E. Nonclinical Toxicology

1. Introduction

DOXIL was developed by LTI to decrease the toxicity and increase the efficacy of doxorubicin. Doxorubicin is an anthracycline antibiotic widely used to treat a wide spectrum of solid tumors, lymphomas, and leukemias.[93,94] The acute toxicity of doxorubicin HCl is characterized by dose-limiting myelosuppression, stomatitis, alopecia, nausea and vomiting. Significant risk of cardiotoxicity is incurred following cumulative doses. Doxorubicin is both carcinogenic and mutagenic.[95,96] Phlebitis is a common observation after long-term intravenous infusion,[97] and extravasation of doxorubicin causes severe necrosis of skin and adjacent tissues.[95]

In order to characterize the safety of DOXIL, a series of nonclinical safety studies have been conducted, including acute toxicity and multiple dose toxicity studies in three species. Intravenous and subcutaneous local tolerance studies, in vitro hemolytic potential, and blood compatibility studies in human blood, plasma, and serum have also been completed. Developmental toxicity studies have been completed in rats and rabbits. In addition, a panel of mutagenicity studies and two safety pharmacology studies have been conducted with placebo Stealth liposomes, are identical in lipid composition to liposomes of DOXIL but lacking doxorubicin. All definitive safety studies were conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations.

Early studies in mice, rats and dogs 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 buffered formulations of DOXIL that differed only in the formulation buffer (DOXIL 2 and DOXIL 3, buffered with tromethamine and histidine, respectively). Pharmacokinetic studies in rats have shown that DOXIL 2 and DOXIL 3 are equivalent; these two equivalent formulations will be referred to as DOXIL. In contrast, the pharmacokinetics of DOXIL 1 and DOXIL are not equivalent in rats or dogs. The studies conducted using DOXIL 1 provide supportive information and are included in the toxicology section of this NDA.

Conventionally formulated doxorubicin HCl (Adriamycin RDF™, Adria Laboratories) was administered for comparative purposes, and placebo liposomes were included as controls. Placebo liposomes were typically dosed at a lipid dose equivalent to that of the high-dosage DOXIL treatment group.

2. Acute Toxicity Studies

Single dose safety studies have been conducted in mice, rats and dogs with DOXIL and in mice and dogs with DOXIL 1. All definitive studies were conducted in compliance with GLP regulations. Pilot studies and studies conducted with DOXIL 1, including a GLP acute mouse study, provided supportive information. A summary of these studies is presented in Table 20, located at the end of this section.

Prior to conducting the definitive acute toxicity studies, pilot studies conducted in mice and rats to compare the acute toxicity of research lots of DOXIL, DOXIL and DOXIL 1 demonstrated no significant differences in their acute toxicity (LTI-30-92-05; LTI-30-92-09). In the GLP rodent acute toxicity study with DOXIL, male Sprague Dawley rats received single intravenous injections of DOXIL 4, 8 or 12 mg/kg and were observed for 28 days post-dosing (LTI-30-92-11). Clinical pathology determinations were undertaken periodically during the recovery period. No necropsies were performed. Approximately 30% treatment-related mortality occurred in the high-dose DOXIL group. Clinical signs of toxicity were dose-related and included: tail and footpad lesions, swelling and inflammation of the penis and scrotum, rough haircoat, alopecia, hypoactivity, hunched posture, and respiratory distress. The primary effect of DOXIL treatment in surviving animals was significant, but reversible, myelotoxicity. Clinical chemistry changes were limited to moderate and reversible dose-dependent increases in blood urea nitrogen (BUN), cholesterol, glucose and chloride and moderate, reversible decreases in alkaline phosphatase, albumin and bilirubin. For comparative purposes, DOXIL 1 was also included in this study; no differences in the acute toxicity of the formulations were observed.

Acute toxicity was evaluated in male beagles following bolus intravenous injections of DOXIL 1.5, 1.8 or 2.1 mg/kg (LTI-30-92-13). Complete physical and clinical evaluations were undertaken during the 4-week recovery period. No necropsies were performed. Two animals were sacrificed prior to the end of the recovery period due to severe cutaneous lesions present at the injection site, possibly due to extravasation of drug. Inflammatory injection site lesions are a known consequence of doxorubicin extravasation. Several dogs developed mild to moderate treatment-related cutaneous lesions on the feet, legs and, rarely, on the abdomen, head and mouth, which had healed by the end of the recovery phase. Alopecia persisted at lesion sites at the end of recovery. Other DOXIL-related adverse effects included mild to moderate myelotoxicity evidenced by decreases in lymphocyte count and red blood cell parameters at most dose levels. Gastrointestinal toxicity, characterized by diarrhea, hemorrhage and histological lesions of the mucosa, had been dose-limiting in several previous studies in dogs. In this study,

however, gastrointestinal toxicity was markedly reduced by decreasing the rate of infusion of the test material from a 60 to 90 second bolus to injection at a rate of 2 mL/min. For comparative purposes, DOXIL 1 was also included in this study; no differences in the acute toxicity of the formulations were observed.

Additional studies of DOXIL 1 acute toxicity in mice and dogs were supportive of the findings with DOXIL in rats and dogs. A GLP acute toxicity study in mice compared the toxicity of DOXIL 1 (6, 12, 18, 24 and 36 mg/kg) and Adriamycin (12 and 24 mg/kg) (LTI-30-92-12). Based on comparative LD_{50} values, Adriamycin is from 2.4- to 3.9-fold more toxic than DOXIL 1 in mice. Other adverse effects, including clinical pathology changes and histopathology findings, were similar in nature, incidence and severity in animals that received equivalent doses of DOXIL 1 or Adriamycin. In a series of non-GLP studies conducted in mice during formulation development to compare the mortality of DOXIL 1 produced by different processes (LTI-30-92-TM-01; LTI-30-92-TM-02; LTI-30-92-02; LTI-30-92-10), DOXIL 1 was less lethal than doxorubicin HCl. There was no effect due to differences between production processes or drug-to-lipid ratio on the acute toxicity of DOXIL 1 in mice. The acute toxicity of DOXIL 1 in dogs (LTI-30-91-05; LTI-30-92-04) yielded similar findings to those reported in the acute dog study with DOXIL. Treatment-related clinical signs of toxicity, including body weight loss, pyrexia and anorexia and histopathological changes (bone marrow hypocellularity, gastrointestinal mucosal and crypt necrosis and cell loss, and thymic and testicular atrophy), were not qualitatively different in animals that received DOXIL 1 or Adriamycin. However, myelotoxicity, as assessed by decreased white blood cell and platelet counts and changes in bone marrow cellularity, was markedly less severe in DOXIL 1-treated animals than in the Adriamycin groups. Reversible gastrointestinal toxicity (gastrointestinal hemorrhaging, diarrhea and histopathological lesions) was equivocally more severe in the DOXIL 1-treated animals, with both onset and recovery delayed by 2 to 3 days. Later studies demonstrated that the gastrointestinal toxicity, which is not a significant toxicity of Adriamycin in humans, could be decreased by reducing the rate of drug administration.

3. Repeat Dose Studies

Repeat dose GLP toxicity studies were conducted in rats and dogs with DOXIL. A GLP repeat dose toxicity study of DOXIL 1 in rats and repeat dose pilot studies with DOXIL 1 in rats and dogs were also conducted. These reports are included in the toxicology section of this NDA for informational purposes. Table 21, located at the end of this section, presents a summary of the repeat dose toxicology studies of DOXIL

In the definitive repeat dose study in rats, male and female Sprague Dawley rats received intravenous injections of DOXIL 0.25, 1.0 or 1.5 mg/kg, Adriamycin 1.0 mg/kg, or Stealth placebo liposomes at a total lipid dose equivalent to DOXIL 1.5 mg/kg (LTI-30-93-08). Treatment was every third day for a total of 13 doses, with the last dose given on Day 37. An additional group of animals received saline on the same treatment schedule.

No adverse effects were associated with the administration of placebo liposomes. Treatment of the DOXIL 1.5 mg/kg group was halted after eight doses (Day 27/28) due to the effect of test material-related dermatologic toxicity on their general health. Adverse effects seen in this group, including clinical signs of toxicity, dermatologic toxicity, body weight and food consumption changes, clinical pathology changes and gross necropsy findings were similar, but more severe than those observed in the DOXIL 1.0 mg/kg group. All surviving animals in this dose group were fully recovered at the recovery necropsy (Day 67/68). With the exception of two males in the Adriamycin group that died just prior to the recovery necropsy on Day 67/68, all other animals survived to the end of the study. With two exceptions, treatment-related clinical pathology and histopathological lesions were qualitatively similar in animals that received DOXIL or Adriamycin; however, the incidence and severity of the changes tended to be higher for Adriamycin-treated animals at equivalent doses of the two drugs.

Dermatologic toxicity was seen only in the DOXIL-treated groups and was characterized as reversible cutaneous lesions, particularly on the feet and legs, of rats given ≥ DOXIL 1.0 mg /kg. Microscopically, the lesions were distinguished by ulceration and inflammation of the epidermis and alopecia. All lesions were fully healed by the end of the recovery period, although alopecia generally persisted at the lesion site.

In contrast, chronic progressive nephropathy was seen only in Adriamycin-treated rats, in 55% and 88% of the animals at the first and second necropsies, respectively. No clinical or histological evidence of renal toxicity was observed in DOXIL-treated animals.

At equivalent doses of DOXIL and Adriamycin, myelotoxicity, as judged by bone marrow cellularity changes, was minimally less severe in DOXIL-treated animals (slight to minimal hypocellularity) than in animals given Adriamycin (slight to moderate severity). Hematologic effects observed (leukopenia and lower erthyron mass) were also generally less severe in DOXIL-treated animals compared to Adriamycin-treated rats. The leukopenia, which was primarily lymphopenia, was fully reversed in both groups. The lower erythron mass was partially reversed in animals given Adriamycin and fully reversed in DOXIL-treated animals.

The cardiotoxicity of DOXIL and Adriamycin also differed. Moderately severe progressive myocyte vacuolation and degeneration were seen in hearts of five of eight of the Adriamycin-treated rats at the second necropsy and in both Adriamycin-treated males that died just prior to that necropsy, compared to minimal changes in just two of nine rats in the DOXIL 1.0 mg/kg group, three of ten in the DOXIL 0.25 mg/kg group and two of ten in the placebo liposome group. Thus, based on clinical pathology and histopathology, DOXIL was less cardiotoxic, nephrotoxic, and marginally less myelotoxic but more dermotoxic than Adriamycin in rats.

An identical repeat dose study conducted with DOXIL 1 in rats was supportive of these conclusions (LTI-30-92-17). DOXIL 1 was less cardiotoxic, myelotoxic, and nephrotoxic than an equivalent or higher dose of Adriamycin, and similar reversible dermal lesions were observed.

These two studies permit the comparison of the effects of multiple administrations of DOXIL (LTI-30-93-08) and DOXIL 1 (LTI-30-92-17) in rats. While qualitatively similar, the adverse effects reported were more severe in animals given DOXIL than in those that received the same dose of the earlier formulation, DOXIL 1. The apparent greater severity of adverse effects in DOXIL-treated rats is believed to be related to differences in the pharmacokinetics of DOXIL and DOXIL 1. Plasma levels of doxorubicin are higher, clearance is lower, plasma half-lives and MRT are longer after treatment with DOXIL than after treatment with DOXIL 1 (see LTI-30-93-06). The longer secondary plasma half-life of DOXIL compared to DOXIL 1 would result in greater plasma and tissue accumulation of doxorubicin when both are dosed on the same schedule. Moreover, based on increases in AUC, the total potential doxorubicin exposure of animals that received DOXIL ranged from 71 to 96% higher than in animals given an equivalent dose of DOXIL 1. Based on comparative AUCs, a dose of DOXIL 1 1.7 to 2.0 mg/kg was equivalent in total exposure to DOXIL 1.0 mg/kg. Other studies have shown that DOXIL was more efficacious at equivalent doses than DOXIL 1, probably because of its significantly longer plasma MRT and increased deposition in tumors.

In a four-week repeat dose safety study of DOXIL in dogs, male beagles received weekly intravenous injections of DOXIL 1.0 mg/kg, Adriamycin 1.0 mg/kg, or DOXIL Stealth placebo liposomes (LTI-30-93-04). For comparative purposes, an additional group of animals was treated with DOXIL 1 1.0 mg/kg. Dogs receiving placebo liposomes exhibited an acute clinical response characterized by transient episodes of depressed activity, prostration, excessive salivation, emesis, defecation, and flushing, which resolved rapidly upon cessation of infusion. The incidence and severity of the response, which was not life-threatening, diminished at the third and fourth doses. A similar

response, though of lesser incidence and severity, was also observed in DOXIL-treated dogs. Decreased food consumption and body weight loss, diarrhea, and inanition were also observed in the DOXIL, DOXIL 1, and Adriamycin groups during the treatment phase.

Dogs that received DOXIL or DOXIL 1 developed similar severe inflammatory lesions of the feet, legs, elbow, head, muzzle, and neck. Microscopically the lesions were characterized by chronic active inflammation, parakeratosis, and/or hair follicle degeneration. Two dogs were sacrificed due to severity of the dermal lesions. Dermal lesions in surviving dogs healed within two weeks of the cessation of treatment, although alopecia persisted. Similar lesions were not seen in animals given Adriamycin. An additional DOXIL 1-treated dog died during the recovery period; no cause of death could be determined. One Adriamycin-treated animal died during the recovery period, with severe thrombocytopenia and leukopenia. All other animals survived until the end of the study.

Mild, reversible reductions in red blood cell count, white blood cell count, and platelet count were seen in surviving animals that received DOXIL, DOXIL 1, or Adriamycin. The hematologic changes tended to have a slightly later onset and to be minimally less severe in the DOXIL and DOXIL 1 treatment groups. The incidence of bone marrow hypocellularity was also decreased in the DOXIL group. Minimal to mild histopathologic alterations of the gastrointestinal tract (congestion and necrosis/cellular debris of the crypts) and lymphocyte depletion in the spleen and lymph nodes were observed in all treated animals. The severity and incidence of these alterations were similar in the DOXIL, DOXIL 1, and Adriamycin-treated animals. All changes fully resolved during the recovery phase.

The comparative toxicity and relative cardiotoxicity of DOXIL and Adriamycin were evaluated in a 10-week repeat dose study (LTI-30-94-07). Male and female beagle dogs (n = 6 per sex) received intravenous injections of DOXIL 0.25, 0.75 or 1.0 mg/kg or Adriamycin 1.0 mg/kg once every three weeks for a total of ten treatments. Additional groups of dogs were treated with saline or Stealth placebo liposomes on the same schedule. Placebo liposomes were administered at the same lipid dose as the high-dose DOXIL treatment group.

There were no treatment-related animal deaths or effects on ophthalmic examinations, ECG results, clinical chemistry, urinalysis, body temperature or heart and respiration rates. A trend towards decreased body weight, body weight gain, and food consumption was observed in the higher dose DOXIL groups. During and after the second dose, dogs

administered placebo liposomes exhibited an acute response characterized by pale mucous membranes, salivation, emesis and periods of hyperactivity followed by hypoactivity and lethargy. The response was diminished, but not eliminated, by a reduction in the dose rate from 2.0 to 0.5 mL/min. Observations in the Adriamycin group and the DOXIL groups included occasional incidence of pale mucous membranes but were generally much less common and severe. The toxicological significance of these observations is not clear; however, the response was not life-threatening, and dogs recovered quickly upon cessation of dosing. Reversible dermal ulcers and persistent alopecia were observed on the feet and limbs in dogs in the DOXIL 0.75 and 1.0 mg/kg groups, but not in the Adriamycin group. The ulcers were characterized microscopically by focal parakeratosis, acantholysis, and chronic active inflammation affecting the skin and underlying dermis.

Microscopic evaluation of the heart revealed vacuolar changes in the myocardium of the hearts of all Adriamycin-treated dogs. Slight to moderate numbers of degenerated, vacuolated myocardial fibers and distinct myocytolysis that increased in severity during the 4-week post-dose recovery period. No DOXIL-treated animals showed any evidence of cardiotoxicity. Mild reversible hypocellularity of the bone marrow, suggestive of treatment-related myelotoxicity, was observed in Adriamycin-treated dogs. Mildly decreased, reversible white blood cell counts, reflected primarily as decreased numbers of neutrophils, were observed in dogs treated with DOXIL or Adriamycin. The results of this study suggest DOXIL was not cardiotoxic and was minimally less myelotoxic than Adriamycin in dogs.

4. Special Toxicity Studies

Two single dose local tolerance studies were conducted to examine the potential of DOXIL to cause injury if accidentally extravasated. Rabbits received single intravenous or subcutaneous injections of 0.1 or 1.0 mL of undiluted DOXIL 2.0 mg/mL, Adriamycin 2.0 mg/mL or Stealth placebo liposomes (LTI-30-93-03 and LTI-30-93-02). Histopathological evaluation of intravenous injection sites revealed that DOXIL, Adriamycin, and placebo liposomes were well tolerated with no gross or microscopic evidence of irritation. In contrast, histopathological evaluation of subcutaneous injection sites showed reversible mild to moderate dose-related inflammation at DOXIL injection sites, compared to moderate to severe inflammation and necrosis at Adriamycin injection sites that showed no signs of resolution during a 4-week recovery period. Subcutaneously injected placebo liposomes were not associated with irritative dermal effects.

In vitro studies were conducted to assess the hemolytic potential of DOXIL and DOXIL placebo liposomes in human blood, as well their compatibility with human serum and plasma (LTI-30-92-15). Neither DOXIL 1.0 mg/mL nor empty Stealth liposomes (1.0 mg/mL; equivalent lipid concentration to DOXIL) induced hemolysis of human erthythrocytes in vitro, nor did either cause coagulation or precipitation of human serum or plasma. A pilot study conducted with DOXIL 1 was supportive of these findings; neither DOXIL 1 (doses up to 0.6 mg/mL) nor DOXIL 1 placebo liposomes caused hemolysis of human erythrocytes in vitro (LTI-30-91-01). An additional hemolytic potential study was conducted with DOXIL liposomes formulated to contain higher amounts of lysophosphatidylcholine (LPC) to determine whether increased amounts of LPC would affect hemolysis of rat blood (LTI-30-94-08). LPC is a degradation product of the phosphatidylcholine component of the liposomes. At low concentrations, LPC binds to and stabilizes cell membranes. At higher concentrations LPC micelles may form in the membrane, resulting in membrane lysis. DOXIL formulations prepared with 0 mg/mL, 0.5 mg/mL, or 0.88 mg/mL LPC caused no hemolysis of rat blood cells.

The acute toxicity of a micellar solution of methoxypolyethyleneglycol- distearoyl-phospatidylethanolamine (MPEG-DSPE) (20 mg/mL) was evaluated in ICR mice to determine the potential of the lipid component of DOXIL to cause toxicity (LTI-30-93-29). Doses of MPEG-DSPE nearly 15-fold higher than that delivered at a toxic dose level of DOXIL were not associated with clinical signs of toxicity.

A summary of these studies is provided in Table 22.

5. Reproductive Studies

The potential developmental toxicity of DOXIL has been evaluated in a definitive GLP study in rats and in a GLP pilot dose-ranging study in rabbits. A summary of these studies is located in Table 23.

In the definitive rat Segment II study, pregnant Sprague Dawley rats received intravenous bolus injections of DOXIL 0.1, 0.5 or 1.0 mg/kg on gestation days (gd) 6, 9, 12 and 15 or Stealth placebo liposomes or saline on the same treatment schedule (LTI-30-94-13). This treatment schedule was utilized to avoid significant accumulation of the long-circulating DOXIL. For comparative purposes, an additional group of pregnant females received Adriamycin 0.2 or 0.4 mg/kg daily between gd 6 and 15.

All rats survived the duration of the study, and no treatment-related clinical signs of toxicity were recorded. No maternal or fetal toxicity was observed in the placebo liposome and DOXIL 0.1 mg/kg treatment groups. Equivalent maternal toxicity,

manifested as reduction in body weight gain and feed consumption, occurred in the DOXIL 0.5 and 1.0 mg/kg groups and in the Adriamycin 0.2 and 0.4 mg/kg groups during the treatment period. Reductions in fetal body weight, increases in fetal resorptions, and retarded ossification of caudal vertebrae and xiphoid centers were seen in the fetuses the DOXIL 1.0 mg/kg treatment group. Delays in ossification are known to be closely related to decreased fetal body weights and fetal growth rate and may be related to maternal toxicity. No evidence of fetal toxicity was noted in either of the Adriamycin treatment groups, although higher dose levels are reported to induce decreased fetal body weights, increased rates of fetal resorptions and soft tissue alterations, including tracheo-esophageal fistulae, small urinary bladders and gut atresia.[98] Higher dose levels of Adriamycin were not utilized in this study because of its significant maternal toxicity. The greater fetal toxicity of DOXIL may be due to the higher daily doses that could be used without inducing comparable maternal toxicity.

The embryotoxicity of DOXIL was confirmed in a GLP pilot study in which pregnant New Zealand White rabbits received intravenous injections of DOXIL 0.5, 1.5 or 2.5 mg/kg on gd 6, 9, 12, 15 and 18 (LTI-30-94-06). An additional group of five females was treated with saline to serve as a negative control.

Decreased body weight gains, followed by body weight losses, were seen in all three DOXIL treatment groups. Absolute and relative food consumption were similarly decreased in these treatment groups. Three females in the high-dose group and one in the mid-dose group died late in gestation (gd 19 to 23). The uterine contents of these four females consisted entirely of resorbed conceptuses. All five females in the low-dose group aborted prior to the end of the study (on gd 21, 23, 24, 25 and 27). The uterine contents of one consisted of three conceptuses that appeared normal, four late resorptions and five early resorptions; the uterine contents of the other four females consisted entirely of resorptions. Surviving females in the mid- and high-dose groups (four and two females, respectively) were necropsied on gd 29. All females had 100% resorptions. No live fetuses or late resorptions were collected, and no examination for gross external alterations could be undertaken. Based on the results of this study, DOXIL is both embryotoxic and an abortifacient in rabbits.

6. Mutagenicity and Genotoxicity Studies

Doxorubicin, the active component of DOXIL, is both mutagenic and carcinogenic, so conducting mutagenicity studies with DOXIL was not deemed necessary. However, four studies were run with Stealth placebo liposomes to confirm their lack of mutagenicity and genotoxicity; a summary of these studies is located in Table 24. Doses of placebo liposomes used in these studies were based on total phosphorus content as a measure of

lipid concentration. Undiluted, the preparations of Stealth placebo liposomes utilized were equivalent in lipid concentration to DOXIL. The high dose of placebo liposomes used in each study was the largest technically possible.

Placebo liposomes did not induce reverse mutations in a standard Ames test in either the presence or absence of an exogenous S9 metabolic activation system (LTI-30-93-19). The principle of the Ames test is the detection of xenobiotic-induced reverse mutations at the histidine locus of *Salmonella typhimurium*, so it could not be conducted in the presence of histidine. DOXIL is formulated with a histidine buffer, and, therefore, an alternate formulation of placebo liposomes buffered with tromethamine was prepared for use in the Ames test. No evidence of the induction of mutations in either the presence or absence of an exogenous mammalian liver S9 metabolic activation system was seen in two independent replicates of the Ames test, using the highest concentration of liposomes technically feasible. This finding was confirmed in two additional in vitro assays. Stealth placebo liposomes formulated with the standard histidine buffer did not induce forward mutations at the thymidine kinase locus in mouse lymphoma L5178Y cells in the presence or absence of S9 (LTI-30-93-20), nor did they induce chromosomal aberrations or polyploidy in Chinese hamster ovary cells with or without exogenous metabolic activation (LTI-30-93-21).

Stealth placebo liposomes were also evaluated in mice for their potential to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) (LTI-30-93-18). Male and female CD-1 mice (n = 15 per sex) received a single intravenous injection of undiluted Stealth placebo liposomes, a 1:2 dilution or a 1:4 dilution of placebo liposomes in saline. The doses of liposomes delivered corresponded in lipid dose to DOXIL doses of 10, 20 and 40 mg/kg. The high dose utilized is approximately equivalent to the LD_{50} of DOXIL in mice. There was no increase in micronucleated PCEs over levels observed in the vehicle control in either sex at any time point. Placebo liposomes were considered negative in the mouse bone marrow micronucleus test.

7. Summary

The acute toxicity of DOXIL following single administration, as characterized in the previously discussed studies, was similar for mice, rats, and dogs. However, dogs were the more sensitive species. There was a dose-related mortality in rats administered DOXIL intravenously. Other signs of toxicity included reversible myelotoxicity and marginal clinical chemistry changes. Dogs administered DOXIL 1.5, 1.8, or 2.1 mg/kg exhibited reversible myelotoxicity, gastrointestinal toxicity, cutaneous lesions and persistent alopecia. Two dogs died as a result of severe injection site reactions to extravasated doxorubicin. Mice administered DOXIL or Adriamycin at 12 and 24 mg/kg

showed similar signs of toxicity (myelotoxicity) at equivalent doses. However, based on comparative LD_{50} values, doxorubicin HCl is from 2.4 to 3.9-fold more toxic than DOXIL 1 in mice.

The toxicity profile of DOXIL following repeated administration was similar for rats and dogs. Multiple intravenous administration of DOXIL 0.25, 1.0 or 1.5 mg/kg to rats was associated with treatment-related dermatologic toxicity, body weight and food consumption changes, alopecia, myelotoxicity (bone marrow cellularity changes), hematologic effects (leukopenia and lower erthyron mass), and minimal pathologic signs of cardiac toxicity. Repeat intravenous administrations of DOXIL 1.0 mg/kg to dogs was associated with dermatologic toxicity of a more severe nature: mild, reversible reductions in red blood cell count, white blood cell count and platelet counts; bone marrow hypocellularity; minimal to mild histopathologic alterations of the gastrointestinal tract; lymphocyte depletion in the spleen and lymph nodes; and mild transient clinical signs associated with the period of infusion. Dermal ulcers, alopecia, mildly decreased white blood cell counts, and reversible bone marrow hypocellularity were also observed in dogs administered multiple intravenous injections of DOXIL 0.25, 0.75 or 1.0 mg/kg. However, no microscopic vacuolar changes in the myocardium of the hearts were observed in these dogs.

DOXIL (0.2 or 2.0 mg) was well tolerated at the site of intravenous injections and caused only moderate dose-related inflammation at subcutaneous sites of injection. DOXIL and empty Stealth liposomes (1.0 mg/mL) tested negative in assays for hemolytic activity. DOXIL 0.1, 0.5, or 1.0 mg/kg caused substantial maternal and fetal toxicity in a rat Segment II reproduction study. DOXIL was also embryotoxic at doses of 0, 5, 1.5, or 2.5 mg/kg in a pilot study in rabbits. The mutagenicity/genotoxicity of DOXIL is presumed due to the known mutagenic/genotoxic effect of doxorubicin; empty liposomes were not mutagenic or genotoxic.

TABLE 20
SUMMARY TABLE OF ACUTE TOXICOLOGY STUDIES OF DOXIL

Report Number	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
			[redacted]	**DOXIL**
LTI-30-92-05	Mouse/ ICR/ 10M	DOXIL: 28, 31		• Toxicity of DOXIL 1 and DOXIL 2 research formulations prepared with 250 mM ammonium sulfate were comparable, based on lethality and body weight loss.
LTI-30-92-09	Rat/ Sprague Dawley/ 10M	DOXIL: 5, 10, 12, 15, 18, 20, 25		• No difference in lethality of DOXIL 1, a research formulation, and DOXIL. • Delayed body weight gain post-dose in DOXIL-treated animals.
LTI-30-92-11	Rat/ Sprague Dawley/ 10M	DOXIL: 4, 8, 12		• Significant, but reversible, myelotoxicity, based on decreased RBC, WBC, Hgb and Hct. • No significant difference between DOXIL 1 and DOXIL. • Dose-related clinical signs of toxicity.
LTI-30-92-13	Dog/ Beagle/ 3	DOXIL: 1.5, 1.8, 2.0		• DOXIL-related cutaneous lesions noted on digits. • Mild to moderate reversible myelotoxicity. • GI toxicity lessened by decreasing dose rate to 2 mL/min. • No significant differences between DOXIL and DOXIL 1.
				DOXIL 1
LTI-30-TM-92-01	Mouse/ ICR/ 10M	DOXIL 1: 26, 28 Dox HCl: 20		• Comparison of 3 different production processes for DOXIL 1. • No difference in mortality among processes. • All DOXIL 1 less lethal than Dox HCl.
LTI-30-TM-92-02	Mouse/ ICR/ 10M	DOXIL 1: 26, 28		• Comparison of 3 different production processes for DOXIL 1. • Mortality correlated with % of free Dox HCl.

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

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TABLE 20 (CONT.)
SUMMARY TABLE OF ACUTE TOXICOLOGY STUDIES OF DOXIL

Report Number	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
				DOXIL 1
LTI-30-92-02	Mouse/ ICR/ 7-9M	DOXIL 1: 27.5, 29.9, 30.4, 33.2 Dox HCl: 20	[redacted]	• Comparison of 2 different production processes for DOXIL 1. • Dose-related increase in mortality with DOXIL 1. • Mortality comparable between DOXIL 1 lots. • Decreased mortality with DOXIL 1 compared to Dox HCl.
LTI-30-92-10	Mouse/ ICR/ 10M	DOXIL 1: 26, 30 Dox HCl: 20		• Alterations in the drug-to-lipid ratio had no effect on mortality • Lethality was decreased with DOXIL 1 compared to Dox HCl.
LTI-30-92-12	Mouse/ CD-1/ 14	DOXIL 1: 6, 12, 18, 24, 36 Dox HCl: 12, 24		• DOXIL 1 less toxic than Dox HCl based on mortality. • Clin path and histopath changes generally similar in nature, incidence and severity in DOXIL 1 and Dox HCl groups.
LTI-30-91-05	Dog/ Beagle/ 2	DOXIL 1: 1.5, 1.88, 2.25 Dox HCl: 1.5, 2.25		• 3/4 died in 2.25 mg/kg DOXIL 1 group; 1/4 at same dose of Dox HCl; 1/4 in 1.8 mg/kg DOXIL 1 group. All deaths due to GI toxicity. • Myelotoxicity less severe in DOXIL 1 groups at comparable doses. • Other adverse effects of DOXIL 1 and Dox HCl equivalent in nature, incidence and severity.
LTI-30-92-04	Dog/ Beagle/ 4-6M	DOXIL 1: 1.7 Dox HCl: 1.7		• No animal deaths. • Myelotoxicity less severe in DOXIL 1 animals, based on hematology and bone marrow cellularity changes. • Reversible GI toxicity more severe after DOXIL 1, possibly related to rate of drug administration.

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

TABLE 21
SUMMARY TABLE OF REPEAT DOSE TOXICOLOGY STUDIES OF DOXIL

Report Number	Study Type	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
Repeat Dose					
LTI-30-93-08	Repeat Dose 13 dose q3d	Rat/ Crl:CD® BR VAF/Plus/ 15M, 15F	DOXIL: 0.25, 1, 1.5 Dox HCl: 1	[redacted]	• Dosing halted in 1.5 mg/kg DOXIL group due to effect of dermal lesions on general health. Death of 1/10 males related to this toxicity. • DOXIL induced dermal lesions at ≥ 1 mg/kg; readily reversible upon cessation of treatment. • DOXIL less cardiotoxic, hematotoxic and nephrotoxic than equivalent dose of Dox HCl. • Other adverse effects similar in nature, incidence and severity in DOXIL and Dox HCl groups. • No effect of placebo liposomes.
LTI-30-93-04	Repeat Dose 4 dose q7d	Dog/ Beagle/ 6M	DOXIL: 1 Dox HCl: 1		• 1 DOXIL and 1 Dox HCl animal died during treatment. • Myelotoxicity milder in DOXIL groups, with later onset, less severe changes and quicker recovery. • DOXIL induced severe inflammatory lesions of feet and legs; readily reversed upon cessation of dosing. • Other adverse effects comparable in DOXIL and Dox HCl dogs. • Dogs given placebo liposomes exhibited transient hypoactivity, flushing, emesis, prostration during 2nd dose. Reduced in incidence and severity at 3rd and 4th doses. • No differences between DOXIL and DOXIL 1

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

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TABLE 21 (cont.)
SUMMARY TABLE OF REPEAT DOSE TOXICOLOGY STUDIES OF DOXIL

Report Number	Study Type	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
LTI-30-94-07	Repeat Dose 10 dose q21d	Dog/ Beagle/ 6M, 6F	DOXIL: 0.25, 0.75, 1 Dox HCl: 1	[redacted]	• Mild to moderate cardiomyopathy in all dogs treated with non-liposomal Dox HCl that worsened during recovery period. • No evidence of cardiotoxicity in any DOXIL-treated dog at interim or final necropsy. • Bone marrow hypocellularity in ribs and femur of Dox HCl-treated animals, with mild decreases in WBC count. Both resolved in 4-wk recovery period. WBC depression only in DOXIL groups, also resolved in recovery period. • Alopecia and mild dermal ulcers seen in 0.75 and 1.0 mg/kg DOXIL groups. Ulcers healed, but alopecia only partially resolved during recovery. • Placebo liposome effect (hypoactivity, emesis, etc.) could be controlled by reducing dose rate from 2.0 to 0.5 mL/min.

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

TABLE 21 (cont.)
SUMMARY TABLE OF REPEAT DOSE TOXICOLOGY STUDIES OF DOXIL

Report Number	Study Type	Species/ Strain/ Group size	Dose[a] (mg/kg)	DOXIL Lot No.	CONCLUSIONS
			DOXIL 1		
LTI-30-92-08	Repeat Dose Pilot 13 dose q3d	Rat/ Sprague Dawley/ 5M	DOXIL 1: 0.5, 1, 2	[redacted]	• Pilot study to determine dosing regimen for GLP rat study. • Maximum dose level must be < 2.0 mg/kg/dose.
LTI-30-92-17	Repeat Dose 13 dose q3d	Rat/ Crl:CD® BR VAF/Plus/ 20M, 20F	DOXIL 1: 0.25, 1, 1.5 Dox HCl: 1		• DOXIL 1 induced reverible cutaneous lesions of extremities at doses ≥1 mg/kg. • DOXIL 1 less cardiotoxic, hematotoxic and nephrotoxic than equivalent doses of Dox HCl. • Other adverse effects comparable in nature, incidence and severity in DOXIL 1 and Dox HCl groups. • No effect of placebo liposomes.
LTI-30-92-07	Repeat Dose 5 dose q7d	Dog/ Beagle/ 1M, 1F	DOXIL 1: 1.33		• Pilot study to set dosing regimen for GLP dog study. • Dosing halted with animals moribund due to treatment-related GI toxicity.
LTI-30-92-16	Repeat Dose 13 dose q7d or q14d	Dog/ Beagle/ 1M, 1F	DOXIL 1: 0.8, 1, 1.2, 1.5 Dox HCl: 1		• Pilot study • Dosing halted in all treatment groups due to effect of treatment-related dermatologic toxicity on general health.

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