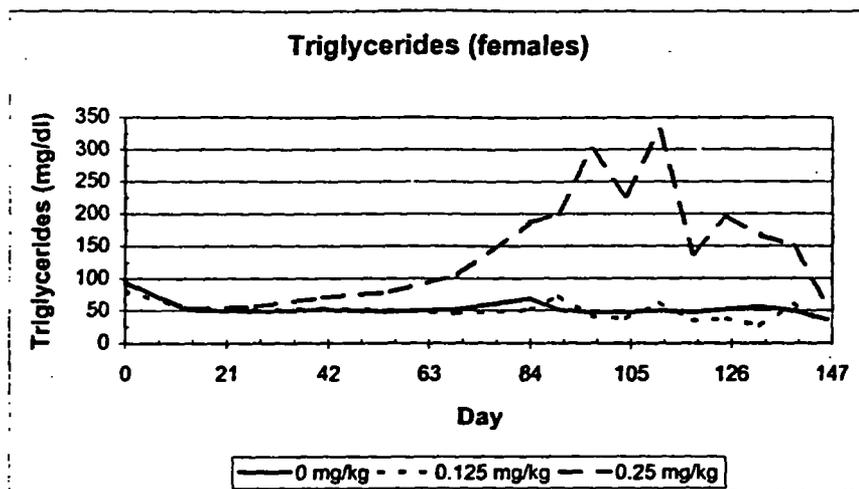


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Organ Weights- no consistent effects

Gross Pathology-

0.25 mg/kg- one male who died prematurely after 15 doses had hydrothorax and ascites; in addition, the pericardial sac was filled with fluid and the heart was pale to brown in color with a rounded apex.

0.125 mg/kg- two males had pinpoint foci on the papillary muscles.

Histopathology

	Day	0 mg/kg		0.125 mg/kg		0.25 mg/kg	
		M	F	M	F	M	F
Nephropathy		2/12	4/12	5/12	4/12	11/12	10/12
	35	0/3 (0)	2/3 (0.2)	1/3 (0.1)	1/4 (0.1)	3/4 (0.3)	1/3 (0.1)
	71	1/3 (0.1)	2/3 (0.2)	0/3 (0)	0/3 (0)	3/3 (1)	3/3 (1)
	85	0/2 (0)	0/2 (0)	2/2 (1)	2/3 (0.2)	2/2 (2.5)	2/2 (1.5)
	146	1/4 (0.1)	0/4 (0)	2/4 (0.5)	1/1 (1)	3/3 (2.2)	4/4 (2.5)
Myocardial Change (interstitial fibrosis or vacuolation of fibers)		0/12	0/12	5/12	4/12	9/12	9/12
	35	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	1/4 (0.3)	1/3 (0.3)
	71	0/3 (0)	0/3 (0)	2/3 (0.7)	1/3 (0.3)	3/3 (1.3)	3/3 (1.8)
	85	0/2 (0)	0/2 (0)	1/2 (0.5)	2/2 (1.3)	2/2 (2.3)	1/2 (0.5)
	146	0/4 (0)	0/3 (0)	2/4 (0.5)	1/1 (1)	3/3 (2.2)	4/4 (1.9)
Testes: Tubular atrophy		1/12		2/12		4/12	
	35	0/3		1/3		1/3	
	71	0/3		1/3		1/3	
	85	0/2		0/2		2/2	
	146	1/4		0/4		0/3	

Limited histopathology on other tissues

Key findings

1. The low dose (0.125 mg/kg) caused mortality in 2/23 rabbits; 4/24 high dose (0.25 mg/kg) rabbits died on the study.
2. Decreased body weight (10-20%) was observed in males starting on about Day 70; female body weights were not as affected.
3. Contrary to the sponsor's interpretation, there was a persistent decrease in white blood cell counts from baseline values starting on Day 7 in males and Day 14 in females. Male WBC counts were depressed 10 to 30 percent during dosing and recovered after day 111, 13 days after the last dose. Female WBC counts were generally depressed from 10 to 20 percent from Day 27 through Day 111.
4. Red blood cell counts were depressed dose dependently at 0.125 and 0.25 mg/kg in males only.
5. At 0.25 mg/kg, there was increased cholesterol, triglycerides, creatinine and chloride (males only).
6. Major target organs included the kidney (tubular casts, glomerular vacuolation), heart (fibrosis and fiber vacuolation) and testes (testicular tubular atrophy) at 0.125 mg/kg.
7. A highest no effect level could not be determined from this study.

Overall Summary

The chronic toxicity of mitoxantrone has been studied in rats, dogs, monkeys and rabbits. The rats, dogs and monkeys were dosed at three week intervals to mimic the clinical exposure, but the rabbits were dosed at one week intervals. In general, the toxic effects observed in the chronic studies are similar to those in the acute studies. The hematopoietic system was the primary target organ of concern. However the heart, kidney, and testes are additional target organs of concern. Neoplasms were also observed in the chronic rat study (see Carcinogenicity section below).

Mitoxantrone caused substantial early mortality in chronic studies at doses below the proposed clinical dose of 12 mg/m². In rats, substantial early mortality (100% in males, 45% in females) was observed in rats administered 0.3 mg/kg (1.8 mg/m²). The mortality was associated with the cumulative dose reaching 4.2 to 4.4 mg/kg (25.2 mg/m² to 26.4 mg/m²) in males and 6.5 to 6.7 mg/kg (39.0 mg/m² to 40.2 mg/m²) in females. In dogs, one out of six dogs administered 0.25 mg/kg (5.15 mg/m²) every three weeks over 30 weeks died. In monkeys, three out of ten animals administered 0.25 mg/kg (3 mg/m²) every three weeks over 44 week died. Rats were more vulnerable to mitoxantrone mortality than dogs or monkeys.

Neutropenia was the most striking toxic effect of mitoxantrone. In the dog and monkey study, where it was possible to do serial white blood cell counts, the graph of the white blood cell counts with time formed a "saw toothed" pattern with a nadir in the count at post dosing day 11 followed by recovery to near pre dose levels just prior to the next dose. At high doses (0.25 mg/kg in dogs), nadir became lower with time, but at lower doses (e.g. 0.125 in dogs or 0.25 mg/kg in monkeys) there appeared to be less cumulative effect. Red blood cell counts were also depressed, although to a lesser extent than white blood cell counts. Hypocellularity of the bone marrow was often observed in neutropenic animals. In addition, lymphoid depletion of the thymus, spleen and lymph nodes were frequently observed.

The heart is another target organ in rats, monkeys, and rabbits. Effects included heart muscle necrosis in rats at 0.3 mg/kg, interstitial fibrosis in monkeys at 0.125 mg/kg and above and myocardial changes (including focal necrosis and thrombi), fibrosis and fiber vacuolation in rabbits at 0.125 mg/kg. In the two year carcinogenicity study in rats, an increased incidence of heart mineralization was observed at 0.1 mg/kg.

Mitoxantrone also affects the kidneys. In the two year carcinogenicity study in rats, glomerulonephritis was the primary cause of premature death in male rats at 0.1 mg/kg every three weeks. In the twelve month rat study, nephrosclerosis was observed at 0.03 mg/kg in males and 0.3 mg/kg in females; hydropic changes with proximal tubule necrosis was observed at 0.3 mg/kg and above; urinalysis alterations at 0.3 mg/kg included proteinuria, occult blood and increased urine volume.

The testes were affected by mitoxantrone treatment. At 0.125 mg/kg diffuse tubular atrophy of the testes and aspermia of the epididymis was observed in dogs. The testes weight was decreased by about 50 percent in these dogs. In monkeys administered 0.25 mg/kg, decreased spermatogenesis in the testes and oligospermia in the epididymis were observed. Finally, testicular tubule atrophy was observed in rabbits at 0.125 mg/kg.

Addendum list:

Addendum 1
Histopathology Inventory for NDA 21-120

Study	84	86	89	91	93-94	104-12	113-116	117-118	120		
Species	Dog	Dog	Monkey	Monkey	Rat	Rat	Dog	Monkey	Rabbit		
Adrenals	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Aorta	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Bone Marrow smear	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Bone (femur)	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Brain	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Cecum	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Cervix											
Colon	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Duodenum	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Epididymis	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Esophagus	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Eye	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Fallopian tube											
Gall bladder	✓	✓	✓	✓			✓	✓	✓		
Gross lesions											
Harderian gland											
Heart	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Hypophysis											
Ileum	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Injection site					✓	✓		✓			
Jejunum	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Kidneys	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Lachrymal gland											
Larynx											
Liver	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Lungs	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Lymph nodes, cervical	✓	✓	✓	✓			✓				
Lymph nodes mandibular	✓	✓	✓	✓			✓				
Lymph nodes, mesenteric	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Mammary Gland	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Nasal cavity											
Optic nerves											
Ovaries	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Pancreas	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Parathyroid	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Peripheral nerve	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Pharynx											
Pituitary	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Prostate	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Rectum	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Salivary gland	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Sciatic nerve					✓	✓		✓			
Seminal vesicles			✓	✓	✓	✓		✓	✓		
Skeletal muscle	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Skin	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Spinal cord	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Spleen	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Sternum											
Stomach	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Testes	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Thymus	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Thyroid	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Tongue	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Trachea	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Urinary bladder	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Uterus	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Vagina	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Zymbal gland											

organ weight obtained

CARCINOGENICITY:

Mouse

Oncogenic Potential of NOVANTRONE Mitoxantrone Hydrochloride (CL 232,315) After Intravenous Administration to Mice Every 21 Days for 2 Years (Final Report) (Report 1)

Study Number: 82241

Volume Numbers: Volume 36, Page 34 to Volume 47, Page 141

Test Facility: Lederle Laboratories, American Cyanamid Co., Pearl River, NY

Study Date(s): 2/8/1983 to 2/15/1985

Date of Submission 3/23/1987

GLP Compliance/Quality Assurance:

QA Report- Yes (X) No ()

Study Type: Two Year Bioassay

Species/strain: Mouse, Crl:COBS CD-1(ICR)BR

Number of animals per group; age at start of study: 60/sex/group, 5 weeks old

Animal housing: Individually in steel wire mesh suspended cages

Drug Lot/Batch number(s): PC 0345

Drug Purity / Stability / Homogeneity: mitoxantrone — %water

Doses:

Group	Dose (mg/kg every 21 days) ^b	Males	Females
1	0 (untreated)	60	60
2	0 (saline)	60	60
3	0.1	60	60
4	0.2	60	60 ^c
5	0.4	60	60

^a Mitoxantrone administration started on February 9, 1983 (day 1). Surviving mice were sacrificed for postmortem examination beginning on February 11, 1985 (day 734).

^b These concentrations resulted in cumulative drug intakes of 3.5, 7.0 and 14 mg/kg for Groups 3, 4, and 5, respectively.

^c One mouse (SAN 518, UAN 91846) escaped and was presumed dead and was not included in the postmortem evaluation.

APPEARS THIS WAY
ON ORIGINAL

- Basis of Dose Selection: Unclear
- Relation to Clinical Use: Same route and mode of administration
- CAC Concurrence: Pre-CAC study
- Restriction Paradigm for Dietary Restriction Studies: None

- Route of Administration: IV
- Frequency of Drug Administration: Every 3 weeks
- Dual Controls Employed: Yes
- Interim Sacrifices: No
- Satellite PK or Special Study Group(s): No
- Unscheduled Sacrifices or Deaths:
- Deviations from Original Study Protocol:

Study Design

Determination	Schedule	Method or Reference
General Observation	Daily	LEDTOX Data Acquisition Program
Body Weight and Food Consumption	Weekly	LEDTOX Data Acquisition Program
Physical Examination	Monthly	LEDTOX Data Acquisition Program
Ophthalmoscopic Examination	-7,175,378,546,699	Indirect Ophthalmoscopy ^a
Hemogram ^b Hematocrit, hemoglobin, RBC, WBC, MCV, MCH, MCHC, WBC differential, reticulocytes platelets	104,177,268,386,393,400, 470,478,484,554,561,568, 638,645,652,722,729,736	Performed by the Clinical Pathology Lab. Dept.973 Method Code 101
Serum Clinical Chemistry Na, K, Cl, Ca, BUN, total bilirubin, cholesterol, creatinine, glucose, inorganic phosphorus, total protein, alkaline phosphatase, SGPT,SGOT, triglycerides	Days 734 to 738	Performed by the Clinical Pathology Lab. Dept.973 Method Code 704
Cytogenetic Evaluation of Bone Marrow	Days 736 and 738	Performed by the Experimental Pathology Dept.971

^a Indirect Ophthalmoscope: American Optical.
^b Group 5 (0.4 mg/kg) males and females were not bled on day 736

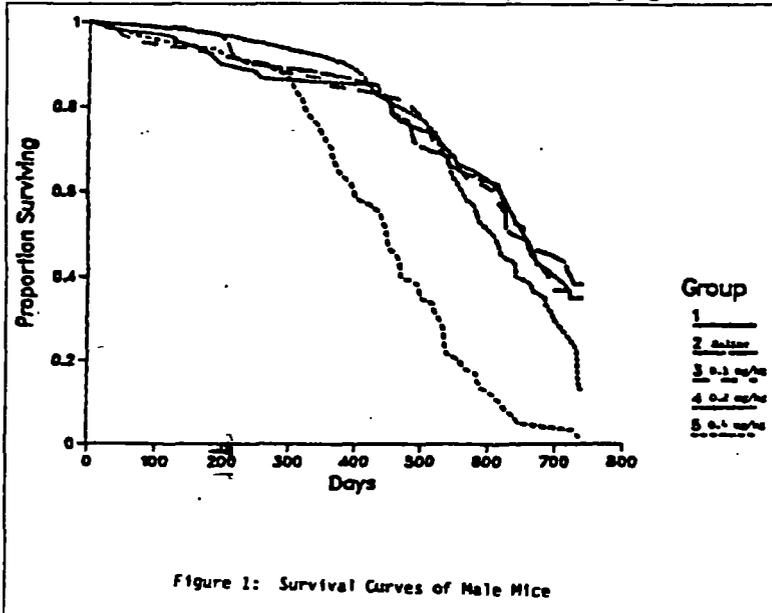
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Determination	Schedule	Method or Reference
Palpable Mass	Day 125 then Monthly to day 716	LEDTOX Data Acquisition Program
Postmortem Examination	Days 734 to 738 (survivors)	Performed by the Experimental Pathology Dept. 971
Urine Blood, bilirubin, ketones, glucose, protein, pH, urobilin- nogen	Days 200 and 716	Manually Recorded
Gross observation, specific gravity, microscopic	Day 200	Microscopic analysis Performed by Clinical Pathology Laboratory, Dept. 973

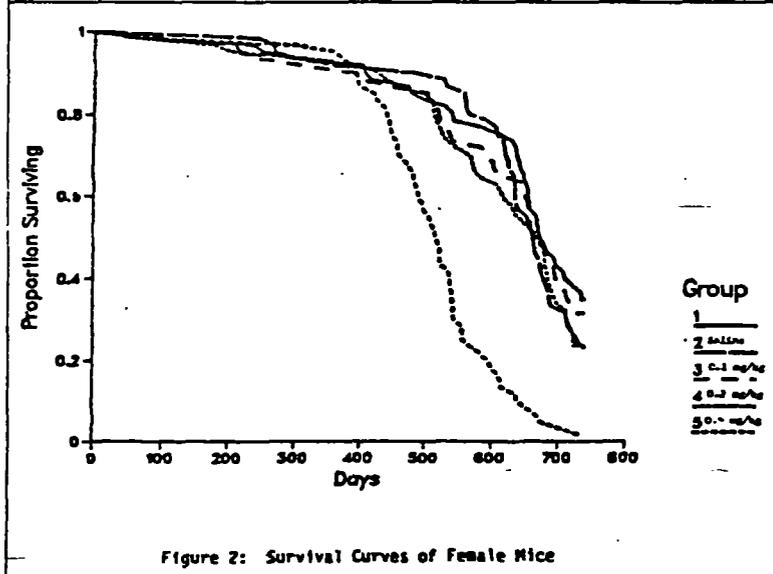
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Study Results and Frequency of Monitoring:

- Clinical Observations: No significant observations reported
- Mortality Substantial early mortality at 0.4 mg/kg

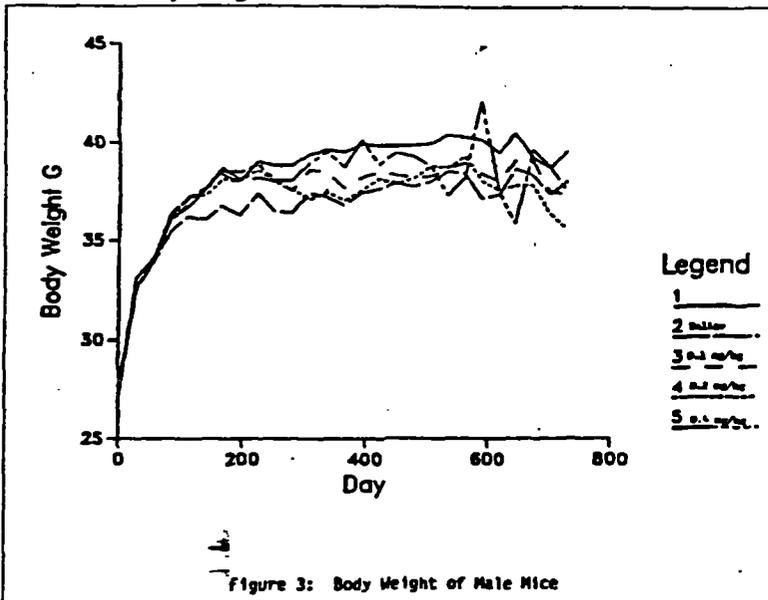


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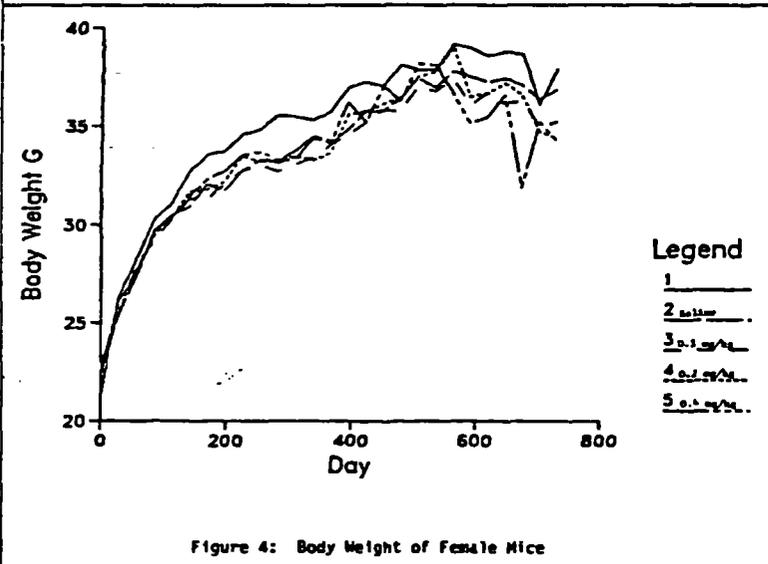


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- Body Weight- No Effects



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- Food Consumption
- Ophthalmoscopy: No effects
- Hematology: No effects reported, but sampling was irregular with respect the doses
- Clinical Chemistry: No effects on clinical chemistry or urinalysis

APPEARS THIS WAY
ON ORIGINAL

- Organ Weights:

Group Number Dose mg/kg		1 0.0	2 0.0 Saline	3 0.1	4 0.2
Prostate-Seminal Vesicles	Absolute Weight	0.641 (6)	0.535 (8)	0.468 ^a (5)	0.653 (2)
	Relative	2.327	1.805 ^a	1.721 ^a	2.077
Testes	Absolute Weight	0.195 (21)	0.175 (23)	0.199 ^b (21)	0.205 (8)
	Relative	0.622	0.555	0.660 ^b	0.668 ^b
Spleen	Absolute Weight	0.116 (21)	0.091 (22)	0.093 (21)	0.075 ^a (8)
	Relative	0.369	0.291	0.310	0.244

- = There was one surviving animal in Group 5.
^a = Statistically significantly different from Control Group 1.
^b = Statistically significantly different from Control Group 2.
^c = For details See Appendix XII-A.
 () = Numbers in parenthesis represents the number of organs weighed.

- Gross Pathology:

- Histopathology:

Non-Tumor

0.1 mg/kg and above- increased atrial thrombosis and vacuolar degeneration of the myocardium; edema, congestion and/or hemorrhage of the subcutis, liver, lungs, lymph nodes and adrenal glands

Tumor

Sex	Males					Females					Total Males	Total Females	Total Male + Females
	1	2	3	4	5	1	2	3	4	5			
Group No. ^a	1	2	3	4	5	1	2	3	4	5			
No. of animals	60	60	60	60	60	60	60	60	59	60	300	299	599
No. of mice with tumors	25	21	32	29	13	38	37	35	42	24	120	176	296
% of mice with tumors	41.6	35.0	53.3	48.3	21.6	63.3	61.6	58.3	71.1	40.0	40.0	58.8	49.4
No. of mice with malignant tumors	19	15	21	21	9	30	26	24	28	13	85	121	206
% of mice with malignant tumors	31.6	25.0	35.0	35.0	15.0	50.0	43.3	40.0	47.4	21.6	28.3	40.5	34.4
No. of mice with multiple tumors ^b	4	4	7	4	2	19	19	15	11	3	21	63	84
No. of primary tumors	29	26	39	33	15	68	55	53	56	27	142	299	441
Mean No. of tumors per tumor bearing mouse	1.16	1.24	1.22	1.14	1.15	1.79	1.49	1.51	1.33	1.13	1.18	1.47	1.35

^a For details see Experimental Design Table 1 and Appendix XIII.
^b Multiple tumors means more than one tumor type in one individual, occurrences of one kind of tumor at different sites (either multicentric or metastatic tumors), or multiple foci of a single type in one organ (i.e., 2 or more fibroadenomas in the mammary gland) were counted as one tumor

Prevalence of Most Common Tumors ^{a,b}										
Sex Group No. Number of Animals	Males					Females				
	1 60	2 60	3 60	4 60	5 60	1 60	2 60	3 60	4 59	5 60
Mammary Gland (N)	9	14	16	8	6	49	51	51	48	44
Adenocarcinoma	0	0	0	0	0	8	3	7	2	5
Fibroadenoma	0	0	0	0	0	1	0	2	1	1
Cystadenoma	0	0	0	0	0	0	0	0	1	1
all mammary tumors	0	0	0	0	0	9	3	9	4	7
Lungs (N)	60	60	60	60	59	60	60	60	59	60
Alveolar Cell Carcinoma	3	5	2	5	2	7	3	2	3	2
Adenoma Alveolar Cell	1	3	0	4	1	1	1	1	0	2
Adenocarcinoma-Alveolar/Bronchiolar	3	3	5	4	1	3	2	5	5	1
Total Adenomas & carcinomas	7	11	7	13	4	11	6	8	8	5
Liver (N)	60	60	60	60	60	60	60	60	59	60
Carcinoma, hepatocellular	8	7	7	12	7	3	2	1	0	1
Hemangioma	3	0	1	0	1	0	0	1	0	0
Adenoma Hepatocellular	0	2	8	3	3	0	1	1	0	0
Adenomas & carcinomas hepatocellular	8	9	15	15	10	3	3	2	0	1
Adrenal Glands (N)	56	60	59	59	59	58	59	59	57	59
Adenoma-Cortical	2	3	6	2	0	0	0	0	0	0
Pituitary Gland (N) ^b	57	53	51	54	50	59	56	57	55	51
Adenoma	0	0	0	0	0	9	6	4	9	1
Uterus (N)	-	-	-	-	-	59	60	60	59	58
Endometrial Polyp	-	-	-	-	-	4	5	3	5	3
Leiomyoma	-	-	-	-	-	2	5	7	2	2
Hemangioma	-	-	-	-	-	1	4	4	1	1
Leiomyosarcoma	-	-	-	-	-	1	2	0	3	0
Cystadenoma, Papillary	-	-	-	-	-	4	2	1	4	3
Sex Group No. Number of Animals	1 60	2 60	3 60	4 60	5 60	1 60	2 60	3 60	4 59	5 60
Hematopoietic Tissue (N)	60	60	60	60	60	60	60	60	59	60
Hematopoietic Tumors ^c	5	2	6	1	0	20	16	11	14	2

^a Derived from Appendices VII-B and XV.

^b For details of treatment refer to Experimental Design Table I.

^c Lymphoma, myeloma and Histiocytic sarcoma.

(N) Number of mice from which tissues were examined

- Not applicable

Excerpt from FDA Statistician Karl K. Lin, Ph.D. Review of this study (Full Text in Appendix)

The data of the two control groups were combined in our analyses. The survival rates at the time of terminal sacrifice for the control, low, medium, and high dose groups were 28%, 28%, 15%, and 3%, respectively, in males, and 23%, 23%, 20%, and 2%, respectively, in females. The intercurrent mortality rates were tested for dose-response relationship according to the method given in the paper of Peto, et al. (1980). The results of the analyses show that there is a significant positive dose-response relationship in intercurrent mortality rate in both males ($p < 0.00001$) and females ($p < 0.00001$).

The Peto prevalence analysis of incidental tumors given in the paper Peto et al. (1980) using time intervals (in weeks) 0-50, 51-80, 81-104, and terminal sacrifices was used to test the positive dose-response relationship in 17 tumor/sex combinations. The 17 selected tumor/sex combinations are those tumor types with five or more occurrences across treatment groups within a sex group. Among the 17 tumor/sex combinations tested, only liver adenoma hepatocellular in male mice shows significant positive dose-response relationship among the treatment groups ($p = 0.0085$).

- Toxicokinetics:

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The dose used in this study (0.1, 0.2, and 0.4 mg/kg; 0.3, 0.6, and 1.2 mg/m²) were lower than what humans would be exposed to in a clinical setting (12 mg/m²). However the early mortality and body weight decreases observed in the high dose suggests that the high dose exceeded the maximum tolerated dose. The mid-dose serves as an adequate back-up dose. The results of this study indicate that mitoxantrone induces hepatocellular cell adenomas in male mice.

- Evaluation of Tumor Findings:

**APPEARS THIS WAY
ON ORIGINAL**

Oncogenic Potential of NOVANTRONE Mitoxantrone Hydrochloride (CL 232,315) After Intravenous Administration to Mice Every 21 Days for 2 Years (Second of Two Studies) Final Report (Report 4)

Study Number: 83188

Volume Numbers: Volume 51, Page 142 to Volume 59, Page 123

Test Facility: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

Study Date(s): 6/24/1983 to 1/15/1986

Date of Submission 9/30/1986

GLP Compliance/Quality Assurance: Yes

QA Report- Yes (X) No ()

Study Type: 2 Year Bioassay

Species/strain: Mice, Crl:COBS CD-1(ICR)BR

Number of animals per group; age at start of study: 60/sex/dose, 5 weeks of age

Animal housing: Individually in stainless steel wire-mesh suspended cages

Drug Lot/Batch number(s): PC 0345

Drug Purity / Stability / Homogeneity:

Doses:

- Basis of Dose Selection: Previous cancer bioassay
- Relation to Clinical Use: Same route and timing of administration
- CAC Concurrence: Pre CAC
- Restriction Paradigm for Dietary Restriction Studies- None
- Route of Administration: IV
- Frequency of Drug Administration: every 21 days
- Dual Controls Employed: No
- Interim Sacrifices: No
- Satellite PK or Special Study Group(s): None
- Unscheduled Sacrifices or Deaths:
- Deviations from Original Study Protocol:

**APPEARS THIS WAY
ON ORIGINAL**

Group	Compound	Dose ^b		No. of Animals	
		mg/kg	mg/m ²	Male	Female
1	Vehicle ^c	0	0	60	60
2	CL 232,315	0.01	0.03	60	60
3	CL 232,315	0.03	0.09	60	60
4	CL 232,315	0.06	0.18	60	60

^a Day 0 of the study was June 24, 1983. Terminal necropsy occurred between July 8, 1985 and July 16, 1985 (study days 745-753).

^b Doses, expressed as free base of CL 232,315.

^c The vehicle was sodium chloride for injection, USP.

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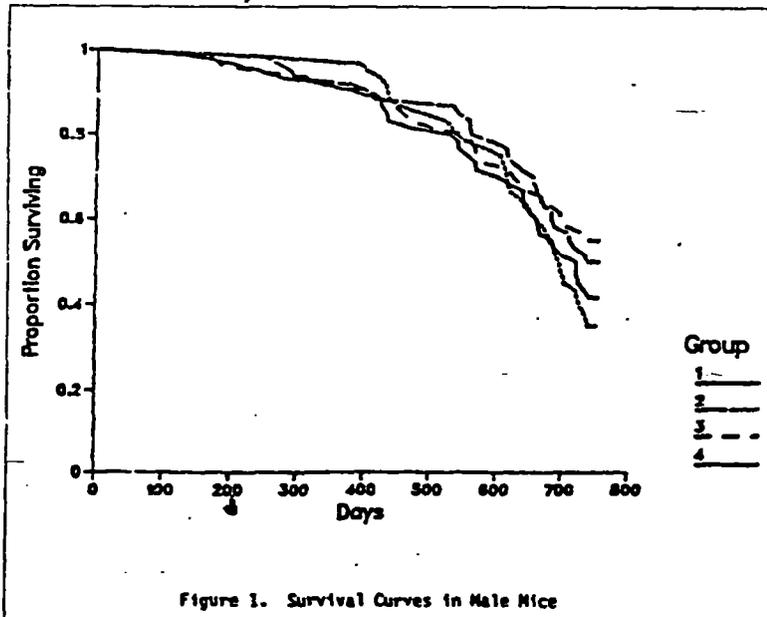
Study Results and Frequency of Monitoring:

Measurements and Observations	When Performed	Method
Daily Observations	Daily Acquisition System	LEDTOX Data
Body Weights and Food Consumption	Weekly	LEDTOX Data Acquisition System
Physical Examinations then monthly	Days -7, 28, 56 Acquisition System	LEDTOX Data
Ophthalmoscopic Examination	Days -10, 186, 368, 552, 714	Indirect Ophthalmoscopy ^a
Blood ^b Hemogram 101	Days 166, 264, 271, 278 then approximately every fourth dosing cycle: 7, 14 and 21 days postdose	Department of Clinical Chemistry
Serum Chemistry ^b Serum Profile 704	Day 746	Department of Clinical Chemistry
Palpable Masses monthly to 733	Day 202 then Acquisition System	LEDTOX Data
Urine Chemstrip ^c Urine Chemistry 301	Days 178, 369, 544 and 720	LEDTOX Data Acquisition System
Sacrifice	Days 745-753	Department of Experimental Pathology

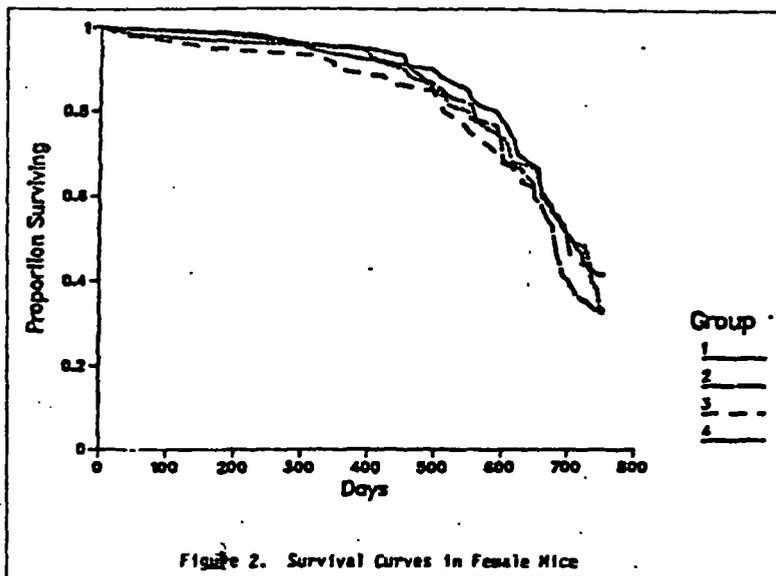
^a Indirect Ophthalmoscope. American Optical
^b All samples drawn from the periorbital sinus of unfasted, unanesthetized animals.
^c Chemstrip 7. Bio-Dynamics/bmc, Indianapolis, Indiana

- Clinical Observations: No effects
- Mortality- No effects

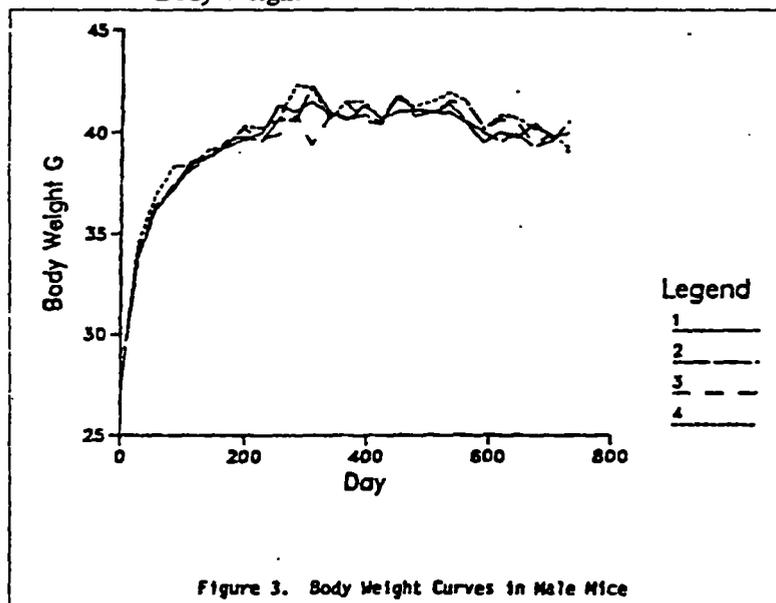
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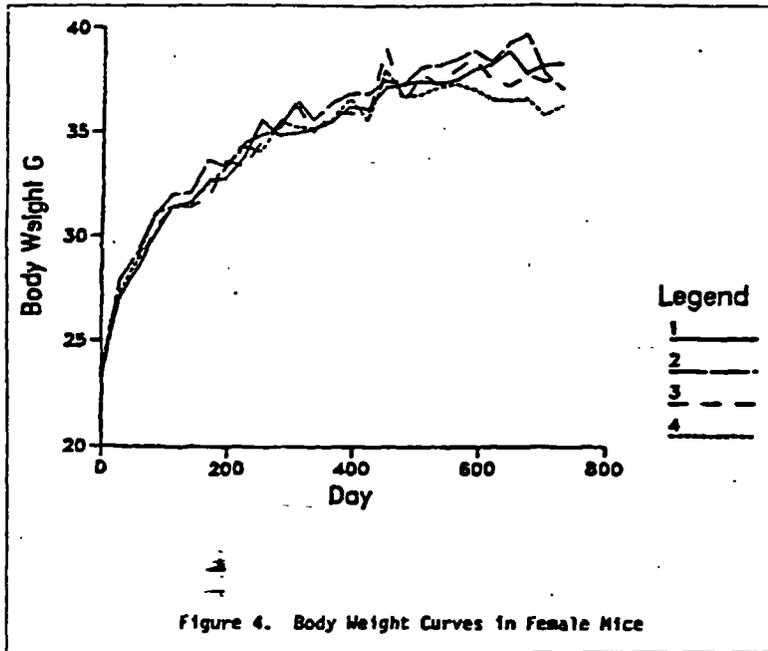


Body Weight



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- Food Consumption- No effects
- Ophthalmoscopy: No effects
- Hematology: No effects
- Clinical Chemistry: No effects
- Organ Weights: No effects
- Gross Pathology: No effects
- Histopathology:
 - Non-Tumor- No effects
 - Tumor

APPEARS THIS WAY
ON ORIGINAL

Overall Occurrence and Multiplicity of Tumors^a

Sex	Male				Female			
	1	2	3	4	1	2	3	4
Group Number ^b								
Number of Animals	60	60	60	60	60	60	60	60
Number of mice with tumors	25	20	24	25	31	29	29	27
Number of mice with malignant tumors	20	10	14	14	21	20	15	19
Number of mice with multiple tumors ^c	5	3	3	3	5	5	4	4
Total number of primary tumors	30	23	27	28	36	34	33	32
Mean number of primary tumors per tumor bearing mice	1.20	1.15	1.12	1.12	1.16	1.17	1.13	1.18

^a Derived from Figures 9-16.
^b For details of treatment, refer to Table 1.
^c More than one tumor type in one mouse. Multicentric tumors counted as one tumor; multiple foci of a single type in one organ were also counted as one tumor.

Prevalence of Most Common Tumors^a

Sex	Male				Female			
	1	2	3	4	1	2	3	4
Group Number ^b								
Mammary Gland: (N)	59	60	60	59	60	60	60	59
- Adenocarcinoma	0	0	0	0	7	1	3	1
Lungs: (N)	60	59	60	60	60	60	60	60
- Adenoma, Alveolar Cell	5	5	7	6	2	5	6	4
- Carcinoma, Alveolar Cell	4	5	5	2	2	4	1	4
- Total Tumors	9	10	12	8	4	9	7	8
Liver: (N)	60	60	60	60	60	60	60	60
- Adenoma, Hepatocellular	4	5	2	6	1	1	1	0
- Carcinoma, Hepatocellular	11	4	5	5	3	0	0	0
- Total Tumors	15	9	7	11	4	1	1	0
Hemopoietic: (N)	60	60	60	60	60	60	60	60
- Lymphoma, Malignant	6	2	2	6	10	8	6	12
- Sarcoma, Histiocytic	0	0	0	0	0	0	0	1
- Total Tumors	6	2	2	6	10	8	6	13
Pituitary: (N)	56	58	58	55	60	58	58	58
- Adenoma	0	0	0	0	4	3	2	3

^a Derived from Appendix XIV.

^b For details of treatment, refer to Table I.

N Number of mice from which tissue was examined.

APPEARS THIS WAY
ON ORIGINAL

Excerpt from FDA Statistician Karl K. Lin, Ph.D. Review of this study (Full Text in Appendix)

The survival rates at the times of terminal sacrifices were 42%, 50%, and 35%, respectively, in males, and 43%, 23%, 42%, and 40%, respectively, in females. These rates were tested for dose-response relationship according to the method given in Peto et al.(1970). The results of the analyses show that there is no significant dose-response relationship (positive or negative) in mortality rate in male and female mice ($p = 0.5553$ and $p=0.9502$, respectively).

Eight tumor/sex combinations with five or more occurrences across treatment groups within a sex group were tested in this study for positive dose-response relationship according to the method of analyzing incidental tumors given in Peto et al (1980). The results of the tests show that there is no significant positive dose-response relationship in the eight tumor/sex combinations tested.

- Toxicokinetics:

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The doses used in this study (0.01, 0.03, and 0.06 mg/kg; 0.03, 0.09, and 0.18 mg/m²) were lower than those used in the previous carcinogenicity study. It is not surprising that there were no effects on survival, body weight, tumor incidence, hematology, or other toxicological parameters. Since the highest dose used in this study is 66-fold lower than the levels to which humans would be exposed in a clinical setting (12 mg/m²), this study is not adequate for evaluating the potential carcinogenicity of mitoxantrone to humans.

APPEARS THIS WAY
ON ORIGINAL

**Oncogenic Potential of Novantrone Mitoxantrone Hydrochloride (CL 232,315)
Administered Intravenously Once Every 3 Weeks to Rats for 25 Months (Report 7)**

Study Number: 81152

Volume Numbers: Volume 59, Page 124 to Volume 74, Page 153

Test Facility: Lederle Laboratories, American Cyanamid Co., Pearl River, NY

Study Date(s): 2/26/1982 to 4/30/1985

Date of Submission

GLP Compliance/Quality Assurance: Yes

QA Report- Yes (X) No ()

Study Type: 2 Year Bioassay

Species/strain: Rat, CrI:COBS CD (SD)

Number of animals per group; age at start of study: One month old rats were used

Group	Compound	Dose ^a		No. of Animals	
		mg/kg	mg/m ²	male	female
1	Vehicle ^b	0	0	70	70
2	Vehicle ^c	0	0	70	70
3	CL 232,315	0.01	0.06	60	60
4	CL 232,315	0.03	0.18	60	60
5	CL 232,315	0.1	0.6	60	60

^a Doses, expressed as anhydrous base of mitoxantrone were administered once every 3 weeks.

^b The vehicle was 0.8% sodium chloride, 0.2% sodium metabisulfite in sterile water (for the first 3 dosing periods) and 0.9% sterile saline for the remainder of the study.

Animal housing: Individually in stainless steel wire-mesh suspended cages

Drug Lot/Batch number(s): PC 0345

Drug Purity / Stability / Homogeneity:

Doses:

- Basis of Dose Selection: Chronic studies
- Relation to Clinical Use: Same route and timing of administration
- CAC Concurrence: Pre-CAC study
- Restriction Paradigm for Dietary Restriction Studies- None
- Route of Administration: IV
- Frequency of Drug Administration: every three weeks
- Dual Controls Employed: Yes
- Interim Sacrifices: No
- Satellite PK or Special Study Group(s): None
- Unscheduled Sacrifices or Deaths:
- Deviations from Original Study Protocol:

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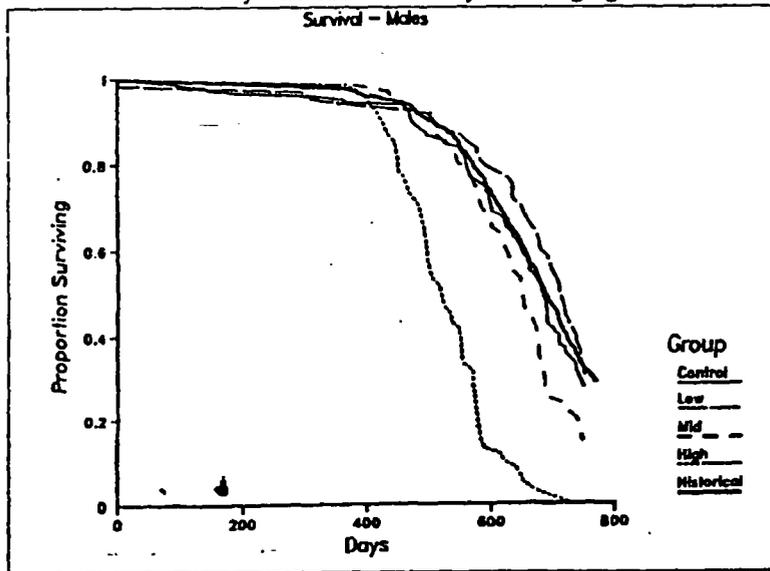
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Study Results and Frequency of Monitoring:

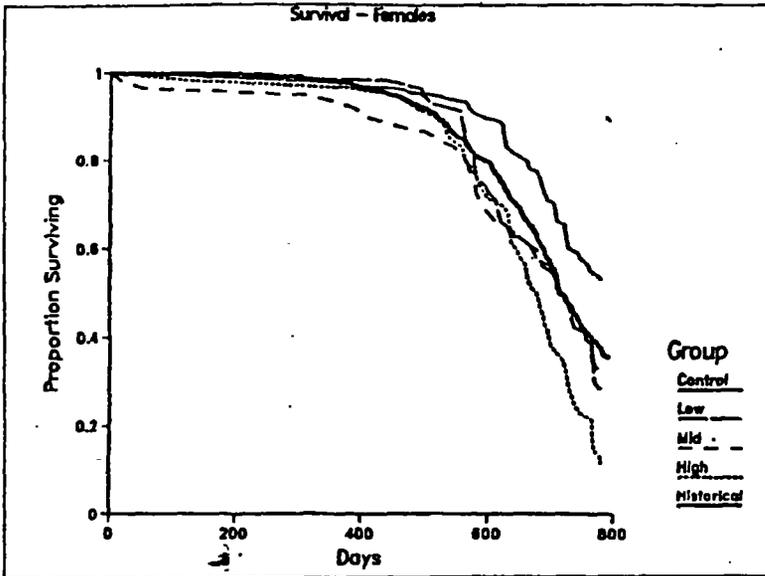
Observations and Measurements	When Performed	Method
General Observations	Daily	Manual recording up to day 646, then LEDTOX Data Acquisition Program
Body Weight and Food Consumption	Weekly	LEDTOX Data Acquisition Program
Physical Examination	Pretest, Weekly	LEDTOX Data Acquisition Program
Ophthalmoscopic Examination	Pretest, every 6 months	Indirect Ophthalmoscopy
Analysis of Eye and Blood Cultures for SDA	Day 55	Serology
Hemogram	Day 241-245, Study Termination	Performed by the Clinical Pathology Lab. Dept. 973 Method Code 101
Serum Clinical Chemistry	Study Termination	Performed by the Clinical Pathology Lab. Dept. 973 Method Code 704
Palpable Mass	Monthly after first mass detected	LEDTOX Data Acquisition Program
Postmortem Examination	Surviving Males (Day 752-761) Females (Day 780-784)	
Cytogenetic Evaluation of Bone Marrow	Study Termination	Described in G.T. 2: 796-810 (1984)

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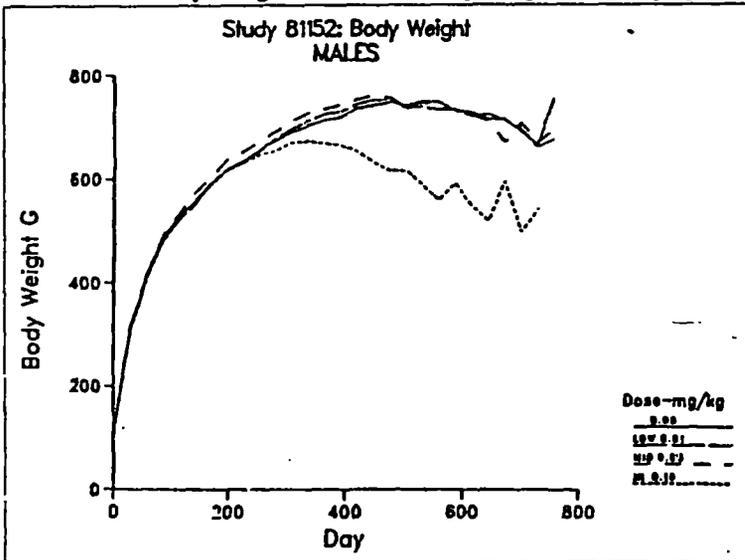
- Clinical Observations:
- Mortality- Increased mortality at 0.1 mg/kg



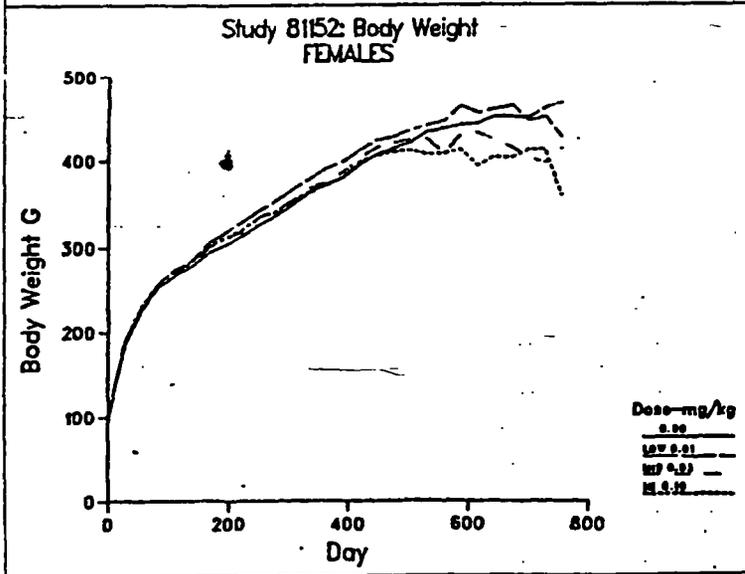
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- Body Weight- Decreased body weight at 0.1 mg/kg in male and 0.03 mg/kg in females



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- Food Consumption- Decreased food consumption in males at 0.1 mg/kg/day
- Ophthalmoscopy: No effects
- Hematology: No effects
- Clinical Chemistry: No effects, but sampling was only done at study termination
- Organ Weights: Organ weights not available for high dose males

Relative organ weights (percent of control)

	0 mg/kg		0.01 mg/kg		0.03 mg/kg		0.1 mg/kg	
	M	F	M	F	M	F	M	F
Kidney	0.869	0.728	0.879 (101)	0.839 (115)	0.915 (105)	0.825 (113)	---	1.102 (151)
Heart	0.359	0.339	0.356 (99)	0.389 (115)	0.428 (119)	0.392 (116)	---	0.447 (132)

- Gross Pathology:
- Histopathology:

Non-Tumor- Sponsor attributed early deaths in the 0.1 mg/kg males to glomerulonephritis

Sex	Group No. ^D	Males					P value ^C		Females					P value		
		1	2	3	4	5	Hetero-	Trend	1	2	3	4	5	Hetero-	Trend	
Number of Animals		70	70	60	60	60	ogeneity		70	70	60	60	60	ogeneity		
Tissue (N) and Findings																
<u>Heart (N)</u>		70	70	60	60	60			69	70	58	60	60			
Accum. Mono. Cells		10	3	1	4	13	.0017	.01828	8	1	6	5	2	.3938	.51917	
Mineralization Myocardium		5	6	2	4	35	.0001	.00005	2	2	0	2	6	.0293	.01297	
Fibrosis		53	43	45	54	49	.0062	.00051	30	40	29	29	31	.0222	.00378	
Vacuolation		57	45	43	54	42	.0060	.00169	31	48	38	38	41	.0978	.00900	
<u>Aorta (N)</u>		70	69	60	60	60			68	70	58	60	60			
Distention		1	0	2	1	16	.0001	.00005	0	1	0	1	1	.6553	.16322	
Myelinization, Media		3	6	12	19	27	.0001	.00005	3	6	2	5	6	.4886	.15312	
Mineralization		3	6	6	9	25	.0001	.00005	4	4	1	6	7	.1064	.03764	
<u>Parathyroid (N)</u>		67	67	60	59	60			69	70	58	58	60			
Hyperplasia		29	38	35	37	53	.0001	.00005	28	19	15	24	31	.0102	.00231	
<u>Stomach (N)</u>		70	69	59	60	58			68	70	58	60	60			
Mineralization Mucosa		4	10	7	9	24	.0001	.00005	4	4	3	6	7	.3800	.05402	
Mineralization Muscularis		3	3	5	5	19	.0001	.00005	2	2	0	2	7	.0088	.00568	
Erosion Mucosa		10	4	2	7	7	.1932	.18130	2	5	4	4	11	.0142	.00248	
Ulceration		5	10	6	6	2	.9313	.66567	0	3	3	3	6	.2437	.21956	
<u>Thymus (N)</u>		61	56	53	56	46			57	52	39	47	49			
Lymphoid Depletion		0	0	0	0	1	.	.	0	0	0	1	0	.	.	
Involution		36	44	35	45	43	.0089	.00147	21	21	21	20	30	.0407	.00972	
<u>Kidney (N)</u>		70	70	60	60	59			69	70	58	60	60			
Hydronephrosis		7	7	4	3	4	.5186	.63996	4	13	6	8	11	.4677	.39959	
Perivasc. Mono. Cells		61	58	50	54	49	.0852	.02194	32	35	24	38	37	.0103	.00755	
Myelinated Glomeruli		0	1	0	0	6	.0001	.00027	0	0	0	0	0	.	.	
Pyelitis Suppurative		3	3	0	2	0	.2668	.88757	3	3	5	4	2	.5352	.51687	
Chronic Glomerulonephritis		62	62	53	59	59	.0107	.00111	53	53	41	44	54	.0121	.00201	
Focal Mineralization																
Renal Pelvis		5	0	3	1	1	.8745	.55203	26	30	17	17	16	.4677	.39959	
<u>Bone Femur (N)</u>		66	69	60	58	59			69	68	57	59	60			
Osteoclastic Resorption		14	13	11	13	40	.0001	.00005	2	1	1	5	12	.0001	.00005	

^A Derived from Appendix XIV
^B For details of treatment refer to Table I
^C Analyzed using procedure described by Paro (15). Asterisks indicate that values are not given here because both sexes have a total of 5 or less affected animals. The two control groups (Nos. 1 and 2) are analysed together.
Accum. Mono. Cells = Accumulation of mononuclear cells
Perivasc. Mono. Cells = Perivascular accumulation of mononuclear cells

Tumor

Overall Occurrence and Multiplicity of Tumors

Sex Group No. ^a	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Number of Animals	70	70	60	60	60	69	70	59	60	60
No. of Rats with Tumors	55	51	45	46	35	64	66	55	54	56
% of Rats with Tumors	78.6	72.9	75.0	76.7	58.3	92.8	94.3	93.2	90.0	93.3
No. of Rats with Malignant Tumors	12	4	9	16	13	13	14	13	6	19
% of Tumor Bearing Rats with Malignant Tumors	21.8	7.8	20.0	34.8	37.1	20.3	21.2	23.6	11.1	33.9
No. of Rats with Multiple Tumors ^b	28	18	18	19	12	49	45	37	35	41
Number of Primary Tumors	95	77	67	79	51	147	138	120	103	121
Mean Number of Tumors per Tumor Bearing Rat	1.73	1.51	1.49	1.72	1.46	2.30	2.09	2.18	1.91	2.16

^a For details of treatment see Table I

^b Multiple tumors means more than one tumor type in one individual. Occurrences of one kind of tumor at different sites (either multicentric or metastatic tumors) or multiple foci of a single type in one organ (e.g. 2 or more fibroadenomas in the mammary gland) were counted as one tumor. Adenomas in different glands of one animal are separate tumors; by definition, adenomas do not metastasize. Adenocarcinomas in different organs are primary to those organs unless the designation "secondary" (Appendix XIX) indicates arrival by metastasis.

Prevalence of Most Common Tumors^a

Sex	Males					P Value ^c Hetero- geneity	Trend	Females					P Value ^c Hetero- geneity	Trend
	1	2	3	4	5			1	2	3	4	5		
Skin (N)	69	69	60	60	59			59	70	58	60	60		
External Auditory Canal	2	0	1	3	8	.0005	.00006	0	1	0	1	8	.0001	.00003
Carcinoma Squamous	0	0	0	1	0	*	*	0	0	0	0	3	*	*
Fibrosarcoma	0	0	0	0	2	*	*	0	0	0	1	2	*	*
Fibroma	7	4	2	2	4	.2694	.31908	1	0	0	0	6	.0001	.00006
Lipoma	1	1	1	1	0	.9529	.36812	0	3	4	1	1	.2470	.34523
Mammary Gland (N)	30	33	26	33	34			67	64	52	55	55		
Adenocarcinoma	0	1	1	0	1	.2949	.19070	6	5	8	1	3	.0703	.85506
Carcinoma	0	0	0	0	0	*	*	2	3	0	0	1	.3041	.86756
Adenoma	0	0	1	0	0	.4235	.37712	19	8	7	9	4	.3509	.84466
Fibroma	1	0	0	0	0	*	*	3	0	0	0	1	*	*
Fibroadenoma	0	1	0	0	0	.7283	.33499	16	22	16	14	18	.5921	.13247
Lipoma	0	0	0	0	0	*	*	1	0	0	1	1	*	*
Thyroid (N)	69	69	60	60	59			69	70	58	59	60		
Adenoma	4	3	3	3	2	.9974	.49134	3	2	3	1	0	.4216	.84135
Carcinoma	1	0	0	1	1	*	*	0	1	1	0	0	*	*
Adenoma Parafollicular	3	4	2	1	0	.3231	.91709	15	5	7	6	2	.1733	.91005
Carcinoma, Parafollicular	0	0	0	2	1	*	*	0	1	0	0	0	*	*
Parathyroid (N)	67	67	60	59	60			69	70	58	58	60		
Adenoma	1	0	0	0	1	*	*	0	1	0	1	0	*	*

^a Derived from Appendix XIV

^b For details of treatment refer to Table I

^c Analyzed using procedure described by Peto (15). Asterisks indicate that values are not given here because one or both sexes have a total of 5 or less affected animals. The two control groups (Nos. 1 and 2) are analyzed together.

(N) Number of rats from which tissue was examined

NA Not applicable

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Sex Group Number ^D	Prevalence of Most Common Tumors ^E										P Value ^C Hetero- Trend genicity		P Value Hetero- Trend genicity	
	Males					Females								
	1	2	3	4	5	1	2	3	4	5				
<u>Liver (N)</u>	59	70	60	60	59	69	70	58	59	60				
Carcinoma hepatocellular	1	0	1	1	0	0	0	0	0	0	*	*	*	*
Cholangiocarcinoma	0	0	0	0	0	0	0	0	1	0	*	*	*	*
<u>Pancreas (N)</u>	68	68	59	60	59	69	70	57	58	60				
Islet Cell Tumor	18	3	6	5	2	7	3	6	3	3	.1592	.93446	.7114	.16798
<u>Thymus/Lymph Node (N)</u>	61	56	52	56	46	57	51	39	47	49				
Leukemia Granulocytic	1	1	1	0	2	0	0	2	1	0	*	*	*	*
Lymphoma	1	0	3	1	1	0	0	2	0	0	.0380	.50017	.1344	.46322
Thyroid	0	1	1	0	0	0	1	0	0	0	*	*	*	*
<u>Spleen (N)</u>	70	70	60	60	59	69	70	57	59	60				
Leukemia, Granulocytic	1	1	1	0	1	0	0	1	1	0	*	*	*	*
Lymphoma	1	0	3	3	0	0	0	1	0	0	.0708	.36380	.1743	.46407
<u>Adrenal Glands (N)</u>	67	70	59	60	59	70	69	57	60	60				
Carcinoma, Cortex	1	0	1	0	1	2	1	1	0	0	*	*	*	*
Medullary Tumor	5	8	3	8	3	1	2	0	2	1	.0354	.04217	.5808	.26270
<u>Testes (N)</u>	70	70	60	58	59									
Interstitial Cell tumor	2	1	2	1	1	NA	NA	NA	NA	NA	.7475	.24794	NA	NA
<u>Pituitary Gland (N)</u>	65	66	57	60	57	69	69	57	59	58				
Adenoma	35	37	28	28	9	58	58	51	48	45	.0115	.99804	.7155	.64773
<u>Bone Marrow (N)</u>	69	70	60	59	59	69	70	58	59	59				
Lymphoma	1	0	2	3	0	0	0	1	0	0	.1147	.33698	.1870	.46417
Leukemia, Granulocytic	0	1	1	0	1	0	0	1	1	0	*	*	*	*

^A Derived from Appendix XIV
^B For details of treatment refer to Table I
^C Analyzed using procedure described by Peto (1971). Asterisks indicate that values are not given here because one or both sexes have a total of 5 or less affected animals. The two control groups (Nos. 1 and 2) are analyzed together.
^(N) Number of rats from which tissue was examined
 NA Not applicable

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Excerpt from FDA Statistician Karl K. Lin, Ph.D. Review of this study (Full Text in Appendix)

The data of the two control groups in this study were combined in the reviewer's analyses. The survival rates at the times of terminal sacrifices for the control, low, medium, and high dose groups were 29%, 37%, 18%, and 0%, respectively. In male rats, and 54%, 30%, 33%, and 15%, respectively, in female rats. The intercurrent mortality rates were tested for dose-response relationship according to the method given in Peto et al. (1980). There is a significant positive dose-response relationship in both male and female rats (both with $p < 0.00001$). The high dose male rats show much higher mortality rates than those of the male rats in other groups during the second year of the study. There is no male rat in the high dose group survived at the end of week 106 for terminal sacrifice. The Peto prevalence analysis of incidental tumors given in the paper Peto et al. (1980) was used to test the positive dose-response relationship in 11 tumor/sex combinations. The 11 selected tumor/sex combinations are those tumor types with five or more occurrences across treatment groups within a sex group. The test results show a significant positive dose-response relationship in skin external auditory canal in both male rats ($p=0.00071$) and female rats ($p=0.000005$), and in skin fibroma in female rats ($p=0.00006$). The results also show a significant negative dose-response relationship in pituitary gland adenoma in male rats. The excessive mortality rates of male rats in the high dose group could have different effects on the above significant positive and negative dose-response relationships in male rats. If the mortality rates of male rats in the high dose groups are lower so that there were animals survived at the end of week 106, some of those male rats may be found to have skin external auditory canal tumor, or pituitary gland adenoma during terminal sacrifices. This will make the significant positive dose-response relationship in skin external auditory canal tumor more significant, and the significant negative dose-response relationship in pituitary gland adenoma less significant in male rats.

- Toxicokinetics: Not done

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The high dose in this study (0.1 mg/kg, 0.6 mg/m²) caused significant early mortality and body weight decreases in male and female rats. Although this dose is below the proposed clinical dose (12 mg/m²), it is above the maximum tolerated dose for rats. The middle dose was (0.03 mg/kg) was about one third the high dose and represents an adequate back up dose. This is an adequate carcinogenicity study.

- Evaluation of Tumor Findings:

An increase incidence of external auditory canal tumors was observed in both male and female rats at 0.3 mg/kg. An increase in skin fibromas was also observed in female rats at 0.3 mg/kg.

Overall Summary:

1. Mitoxantrone was tested for carcinogenicity in two mouse studies and one rat study.
2. In the first mouse study, an increase in the incidence of hepatocellular adenoma in male mice at a dose of 0.1 mg/kg (0.03 fold the recommended human dose, on a mg/m² basis).
3. No carcinogenic effects were observed in the second mouse study, however the doses used in this study were below the maximum tolerated dose so that this study could not be considered an adequate test of carcinogenic potential.
4. In the rat study, an increased incidence of external auditory canal tumors and fibromas was observed at 0.03 mg/kg (0.02 fold the recommended human dose, on a mg/m² basis).
5. In a chronic one year toxicity study in which rats were treated every 21 days (see page 26), 5/24 female rats in the 0.3 mg/kg group had auditory canal tumors. In addition, 1/17 female rats in the 0.6 mg/kg group had auditory canal tumors, despite high early mortality (all rats had died or were sacrificed by week 42 of the study). Finally, 1/18 male rats in the 0.3 mg/kg group had auditory canal tumors, despite high early mortality (all rats had died or were sacrificed by week 45 of the study). The higher doses in this study had very high early mortality so that only a few rats reached the six month timepoint.
6. Mitoxantrone is genotoxic in bacterial and mammalian test systems (both in vivo and in vitro) (see genotoxicity section below).
7. Mitoxantrone is a topoisomerase II inhibitor. Topoisomerase II inhibitors, in combination with other antineoplastic agents, have been associated with the development of acute leukemia in humans.
8. Since mitoxantrone was carcinogenic in experimental animals at doses below the proposed clinical dose, mitoxantrone has the potential to be carcinogenic in humans.

Addendum list:

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**Addendum 1
Histopathology Inventory for IND #**

Study	1	4	7	
Species	Mouse	Mouse	Rat	
Adrenals	✓	✓	✓	
Aorta	✓	✓	✓	
Bone Marrow smear	✓	✓	✓	
Bone (femur)	✓	✓	✓	
Brain	✓	✓	✓	
Cecum	✓	✓	✓	
Cervix				
Colon	✓	✓	✓	
Duodenum	✓	✓	✓	
Epididymis	✓	✓	✓	
Esophagus	✓	✓	✓	
Eye	✓	✓	✓	
Fallopian tube				
Gall bladder	✓	✓	✓	
Gross lesions	✓	✓	✓	
Harderian gland	✓	✓	✓	
Heart	✓	✓	✓	
Hypophysis				
Ileum	✓	✓	✓	
Injection site	✓	✓	✓	
Jejunum	✓	✓	✓	
Kidneys	✓	✓	✓	
Lachrymal gland				
Larynx				
Liver	✓	✓	✓	
Lungs	✓	✓	✓	
Lymph nodes, cervical	✓	✓	✓	
Lymph nodes mandibular	✓	✓	✓	
Lymph nodes, mesenteric	✓	✓	✓	
Mammary Gland	✓	✓	✓	
Nasal cavity				
Optic nerves				
Ovaries	✓	✓	✓	
Pancreas	✓	✓	✓	
Parathyroid	✓	✓	✓	
Peripheral nerve	✓	✓	✓	
Pharynx				
Pituitary	✓	✓	✓	
Prostate	✓	✓	✓	
Rectum	✓	✓	✓	
Salivary gland	✓	✓	✓	
Sciatic nerve				
Seminal vesicles	✓	✓	✓	
Skeletal muscle	✓	✓	✓	
Skin	✓	✓	✓	
Spinal cord	✓	✓	✓	
Spleen	✓	✓	✓	
Sternum	✓	✓	✓	
Stomach	✓	✓	✓	
Testes	✓	✓	✓	
Thymus	✓	✓	✓	
Thyroid	✓	✓	✓	
Tongue	✓	✓	✓	
Trachea	✓	✓	✓	
Urinary bladder	✓	✓	✓	
Uterus	✓	✓	✓	
Vagina	✓	✓	✓	
Zymbal gland				

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REPRODUCTIVE TOXICOLOGY:

CL 232,315: Effects of Intravenous Administration Upon Reproductive Function and Fertility in the Rat (Report 143)

Study No: and number: 15167

Amendment #, Volume # and Page #: Volume 30, Page 1-343

Site and testing facility: _____

GRP compliance:

QA- Reports Yes (X) No ():

Lot and batch numbers: PC 0345

Protocol reviewed by Division Yes () No ():

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Methods:

- Species/strain: Rats, Sprague-Dawley
- Doses employed: 0.0033, 0.01, 0.03 mg/kg
- Route of Administration: IV
- Study Design:

Generation	Sex	Number per group	IV Dose ^a (mg/kg/day)	Treatment	Observations and measurements	Time of sacrifice	Postmortem examination
F ₀	M	28	0.0033 0.01 0.03	daily for 71 days before pairing, through mating period, and until sacrifice after pregnancy of F ₀ females was confirmed	OB; BW; FC; VI; RBC; WBC	upon confirmation of pregnancy of F ₀ females	gross; weight of testes, epididymides, seminal vesicles, prostate glands
F ₀	F	14	0.0033 0.01 0.03	daily for 15 days before pairing, through mating period, and until sacrifice day 21 of pregnancy	OB; EST; BW; FC; VI; RBC; WBC	day 21 of pregnancy	gross; reproductive tract; fetuses (skeletal and visceral evaluation)
		14	0.0033 0.01 0.03	daily for 15 days before pairing, through mating period, and until weaning at day 25 postpartum	OB; EST; BW; FC; VI; evaluation of pregnancy length, parturition, litter and neonate data	day 25 postpartum	gross

^a The study employed control groups which received vehicle.

^b The following were recorded: number of corpora lutea in each ovary; number and distribution of implantations, resorptions, and live and dead fetuses; weight and sex of individual fetuses; individual placenta weights; external abnormalities of individual fetuses; internal morphologic abnormalities.

BW - body weight once (M) or twice (F) weekly

EST - assessment of estrus cycle before pairing

FC - food consumption weekly

OB - daily observation of physical appearance and behavior

RBC - red blood cell count of 5/sex/group just before sacrifice

WBC - white blood cell count and differential of 5/sex/group just before sacrifice

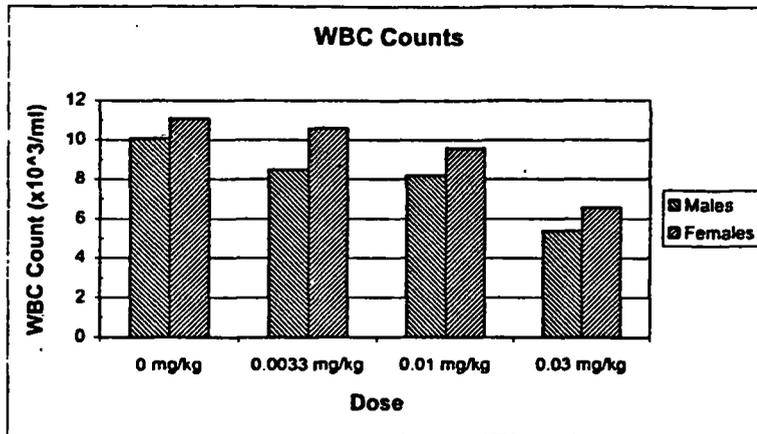
VI - water intake weekly before pairing

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26 rats/sex/group of the F1 generation were randomly selected and mated at 9 to 10 weeks of age. The resulting litters were assessed for fertility, terata, and effects on development of the F2 generation.

Results:

- Clinical signs: no clinical signs, Decreased WBC at 0.03 mg/kg in males and 0.01 mg/kg in females



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- Mortality: 2 control males died prematurely; no deaths in treated rats
- Body weight: 0.03 mg/kg females had reduced body weight gain during gestation
- Food consumption: No Effects
- Toxicokinetics: Not done
- Fertility in Males
 - In-life observations: no effects on pre-coital interval, mating performance, conception rate or fertility index
 - Terminal and Necroscopic evaluations: slight decrease in absolute and relative weights of epididymides at 0.03 mg/kg (6 and 7%, respectively)
- Fertility and Early Embryonic Development in Females
 - In-life observations: No effects on estrus cycle regularity or female fertility
 - Terminal and Necroscopic evaluations: No effects on incidence of terata or on litter sizes
- Embryo-fetal Development
 - In-life observations:
 - Terminal and Necroscopic evaluations:
 - Dams: no effects on ovulation
 - Offspring: no effects on implantation, survival, growth and development *in utero*
- Prenatal and postnatal development, including maternal function
 - In-life observations:
 - Dams: no effects on maternal behavior; Slight decrease in maternal weight (96% of control weight) on Gestation Day 21
 - Offspring: litter size at birth was reduced in females receiving 0.03 mg/kg (11.8 vs 13.3 in controls, 87% of control), but subsequent viability was unaffected and post-natal development; no effects on post natal development or survival
 - Terminal and Necroscopic Evaluations:
 - Dams:
 - Offspring:

Summary and Evaluation:

1. There was a dose dependent decrease in white blood cell count in mitoxantrone treated rats. The white blood cell count at 0.03 mg/kg was 53 and 59% of control values in males and females, respectively. This suggests that the doses used in this study were sufficient to cause slight toxicity in the F0 generation.
2. Only minor decrease in litter size was observed at 0.03 mg/kg, however subsequent development was unaffected. Physical and behavioral development were unaffected by mitoxantrone.
3. No effects on the F1 generation fertility or development of the F2 generation were observed.

A Teratology Study (Segment II) of CL 232,315 Administered Intravenously to Pregnant Rats (Study 146)

Study No: and number: 80145

Amendment #, Volume # and Page #: Volume 31, Page 72-178

Site and testing facility: Lederle Laboratories, American Cyanamid Co, Pearl River, NY

GRP compliance: No

QA- Reports Yes (X) No ():

Lot and batch numbers: PC 0345

Protocol reviewed by Division Yes () No (X):

Methods:

- Species/strain: Rats, CrI:COBS CD (SD)
- Study Design:

Group	Treatment ^a	Daily Dose (mg/kg)	Mated ^b Females
I	Vehicle ^c	0	44
II	CL 232,315	0.05	22
III	CL 232,315	0.10	22
IV	CL 232,315	0.20	22

^a Administered intravenously, once daily, on day 6-15 of pregnancy, at a dosing volume of 1 ml/100 g of body weight.

^b Females were cohoused with males (1:1) until mating was confirmed by the presence of a copulatory plug. This day was defined as day 0 of pregnancy.

^c Normal saline 0.9% (sodium chloride injection, U.S.P.).

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- Parameters and endpoints evaluated:

Procedure	Subject	Days(s) of Pregnancy
Selection	Females	Prior to mating
Randomization	Dams	Prior to day 6
Body weight measurement	Dams Fetuses	0, 6, 10, 13, 16 and 21 21
Food consumption measurement	Dams	0-6, 6-10, 10-13, 13-16, and 16-21
Compound preparation	...	6-15
Compound administration	Dams	6-15
Adjustment of dose	10, 13
Clinical observations	Dams	0, 6-16, 21 and additionally as required
Sacrifice and postmortem examination	Dams	21
Gross external examination	Fetuses	21

- Statistical evaluations: