V. SUMMARY OF THE NONCLINICAL PHARMACOLOGY AND TOXICOLOGY SECTION

A. Introduction

DOXIL® (Stealth® liposomal doxorubicin hydrochloride) Injection is a long-circulating liposomal formulation of doxorubicin proposed for treatment of patients with AIDS-related Kaposi's Sarcoma (KS) who have failed systemic combination chemotherapy due to disease progression or unacceptable toxicity. Stealth liposomes are formulated with surface-bound segments of methoxypolyethylene glycol (MPEG), which reduce uptake by hepatic macrophages and result in a long circulation time compared to conventional liposomes.

DOXIL is considered to be superior to conventional doxorubicin with respect to improved tumor activity and decreased toxicity. A number of pharmacology and pharmacokinetic studies have been conducted to evaluate this claim. In addition to the pharmacologic safety, the general and reproductive toxicity, genotoxicity, and hemolytic and irritative potential of DOXIL have been investigated in vitro and in animal bioassays. In a number of these studies, doxorubicin HCl and empty liposomes were compared. The results and conclusions of these studies are presented in this section of the Overall NDA Summary. Studies conducted by Liposome Technology, Inc. (LTI) are listed at the end of each section where they are described; complete study reports are in the Clinical Data Section of the NDA. Literature references cited are listed at the end of this Overall NDA Summary in Section X.

This Nonclinical Pharmacology and Toxicology Summary is divided into six segments:

- This introduction;
- Nonclinical efficacy studies;
- General pharmacology;
- Nonclinical Absorption, Distribution, Excretion and Metabolism, (ADME) Summary;
- Nonclinical toxicology; and
- Final summary.

B. Nonclinical Efficacy Studies

1. Mechanism of Doxorubicin Action

The exact mechanism of the antitumor activity of doxorubicin and related anthracyclines is not precisely known, but it is correlated to interference with the cellular synthesis of macromolecules. Among proposed mechanisms are the intercalation of the aglycone moiety of the anthracycline between adjacent base pairs in the DNA double helix,[58-60] leading to inhibition of DNA, RNA and protein synthesis; alteration of cell membrane function;[61-63] the induction of DNA strand breaks;[64,65] and anthracycline-mediated free radical generation with associated DNA alkylation and degradation .[66-68] None of these mechanisms alone is considered adequate to fully explain all of the cytotoxic properties of the drug,[66,69] although it is generally believed that the inhibition of DNA and RNA synthesis is responsible for the majority of its cytotoxic action.

2. Effects of DOXIL on Transplantable Tumors in Mice

In order to characterize the therapeutic effectiveness of DOXIL® Injection, LTI has conducted a series of efficacy studies in murine tumor models and with xenografts of human tumors implanted in immune deficient mice. Early studies were conducted with the original formulation of DOXIL, known as DOXIL 1, which was unbuffered and stored frozen. Later studies were conducted with either of two liquid formulations of DOXIL that differed from each other only in the formulation buffer (DOXIL 2 and DOXIL 3, buffered with tromethamine and histidine, respectively, see Table 8). A summary of studies designed to measure the anti-tumor activity of DOXIL is presented at the end of this section in Table 9, including test models employed, routes of drug administration and tumor cell implantation, dose range, other drugs and reference to LTI reports.

TABLE 8
Summary of DOXIL Formulations used in Nonclinical Studies

	Frozen Formulation	Liquid Formulations	
Component	**DOXIL 1**	**DOXIL 2**	**DOXIL 3**
Doxorubicin HCl	[redacted]mg/mL	[redacted]mg/mL	2.0 mg/mL
HSPC	[redacted] mg/mL	[redacted] mg/mL	9.58 mg/mL
MPEG-DSPE	[redacted] mg/mL	[redacted] mg/mL	3.19 mg/mL
Cholesterol	[redacted] mg/mL	[redacted] mg/mL	3.19 mg/mL
Sucrose[a]	[redacted]mg/mL	[redacted] mg/mL	94 mg/mL
[redacted]	[redacted]	[redacted] mg/mL	--
Histidine	[redacted]	[redacted]	1.55 mg/mL
Ammonium Sulfate[a]	[redacted]mg/mL	[redacted]mg/mL	2 mg/mL
[redacted]	[redacted] mg/mL	[redacted]	--
[redacted]	[redacted]mg/mL	[redacted]	--
Water for injection	qs [redacted]mL	qs [redacted]mL	qs 1 mL
Formulation pH	[redacted]	[redacted]	6.5

[a] [redacted]

Pharmacokinetic studies in rats have shown that DOXIL 2 and DOXIL 3 are equivalent (see ADME summaries), and this summary will not distinguish between the two, which will be referred to collectively as DOXIL hereafter. In contrast, the pharmacokinetics of DOXIL 1 and DOXIL 2/3 are not equivalent in rats or dogs, and the studies conducted with DOXIL 1 are provided in the efficacy section of this NDA as supportive information. Efficacy studies have been conducted with all three formulations.

Most studies reported here include conventionally formulated doxorubicin HCl (Adriamycin RDF™, Adria Laboratories) for comparative purposes. Two studies also included doxorubicin HCl encapsulated in conventional, or non-Stealth, liposomes for comparison of activity.

a. Studies with DOXIL

The efficacy of DOXIL has been evaluated in the murine C26 colon carcinoma model, two murine mammary tumor models and two human xenograft models (the ovarian carcinoma HEY and the prostatic carcinoma PC-3) in nude mice. In addition, the effect of altering liposome size on efficacy was evaluated in the murine C26 colon carcinoma model.

The therapeutic effect of DOXIL administered as a once or twice weekly bolus intravenous injection was evaluated in Balb/c mice that had been inoculated subcutaneously with 10^6 C26 tumor cells (LTI-30-93-22). Beginning 10 days after tumor implantation, mice received DOXIL 3 or 6 mg/kg once weekly for three weeks or DOXIL 1.5 or 3 mg/kg twice weekly for 3 weeks. An additional group received DOXIL 6 mg/kg twice weekly for 1.5 weeks (three treatments). Control animals received saline. Mean survival time (MST) was longest (74 days), inhibition of tumor growth was greatest, and onset of tumor regression was earliest in animals treated once weekly with DOXIL 6 mg/kg. Treatment with lower doses resulted in some increase in MST, but had little effect on tumor growth.

Altering the size of the liposomes had little effect on efficacy in the mouse C26 colon carcinoma model (LTI-30-94-12). Three sizes of doxorubicin-containing Stealth liposomes, with mean liposome diameters of [A*] nm (Dox-[A*]), [B*] nm (Dox-[B*]) and [C*] nm (Dox-[C*]), were compared. Dox-[A*] is equivalent to DOXIL, which has a mean particle size of approximately 100 nm. Nine days after implantation of 1×10^6 C26 tumor cells, Balb/c mice were treated intravenously with 6 mg/kg of the three preparations once weekly for three weeks. The MST of untreated control animals was 31 days and no control animals survived longer than 40 days. MST in the Dox-[A*], Dox-[B*] and Dox-[C*] treatment groups were 91, 66 and 83 days, respectively. Four animals each in the Dox-[A*] and Dox-[C*] groups remained tumor-free at study termination 120 days after tumor cell inoculation. Tumor growth rate and volume was decreased compared to controls in animals treated with the liposomes, regardless of liposome size. Tumor volume was decreased, in some cases to the point of no measurable tumor, in animals administered the three liposome size variants. Based on the results of this study, altering the size of doxorubicin-containing Stealth liposomes in the range of approximately 100 to 150 nm had no biologically significant effect on anti-tumor efficacy in the murine C26 colon carcinoma model.

The efficacies of DOXIL and the earlier formulation, DOXIL 1, were evaluated in Balb/c mice inoculated with C26 tumor cells (LTI-30-93-17). MST was increased relative to control in animals treated with DOXIL 3, 6, or 9 mg/kg, DOXIL 1, 6, or 9 mg/kg, and Adriamycin 6 mg/kg. At the 3 and 9 mg/kg dose levels, the MST was greater in the DOXIL group compared to the DOXIL 1 group. MST was similar in animals treated with 6 mg/kg DOXIL, DOXIL 1, or Adriamycin. Both formulations of DOXIL (at a dose of 9 mg/kg) inhibited tumor growth relative to control, but DOXIL was again more effective than DOXIL 1, leading to the conclusion that DOXIL was therapeutically

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superior to DOXIL 1. This difference may be related to the longer circulation time and greater area under the plasma-concentration curve (AUC) of DOXIL.

The activity of DOXIL and DOXIL 1 was compared in the murine MC2 and 2105 mammary tumor models (LTI-30-93-13). Both DOXIL 1 or DOXIL 3 or 6 mg/kg were more effective than Adriamycin 6 mg/kg at inhibiting growth of the slow growing MC2 tumor and the fast growing 2105 tumor. The incidence of 2105 tumors was not reduced by any treatment. Both DOXIL formulations significantly reduced the incidence of the MC2 tumors compared to placebo. DOXIL was slightly, but not significantly, more effective at decreasing tumor incidence than DOXIL 1.

DOXIL efficacy was also studied in two different xenografts of human tumors in nude mice. Mice were implanted subcutaneously or injected intraperitoneally with the human ovarian carcinoma HEY. DOXIL 6 or 9 mg/kg was significantly better than the same dose of Adriamycin at inhibiting tumor growth and effecting cures in mice bearing subcutaneous implants. Overall, 70% of mice treated with DOXIL 9 mg/kg survived until study termination with no evidence of tumor, compared to just 18% of the mice that received the same dose of Adriamycin. After intraperitoneal injection of tumor cells, 80% of control mice developed palpable tumors between 18 and 25 days after injection. All ten DOXIL-treated (9 mg/kg), compared to four of ten Adriamycin-treated mice, were tumor-free at necropsy on Day 70. The other six Adriamycin mice died due to drug toxicity between Days 7 and 52. In this ovarian carcinoma model, DOXIL was more effective and less toxic than conventionally formulated doxorubicin HCl.

In a second xenograft study in nude mice, mice were implanted subcutaneously with xenografts of the human prostatic carcinoma PC-3 (LTI-30-93-15). DOXIL 6 or 9 mg/kg was more effective than the same dose of Adriamycin with respect to inhibition of tumor growth and increase in tumor-free survival. No Adriamycin-treated mice survived until study termination, and, at a dose of 9 mg/kg, all mice died by Day 36 due to drug-related toxicity. In a replicate study of identical design, the tumor implants grew more rapidly, and no mice were cured by either DOXIL or Adriamycin. However, tumor growth inhibition was significantly greater with DOXIL, and MST was increased compared to equivalent doses of Adriamycin.

The localization of doxorubicin after treatment with DOXIL or Adriamycin in tumor, liver and kidney was also determined using laser confocal scanning microscopy to quantitate the fluorescence of doxorubicin. Doxorubicin levels in all three tissues were higher after DOXIL treatment than after Adriamycin treatment. One hour after injection

the doxorubicin concentration of tumor tissue was nearly three-fold higher in DOXIL-treated animals, and, by 24 hours after treatment, tumor drug levels were nearly 80-fold higher in DOXIL animals. Drug levels persisted in tumors of DOXIL-treated animals with time. AUC values calculated for doxorubicin concentration in the tumor gave relative values of 36.5 and 919 in the Adriamycin and DOXIL animals, respectively, representing a 25-fold increase in the drug concentration in the tumor.

b. Studies with DOXIL 1

The earlier formulation of DOXIL, known as DOXIL 1, was also tested in murine tumor models, including the C26 colon carcinoma, the MC2 mammary tumor model, the metastatic mammary tumor model and the P388 lymphocytic leukemia model, as well as in a human xenograft model of non-small cell lung cancer in severe combined immunodeficient (*scid*) mice. DOXIL and DOXIL 1 are not pharmacokinetically equivalent (see ADME summaries), and DOXIL was marginally more effective than DOXIL 1 in the C26 colon carcinoma model and the MC2 and 2105 mammary tumor models (LTI-30-93-13; LTI-30-93-17). Studies using DOXIL 1 are included in the NDA nonclinical efficacy section for informational purposes. Briefly, the activity of DOXIL 1 was compared to that of Adriamycin in the murine C26 colon carcinoma model (LTI-30-91-03; LTI-30-93-11), several murine mammary tumor models (LTI-30-92-03), a murine model of mammary tumor metastasis (LTI-30-93-12), the murine P388 lymphocytic leukemia model (LTI-30-91-02), and a human xenograft study (human non-small cell lung tumor in *scid* mice) (LTI-30-93-16). DOXIL 1 was more effective at slowing or arresting tumor growth, prolonging MST, and preventing the development of spontaneous metastases from intramammary implants of tumors.

3. Summary

The efficacy of DOXIL has been evaluated in a variety of different tumor models, including several human xenograft models (Table 9). In every model examined DOXIL was more effective than the same doses of Adriamycin at inhibiting or halting tumor growth, at effecting cures and/or at prolonging survival times of tumor-bearing animals. Most often, all three endpoints were improved by DOXIL, and in no case was DOXIL less effective than Adriamycin. DOXIL was more active in both solid and dispersed tumors, and was more effective than Adriamycin in preventing spontaneous metastases from intramammary implants of two different mammary tumors in mice. These findings are also supported by studies done with DOXIL 1 in several murine tumor models and a human xenograft model.

In general, the efficacy of Adriamycin in these models was limited by its toxicity at high doses. Typically, DOXIL could be used at a higher dose, offering an increased therapeutic advantage. Pharmacokinetic and tissue distribution studies suggest that the greater persistence, particularly in tumor tissue, achieved with DOXIL compared to conventional doxorubicin also contributes a therapeutic advantage. The efficacy of DOXIL compared to that of conventional liposomal (non-Stealth) doxorubicin HCl indicated that DOXIL was significantly more effective than conventional liposomal doxorubicin HCl, demonstrating the impact of the long-circulating Stealth liposome. Based on the results of these nonclinical studies, DOXIL appears to be an effective agent for the treatment of both solid and dispersed tumors.

TABLE 9
SUMMARY TABLE OF PHARMACOLOGY STUDIES OF DOXIL

Report Number	Tumor Type	Species/ Strain/ Group size	# of Doses	Dose[a] (mg/kg)	Doxil Lot No.	CONCLUSIONS
				DOXIL		
LTI-30-93-22	C26 colon carcinoma (sc)	Mouse/ Balb/c/ 5 or 8	3 or 6	DOXIL: 1.5, 3, 6, 9, 12, 15	[redacted]	• MST and inhibition of tumor greatest in once weekly 6 mg/kg group. • Lower doses or more frequent dose were less effective at inhibiting tumor growth or increasing MST.
LTI-30-94-12	C26 colon carcinoma	Mouse/ Balb/c/ 5	3	DOXIL: 6		• No consistent significant difference among three liposome size variants ([redacted] nm diameter) used to evaluate effect of liposomal diameter on efficacy.
LTI-30-93-17	C26 colon carcinoma (sc)	Mouse/ Balb/c/ 10	3	DOXIL: 3, 6, 9 Dox HCl: 6		• DOXIL and DOXIL 1 showed greater effectiveness than Dox HCl at inhibition of tumor growth.
LTI-30-93-13	MC2/2105 mammary tumor (sc)	Mouse/ C3H/He/ 10	4	DOXIL: 3, 6 Dox HCl: 6		• DOXIL and DOXIL 1 showed greater effectiveness than Dox HCl. • DOXIL was slightly more effective than DOXIL 1 at 6 mg/kg dose level.
LTI-30-93-14	Human ovarian carcinoma xenograft (sc, ip)	Mouse/ Swiss nude *nu/nu/* 3	3	DOXIL: 6, 9 Dox HCl: 6, 9		• DOXIL was more effective than Dox HCl at inhibiting growth of sc tumors and curing tumor (70% vs. 18% cures). • DOXIL inhibited growth of ip tumors.
LTI-30-93-15	Human prostatic carcinoma xenograft (sc)	Mouse/ Swiss nude *nu/nu/* 4	4	DOXIL: 6, 9 Dox HCl: 6, 9		• Greater inhibition of tumor growth by DOXIL compared to equivalent dose of doxorubicin. • Greater increase in MST by 9 mg/kg DOXIL treatment (62.5 d) compared to equal dose of doxorubicin (32.4 d) • Tumor AUC of doxorubicin conc. was 25-fold higher in DOXIL animals.

[a] Dose route was intravenous bolus unless otherwise indicated.

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TABLE 9 (cont.)
SUMMARY TABLE OF PHARMACOLOGY STUDIES OF DOXIL

Report Number	Tumor Type	Specie/ Strain/ Group size	# of Doses	Dose[1] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
DOXIL 1						
LTI-30-91-03	C26 colon carcinoma	Mouse/ Balb/c/ 10	3	DOXIL 1: 6, 9 Dox HCl: 6	[redacted]	• DOXIL 1 significantly better at inhibiting tumor growth than Dox HCl. • MST in both DOXIL 1 dose groups >120 days, compared to 49 days in 6 mg/kg Dox HCl group.
LTI-30-93-11	C26 colon carcinoma (sc)	Mouse/ Balb/c/ 10	3	DOXIL 1: 3, 6, 9 Dox HCl: 6		• DOXIL 1 completely inhibited tumor growth at doses ≥ 6 mg/kg; partial inhibition at 3 mg/kg. • Dox HCl had no significant effect on tumor growth.
LTI-30-92-03	MC2/MC65 mammary tumor (sc)	Mouse/ C3H/He/ 10	3 or 4	DOXIL:1 6, 9 Dox HCl: 6, 9 Lip-Dox: 6, 9		• DOXIL 1 more effective than Dox HCl or non-Stealth liposomal Dox HCl in treating 3 types of implanted tumors. • DOXIL 1 was more effective in treating slower growing tumors compared to faster growing tumors. • DOXIL 1 less effective in treating well established tumors, but still incr. MST. • DOXIL 1 treatment did not induce dox-resistant tumor cells.
LTI-30-93-12	MC19/MC65 mammary tumor metastasis	Mouse/ C3H/He/ 10	4	DOXIL 1: 6, 9 Dox HCl: 6		• DOXIL 1 more effective than Dox HCl in preventing spontaneous development of metastases of both tumor types. • No metastases in DOXIL 1-treated MC19, compared to 67% of Dox HCl group. • 1/20 metastases in DOXIL 1-treated MC65, compared to 25% of Dox HCl group.

[1] Dose route was intravenous bolus unless otherwise indicated.

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TABLE 9 (cont.)
SUMMARY TABLE OF PHARMACOLOGY STUDIES OF DOXIL

Report Number	Tumor Type	Species/ Strain/ Group size	# of Doses	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
				DOXIL 1		
LTI-30-91-02	P388 lymphocytic leukemia (ip)	Mouse/ CDF_1/ 6	1	DOXIL 1: 0.6, 1, 2, 4, 10, 15, 22, 30 Dox HCl: 0.6, 1, 2, 4, 10, 15, 22	[redacted]	• Both DOXIL 1 and Dox HCl were associated with a dose-dependent increase in MST • MSTs of DOXIL 1-treated groups were longer than that of Dox HCl groups at all dose levels ≥ 4 mg/kg.
LTI-30-93-16	Human lung tumor xenograft (sc)	Mouse/ C.B-17 *scid scid/scid/* 3-8	2 or 10-16	DOXIL 1: 0.5, 1, 2, 3, 6, 9 Dox HCl: 3, 6, 9 Lip-Dox: 0.5, 1, 2		• DOXIL 1 caused arrest of tumor growth, but some toxicity at 3 mg/kg after multiple weekly injections. DOXIL 1 at 2 mg/kg was effective and caused no observable toxicity. • Treatment with Dox HCl had only slight effect on tumor growth. • Non-Stealth liposomal Dox HCl was ineffective in arresting tumor growth but did decrease growth rate slightly.

[a] Dose route was intravenous bolus unless otherwise indicated.

Nonclinical Efficacy Study Reports Referenced

DOXIL Pharmacology Studies:

Report Number: LTI-30-93-13
Report Title: Comparison of the Therapeutic Effects of DOXIL Lot 0175 and DOXIL Lot 0177 in the Mouse Mammary Carcinoma Model.
Report Date: [redacted] 19[redacted]
Report Author: [redacted]
Study Site:
DOXIL Lot No.:

Report Number: LTI-30-93-14
Report Title: Therapy of Human Ovarian Carcinoma Xenografts Using Doxorubicin Encapsulated in Sterically Stabilized Liposomes.
Report Date: [redacted] 19[redacted]
Report Author: [redacted]
Study Site:
DOXIL Lot No.:
Placebo Liposome Lot No.: NA

Report Number: LTI-30-93-15
Report Title: Tissue Distributions and Therapeutic Effects of Intravenous Liposomal and Free Doxorubicin Against Human Prostatic Carcinoma Xenografts.
Report Date: [redacted] 19[redacted] (Amended)
Report Author: [redacted]
Study Site:
DOXIL Lot No.:

Report Number: LTI-30-93-17
Report Title: Comparison of the Therapeutic Effects of DOXIL Lot 0175 and DOXIL Lot 0177 in the Mouse Colon Carcinoma C26.
Report Date: [redacted] 19[redacted]
Report Author: [redacted]
Study Site:
DOXIL Lot No.:

Nonclinical Efficacy Study Reports Referenced (continued)

Report Number: LTI-30-93-22
Report Title: Effect of Treatment Frequency on the Toxicity and Efficacy of DOXIL (Stealth liposomal doxorubicin HCl) Injection in the Mouse Colon Carcinoma C26 Tumor Model.
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

Report Number: LTI-30-94-12
Report Title: Effect of Particle Size on the Efficacy of Liposomal Doxorubicin in the Mouse Colon Carcinoma C26 Tumor Model
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

DOXIL 1 Pharmacology Studies:

Report Number: LTI-30-91-02
Report Title: Antitumor Activity of Stealth Liposomal Doxorubicin Hydrochloride Injection in the Murine P388 Lymphocytic Leukemia Model
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

Report Number: LTI-30-91-03
Report Title: Comparison of the Antitumor Activity of Epirubicin HCl and Doxorubicin HCl Encapsulated in Long Circulating Liposomes Incorporating a Polyethylene Glycol-Derivatized Phospholipid Against the Mouse C26 Colon Carcinoma.
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

Nonclinical Efficacy Study Reports Referenced (continued)

Report Number: LTI-30-92-03
Report Title: Doxorubicin Therapy of Primary Mouse Mammary Carcinomas
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

Report Number: LTI-30-93-11
Report Title: Doxorubicin Therapy of Mouse Colon Carcinoma C26
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

Report Number: LTI-30-93-12
Report Title: Doxorubicin Therapy to Prevent the Development of Metastases from Mouse Mammary Carcinomas
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]
Placebo Liposome [redacted]

Report Number: LTI-30-93-16
Report Title: Arrest of Human Lung Tumor Xenograft Growth in Severe Combined Immunodeficient Mice Using Doxorubicin Encapsulated in Sterically Stabilized Liposomes.
Report Date: [redacted]
Report Author: [redacted]
Study Site: [redacted]
DOXIL Lot No.: [redacted]

C. General Pharmacology

As part of the characterization of the overall safety profile of DOXIL, Stealth placebo liposomes were evaluated in two safety pharmacology studies, including a neurobehavioral evaluation in rats and a cardiovascular effect study in dogs.

A summary of these studies is presented in Table 10, located at the end of this section.

1. Neurologic Effects

The potential for liposome-related neurobehavioral changes was evaluated in rats (LTI-30-93-25). The preparations of Stealth placebo liposomes utilized were equivalent in lipid concentration to DOXIL and consisted of an identical lipid formulation, but lacked doxorubicin. The liposomes were dosed undiluted at the maximum recommended dose volume in rats (15 mL/kg), so that the lipid dose delivered was the highest possible. Groups of 10 rats each received single intravenous injections of undiluted Stealth placebo liposomes, a 1:2 dilution of the stock placebo liposome solution with physiological saline or a 1:10 saline dilution. Lipid doses of the placebo liposome groups were equivalent to the lipid doses in DOXIL 30, 15 and 3 mg/kg, respectively. The lipid doses delivered to the high- and mid-dose groups were in excess of the lipid doses that are administered at the LD_{50} of DOXIL in rats (approximately 11 mg/kg). Neurological signs and symptoms were evaluated approximately 5 minutes post-dose using a functional observation battery (FOB), a motor activity assessment and a startle response test. The FOB included indices of autonomic function, muscle tone and equilibrium, sensorimotor reactivity, thermal sensitivity and central nervous system function. No animals died during the course of this study. No clinical signs of toxicity were evident in any treatment group. No evidence of any behavioral or functional effects related to treatment with placebo liposomes was seen. Responses across dose groups were within normal limits and were consistent with behavioral profiles reported for the Sprague-Dawley rat. Based on the results of this study, Stealth placebo liposomes were judged to have no neurobehavioral effects.

2. Cardiovascular Effects

A cardiovascular safety pharmacology study was conducted in dogs to further evaluate the acute response seen during administration of Stealth placebo liposomes after multiple doses in dogs. Before conducting the definitive study, a small pilot study was performed in dogs with implanted radiotelemetry transmitters for monitoring blood pressure and heart rate (LTI-30-94-14). Three beagles (one female and two males) were treated with Stealth placebo liposomes (2.0 mL/kg at a dose rate of 2.0 mL/min) on Days 1, 8 and 11.

On Days 8 and 11, one dog of each sex was pretreated with the antihistamines Benadryl® and Zantac® to evaluate their potential effect on the acute response. White blood cell and platelet counts and histamine levels post-treatment were also monitored. Treatment with placebo liposomes induced transient moderate hypotension in all dogs. There was no evidence of compensatory cardiac acceleration. White blood cell and platelet counts were decreased below normal expected levels at 5 minutes post-treatment, but were fully resolved or resolving by 1 hour post-dose. Histamine levels were variable and not correlated with antihistamine pretreatment. Pretreatment with antihistamines had no definitive effect on any parameter assessed. Because of the equivocal results of this pilot study, a larger GLP study was undertaken.

The definitive cardiovascular safety pharmacology study (LTI-30-94-10) was conducted in dogs to further evaluate the acute response that was induced in two earlier multiple dose studies (LTI-30-94-07; LTI-30-93-04) by repeated dosing with Stealth placebo liposomes. Nine male beagle dogs were implanted with radiotelemetry transmitters to monitor systolic, diastolic and mean arterial blood pressure, heart rate and EKG, and equipped with ambulatory infusion pumps. Initially, all dogs received saline to establish baseline values. Secondly, all dogs received 1.0 mL/kg DOXIL placebo liposomes at a dose rate of 1.0 mL/min. One week later, groups of three dogs each were treated with 1.0 mL/kg of placebo liposomes at dose rates of 0.25, 0.5 or 1.0 mL/min. Finally, four dogs were pretreated with Benadryl and Zantac 30 minutes prior to treatment with placebo liposomes (1.0 mL/kg, 1.0 mL/min) and the remaining dogs were pretreated with saline.

Infusion of placebo liposomes was characterized by a statistically and physiologically significant decrease in blood pressure (19 to 70%) that began immediately after the start of treatment and resolved quickly after the end of treatment. No consistent compensatory acceleration in heart rate was observed. The extent of the blood pressure decrease was greater after the second treatment with placebo liposomes than after the first and was not affected by dose rate, although the duration of the effect was inversely related to rate. Pretreatment with antihistamines decreased, but did not eliminate, the drop in blood pressure. Antihistamine pretreatment had no effect on the incidence or severity of clinical signs of toxicity, including hypoactivity, flushing, diarrhea and emesis. The biological significance of this finding is not readily apparent, but may be related to histamine release in response to the infusion of relatively large amounts of lipid.

TABLE 10
SUMMARY TABLE OF GENERAL PHARMACOLOGY Studies

Report Number	Study Type	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
					Placebo Liposomes
LTI-30-93-25	Safety Pharmacol. (Neuro-behavioral)	Rat/ Sprague Dawley/ 10M	Placebo Liposomes: 1.5, 7.5, 15 mL/kg[b]	[redacted]	• Stealth liposomes did not induce any adverse neurobehavioral effects or evidence of neurotoxicity.
LTI-30-94-14	Safety Pharmacol. Pilot (Cardio-vascular)	Dog/ Beagle/ 2M, 1F	Placebo Liposomes: 2 mL/kg[b]		• Stealth placebo liposomes induced moderate hypotension that resolved upon cessation of dosing. No compensatory cardiac acceleration was observed. • White blood cell and platelet counts decreased below normal range at 5 min post-dose, and rebounded within 1 hr, suggesting sequestration of cells may be cause. • Histamine levels were not related to liposomes or antihistamine pretreatment. • Antihistamine pretreatment had no consistent effect.
LTI-30-94-10	Safety Pharmacol. (Cardio-vascular)	Dog/ Beagle/ 9M	Placebo Liposomes: 1 mL/kg[b]		• Physiologically significant decrease in blood pressure (19-70%) immediately after start of dosing that resolved rapidly after end of dosing. No consistent compensatory cardiac acceleration. • No effect of dose rate (0.25-1.0 mL/min) on extent of hypotension, but duration inversely related. • Antihistamine pretreatment (Benadryl and Zantac) reduced extent of hypotensive response but had minimal effect on clinical signs (hypoactivity, flushing of gums, emesis, diarrhea).

[a] Dose route was intravenous bolus unless otherwise indicated.
[b] Dose volume of Stealth Placebo liposomes. Equivalent in lipid concentration to undiluted DOXIL.

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General Pharmacology Reports Referenced

General Pharmacology Studies:

Report Number: LTI-30-93-25[redacted]
Report Title: Neurobehavioral Evaluation of DOXIL Placebo Liposomes in Sprague Dawley Rats.
Report Date: [redacted] 19[redacted]
Study Director: [redacted]
Study Site: [redacted]
Placebo Liposom[redacted]

Report Number: LTI-30-94-14[redacted]
Report Title: Pilot Cardiovascular Evaluation Study of DOXIL Placebo Liposomes in Dogs.
Report Date: [redacted] 19[redacted]
Study Director: [redacted]
Study Site: [redacted]
Placebo Liposom[redacted]

Report Number: LTI-30-94-10 ([redacted]
Report Title: Cardiovascular Evaluation of DOXIL Placebo Liposomes in Beagle Dogs Receiving Multiple Intravenous Doses.
Report Date: [redacted] 19[redacted]
Study Director: [redacted]
Study Site: [redacted]
Placebo Liposome[redacted]