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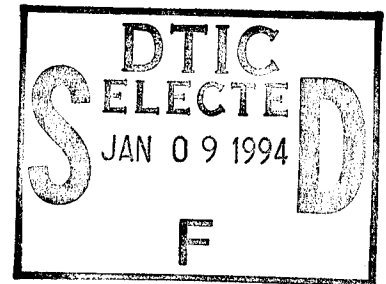
TITLE: PRECLINICAL TOXICOLOGY STUDIES FOR NEW DRUGS AND VACCINES

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13. ABSTRACT (Maximum 200 words) During the reporting period, Two Week Oral Toxicity Studies of WR242511 and WR269410 in Rats, <i>In Vitro</i> Mutagenicity Tests of WR242511 and WR269410, Four Week Oral Toxicity Studies of WR242511 and WR269410 in Dogs, Thirteen Week Oral Toxicity Studies of WR238605 and WR6026 with a Thirteen Week Recovery Period in Dogs, a Thirteen Week Oral Toxicity Study of WR269410 in Rats, and Acute Oral and IP Toxicity Studies of WR242511 and WR269410 in Rats were completed and study reports submitted. Revised draft reports were submitted for Thirteen Week Oral Toxicity Studies of WR238605 with a Thirteen Week Recovery Period in dogs and rats. WR242511 and WR269410 were studied in Thirteen Week Oral Toxicity Studies in Rats and a Four Week Oral Toxicity Study in Dogs, and draft reports were submitted. One Week Intramuscular Dose Range-Finding Studies of Co-administered HI-6 Dichloride Monohydrate and Atropine Citrate in dogs and rats were also conducted, and draft reports submitted. Lastly, draft reports were submitted for a Thirteen Week Oral Toxicity Study in Rats, a Dose Range-Finding Developmental Toxicity Study in Rats and a Four Week Oral Toxicity Study in Dogs on WR242511. In addition, <i>In Vitro</i> Mutagenicity Tests of HI-6 Dichloride, a Preliminary Toxicity Study of Ampicillin in Rabbits, a Four Week Toxicity Study of WR269410 in Dogs, Dose Range-Finding and Developmental Toxicity (Segment II) Studies of WR242511 in Rabbits, a Developmental Toxicity (Segment II) Study of WR242511 in Rats, Two Week Toxicity Studies of HI-6 Dichloride in Dogs and Rats, Dose Range-Finding and Developmental Toxicity (Segment II) Studies of WR238605 and WR6026 in Rats, a Four Week Toxicity Study of Halofantrine in Mice and a Dose Range-Finding Developmental Toxicity Study of WR6026 in rats were initiated during the reporting period.			
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FOREWORD

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X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

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B. M. Lane
PI - Signature 12/7/94
Date

1. List of Study Reports:

<u>Study Report No.</u>	<u>Task Order</u>	<u>Title of Study</u>	<u>Date of Study Report</u>
104	UIC-7	Acute Oral and Intraperitoneal Toxicity Study of WR242511 and WR269410 in Rats	3/22/94
106	UIC-7	Two Week Oral Dose Range-Finding Toxicity Study of WR242511 in Rats	3/22/94
112	UIC-7	Two Week Oral Dose Range-Finding Toxicity Study of WR269410 in Rats	3/22/94
-	UIC-7	<i>In Vitro</i> Mutagenicity Testing of WR242511 Tartrate	7/14/94
-	UIC-7	<i>In Vitro</i> Mutagenicity Testing of WR269410	7/14/94
133	UIC-7	Four Week Oral Dose Range-Finding Study of WR242511 in Dogs	9/26/94
135	UIC-7	Four Week Oral Dose Range-Finding Study of WR269410 in Dogs	9/26/94
097	UIC-5	Thirteen Week Oral Toxicity Study of WR238605 with a Thirteen Week Recovery Period in Dogs	10/5/94
098	UIC-5	Thirteen Week Oral Toxicity Study of WR238605 with a Thirteen Week Recovery Period in Rats	10/5/94
113	UIC-7	Thirteen Week Oral Toxicity Study of WR269410 in Rats	10/5/94

1A. List of Draft Reports:

<u>Draft Report No.</u>	<u>Task Order</u>	<u>Title of Study</u>	<u>Submitted Date of Draft Report</u>
097	UIC-5	Thirteen Week Oral Toxicity Study of WR238605 with a Thirteen week Recovery Period in Dogs	05/09/94

098	UIC-5	Thirteen Week Oral Toxicity Study of WR238605 with a Thirteen Week Recovery Period in Rats	05/17/94
107	UIC-7	Thirteen Week Oral Toxicity Study of WR242511 in Rats	05/31/94
113	UIC-7	Thirteen Week Oral Toxicity Study of WR269410 in Rats	06/22/94
133	UIC-7	Four Week Oral Dose Range-Finding Study of WR242511 in Dogs	07/12/94
135	UIC-7	Four Week Oral Dose Range-Finding Study of WR269410 in Dogs	07/12/94
129	UIC-8	One Week Intramuscular Preliminary Dose Range-Finding Study of Co-Administered HI-6 Dichloride Monohydrate and Atropine Citrate in Dogs	07/20/94
131	UIC-8	One Week Intramuscular Preliminary Dose Range-Finding Study of Co-Administered HI-6 Dichloride Monohydrate and Atropine Citrate in Rats	07/20/94
107	UIC-7	Thirteen Week Oral Toxicity Study of WR242511 in Rats	10/03/94
143	UIC-7	Dose Range-Finding Developmental Toxicity Study of WR242511 in Rats	10/06/94
134	UIC-7	Four Week Oral Toxicity Study of WR242511 in Dogs	10/21/94

2. A short summary of the types of studies conducted and a brief conclusion of the drugs studied in the reporting year.

Task Order UIC-3

The purpose of this project was to examine the mutagenicity of HI-6 Dichloride. The following five *in vitro* studies were conducted at Microbiological Associates, Inc. via a subcontract mechanism.

Salmonella/Mammalian-Microsome Plate Incorporation
Mutagenicity Assay (Ames Test)

L5178Y TK +/- Mouse Lymphoma Mutagenesis Assay

CHO/HGPRT Mutation Assay

Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells

Chromosomal Aberrations in Human Blood Lymphocytes

HI-6 Dichloride was shown to be positive in the two chromosomal aberrations tests while being negative in the remaining three assays which tested for point mutations. Accordingly, an *in vivo* cytogenetics test in rats was conducted as a confirmatory study. In that study, two samples of HI-6 Dichloride were tested; one which was synthesized using bischloromethylether (BCME), and one which was synthesized by a non-BCME route. Both samples were negative in the *in vivo* assay. It is therefore concluded that the overall mutagenic hazards associated with exposure to HI-6 Dichloride are negligible.

Study No. 097 (Task Order UIC-5)

This study evaluated the toxicity of WR238605 in dogs following thirteen weeks of daily oral (gavage) administration. A thirteen week recovery period was included for all groups. Dose levels studied were 0 (vehicle control), 0.1, 2.0 and 6.0 mg base/kg/day. The primary toxic effects of WR238605 were seen in the lungs, RBCs, and liver. Drug treatment was associated with hemolytic anemia which was supported by reticulocytosis, bone marrow hypercellularity, decrease in bone marrow M/E ratio, splenomegaly, extramedullary hematopoiesis, and hemosiderosis in the liver and spleen. Mild hepatotoxicity as evidenced by hepatocyte necrosis (high dose males) was supported by altered clinical chemistry values. Apparent congested retinal vessels was seen in one high dose female, which was no longer evident by the end of the recovery period. Possible generalized or secondary toxic effects related to the stress produced by the anemic and/or methemoglobinemic state included decrease in weight gain; neutrophilic and monocytic leukocytosis; and depletion of thymic lymphocytes. Methemoglobinemia was manifested by clinical signs of cyanosis (blue gums, tongue, and sclera). Lung lesions induced by WR238605 included alveolar proteinosis and subacute inflammation. The above described toxic effects were generally seen at the high and mid dose levels, although mild cyanosis and hemosiderosis secondary to hemolytic anemia, subacute inflammation in the lungs and liver, and bone marrow hypercellularity were also seen to a limited extent in low dose animals. WR238605 toxicity was essentially reversible, except for the lung lesions and the microscopic changes secondary to the observed hemolytic anemia, e.g. hemosiderosis.

Study No. 098 (Task Order UIC-5)

This study evaluated the toxicity of WR238605 in rats following thirteen weeks of daily oral (gavage) administration. A thirteen week recovery period was included for all groups. Dose levels studied were 0 (vehicle control), 0.5, 6 and 18 mg base/kg/day. The primary toxic effects were seen in the RBCs, lungs and liver. Significant methemoglobin production was observed in mid and high dose animals, but was reversible. Microscopic lesions in the spleen, kidney, and bone marrow were secondary to mild hemolytic anemia. Toxicity again was limited to the two highest dose levels. Decreased food consumption, decreased body weight gains, methemoglobin production and mild anemia were observed at the mid and high dose levels, but were readily reversible after treatment cessation. Increases in serum ALT, AST, and/or LDH and decreased A/G ratios in high dose animals and possible mid dose males suggested mild hepatotoxicity, however, histopathologic lesions were not seen. Leukocytosis possible secondary to stress and consisting of increased numbers of lymphocytes, mature neutrophils, and/or monocytes were seen in the treatment period at the two highest dose levels and was reversible after cessation of treatment. Because the aforementioned toxic responses were limited to mid and high dose animals, a no-adverse effect level of WR238605 was assessed to be 0.5 mg base/kg/day.

Study No. 139 (Task Order UIC-6)

This study evaluated the toxicity of microencapsulated ampicillin anhydrate following a single exposure in New Zealand White rabbits when applied into a surgically, aseptically-produced, deep muscle wound. The study included four groups of animals which were either sham-treated (surgery only), treated with 500 mg of microcapsule placebo (MP), treated with 250 mg of microencapsulated ampicillin anhydrate (MAA) or treated with 500 mg of MAA (core weight was 28.2 wt % ampicillin). No apparent test article-related changes were observed in the study. Myocardial changes seen in one high dose MAA-treated animal were probably spurious. Other microscopic changes and alterations in body weights and clinical pathology parameters were considered to be related to the surgical procedure or the implantation of a foreign material into the thigh wound, and not related to ampicillin treatment. Plasma ampicillin levels were detected in seven of eight MAA-treated rabbits 1 hour after treatment and generally declined thereafter at the later time points tested. Based upon the aforementioned findings, the no observed effect level (NOEL) was probably 500 mg microencapsulated ampicillin anhydrate, the high dose of MAA in the present study. This dose level is therefore suitable as the high dose in the subsequent main toxicity study in rabbits.

Study No. 104 (Task Order UIC-7)

This study examined the acute oral and intraperitoneal toxicity of WR242511 Tartrate and WR269410 in rats. The dose levels were selected on the basis of range-finding tests.

After dosing, the animals were weighed weekly, observed daily for 14 days, and the survivors were necropsied on Day 14. Nonsurvivors were also necropsied. The acute oral LD50 of WR242511 Tartrate in male rats, administered in 1% Methylcellulose/0.4% Tween 80 by gavage, was approximately eight-fold lower than in female rats (males; 16.3 mg base/kg and females; 135 mg base/kg). The LD50 values were not significantly different between the sexes (males; 23 mg base/kg and females; 30 mg base/kg). Thus, the LD50 of WR242511 Tartrate was unaffected by sex when administered by intraperitoneal injection, but affected by sex when administered orally.

The acute oral LD50 of WR269410 in male rats, administered in 1% Methylcellulose/0.4% Tween 80 by gavage, was approximately three-fold greater than in female rats (males; 420 mg/kg and females; 147 mg/kg). Due to a physical inability to intraperitoneally administer WR269410 dosage formulations in 0.1% Methylcellulose/0.4% Tween 80 at high enough concentrations to produce lethality, WR269410 was administered intraperitoneally as a solution in polyethylene glycol 200 (PEG 200). The calculated LD50 value of intraperitoneally administered WR269410 in males was 155 mg/kg. An LD50 value in females was estimated to be approximately 70 - 80 mg/kg.

The results of this study suggest that WR242511 Tartrate is more acutely toxic than WR269410 when administered either orally or intraperitoneally. Based on the oral LD50 data and after consultation with the Sponsor, the following dose levels were suggested to be used in the two week oral dose range-finding studies in rats of WR242511 Tartrate; 0, 0.5, 2.0, and 6.2 mg base/kg/day, and of WR269410; 0, 2.0, 6.0, 18.0 mg/kg/day.

Study No. 106 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 Tartrate in rats following two weeks of daily oral administration by gavage. Dose levels studied were 0 (vehicle control), 0.5, 2.0 and 6.2 mg base/kg/day. The primary toxic effects of WR242511 Tartrate included anemia, hepatotoxicity and leukocytosis. Females were more sensitive than males to the anemic state whereas the reverse was true for hepatotoxicity. Anemia was seen in mid and high dose females, whereas hepatotoxicity was only observed in high dose males and may have been associated with the death of one high dose male on Day 13. Generalized leukocytosis occurred in the high dose animals and in mid dose females. Toxicity was not apparent in low dose animals. Significant methemoglobinemia was noted in mid and high animals, and possible at the low dose. As this is the desired pharmacologic effect of WR242511 Tartrate, its occurrence was not considered indicative of toxicity. The purpose of this study was to select dose levels for a three month toxicity study in rats. It is anticipated that significant toxicity would occur at the high dose, marginal or no toxicity would be observed at the mid dose, and no toxicity would occur at the low dose level. On this basis, the following three dose level ranges were suggested: 0.5, 1 - 1.5, and 2 - 4.5 mg base/kg/day.

Study No. 107 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 Tartrate in rats following thirteen weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 0.5, 1.5 and 4.5 mg base/kg/day. The primary treatment-related toxic effects of WR242511 were seen in the liver, lungs and RBCs. Males appeared more sensitive than females to the hepatotoxic effects of WR242511 administration. Microscopic liver lesions (hepatocyte degeneration and necrosis), and elevations in serum ALT and/or SDH levels were observed in mid and high dose males. Increased triglyceride and cholesterol levels in high dose females, and increased cholesterol levels in high dose males also suggested potential hepatocellular toxicity. Increases in total bile acids and alkaline phosphatase levels suggested hepatobiliary changes in high dose animals. Pulmonary microscopic lesions (alveolar histiocytosis) were observed in all WR242511-treated groups. These dose-related effects (hepatocyte degeneration and necrosis, and alveolar histiocytosis) probably contributed to the early deaths of seven out of ten high dose males. Treatment-related mild anemia was observed in mid dose and high dose animals. Hemosiderosis in the spleen of high dose females was probably secondary to mild hemolytic anemia. Significant methemoglobin production was also observed in mid and high dose animals. The lesser methemoglobinemic response seen in high dose males compared to high dose females may have been secondary to the greater hepatotoxic effect in males, resulting in a reduction in the production of a direct methemoglobin-forming metabolite. Thymic lymphocyte depletion in high dose males was apparently secondary to stress produced by test article administration, but possibly could also be a direct treatment-related effect. Mild leukocytosis possibly secondary to stress and consisting of increased number of lymphocytes, neutrophils, monocytes, and/or eosinophils was seen in high dose animals and mid dose males. Thrombocytopenia was observed in all WR242511-treated groups. Because alveolar histiocytosis, thrombocytopenia, and hematology changes were seen at the low dose level, a no-adverse effect level of WR242511 could not be determined.

Study No. 112 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in rats following two weeks of daily oral administration by gavage. Dose levels studied were 0 (vehicle control), 2.0, 6.0, and 18.0 mg/kg/day at study initiation. On Day 7, the mid dose level (6.0 mg/kg/day) was elevated above the high dose to 30 mg/kg/day for the second treatment week due to a lack of significant toxicity at the high dose during the first week of treatment. The primary toxic effect of WR269410 was hemolytic anemia, which was supported by macrocytosis, reticulocytosis, Heinz bodies, splenomegaly and extramedullary hematopoiesis. Females were more sensitive than males to the anemic state. Anemia was seen in males at the two higher doses, but was apparent in all female treatment groups. Methemoglobinemia, the expected pharmacologic effect, was also observed at all three dose levels. As this is the desired pharmacologic effect of WR269410, its occurrence was not considered indicative of toxicity. Cardiomegaly, possible secondary to the methemoglobinemic and anemic state, was seen only in females at 6.0/30.0

mg/kg/day. The purpose of this study was to select dose levels for a three month toxicity study in rats. It is anticipated that significant toxicity would occur at the high dose, marginal or no toxicity would be observed at the mid dose, and no toxicity would occur at the low dose level. On this basis, the following dose levels are suggested: 0, 1, 2.5 and 6 mg/kg/day. After consultation with the Sponsor, the following dose levels were chosen for the three month toxicity study in rats: 0, 1, 3, and 10 mg/kg/day.

Study No. 113 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in rats following thirteen weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 1, 3 and 10 mg/kg/day. The primary toxic affect of WR269410 treatment was hemolytic anemia. The diagnosis of anemia was supported by anisocytosis, polychromasia, macrocytosis, reticulocytosis, Heinz bodies, Howell-Jolly bodies, splenomegaly, splenic extramedullary hematopoiesis, and hemosiderosis in the liver and spleen. Dose-related anemia and methemoglobinemia were observed in all WR269410-treated groups. Slight leukocytosis possibly secondary to stress and consisting of increased numbers of mature neutrophils was seen in the high dose males, but not females, or other dose groups. Because subtle effects of the aforementioned anemia including secondary histologic changes (splenic hemosiderosis and extramedullary hematopoiesis) were observed in low dose animals, a no-observed effect level of WR269410 could not be determined in the present study.

Study No. 133 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. The primary toxic effects of WR242511 tartrate were seen in the RBCs, liver and the lung. Anemia and methemoglobinemia were observed in all the dose levels tested. Compensatory changes to the anemic state included macrocytosis, reticulocytosis and increased nucleated RBCs. Decreases in body weight and food consumption were seen at the high dose level and possibly the mid dose level. Microscopic lesions were seen in the liver and the lung at all dose levels tested. Hepatocellular swelling was supported by decreases in the A/G ratio and increases in haptoglobin levels in mid and/or high dose animals. Pulmonary lesions (alveolar proteinic exudates, macrophage infiltration and acute alveolar inflammation) and mild to moderate thrombocytopenia were seen at all dose levels. Leukocytosis consisting of increased mature neutrophils, possibly secondary to stress, was seen in high animals and possibly in the mid dose female. On the basis of the findings from this study and after consultation with the Sponsor, the following three dose levels have been selected for use in the four week oral toxicity study: 0.1, 0.3 and 1.0 mg base/kg/day.

Study No. 134 (Task Order UIC-7)

This study evaluated the toxicity of WR242511 tartrate in dogs following four weeks of daily administration by gelatin capsule. Dose levels studied were 0, 0.1, 0.3 and 1.0 mg base/kg/day. The primary toxic effects of WR242511 were seen in the RBCs, lungs and platelets. Although subtle, hemolytic anemia was supported by reticulocytosis, secondary splenic extramedullary hematopoiesis and bone marrow hyperplasia in high dose animals. A slight, statistically insignificant decrease in body weight (-0.6 kg) was also seen in high dose males and females. Methemoglobinemia, the desired pharmacologic effect, accompanied by clinical signs of cyanosis (blue gums, tongue and sclera), and mild to moderate thrombocytopenia were observed in mid and high dose animals. WR242511 treatment induced interstitial pulmonary inflammation in seven out of eight high dose animals. Minimal, but significant increases in serum AST, globulin, and triglyceride levels in high dose males and decreases in albumin levels and A/G ratio in both high dose males and females, not accompanied by corresponding histopathologic changes in the liver, suggests that WR242511 is marginally hepatotoxic. Additionally, increased serum haptoglobin levels, indicative of an acute phase reaction, were observed in mid dose males and high dose animals. Because the aforementioned toxic responses were limited to the mid and high dose levels, the no-observed effect level (NOEL) of WR242511 tartrate was 0.1 mg base/kg/day.

Study No. 135 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in dogs following four weeks of daily administration by gelatin capsule. Because neither frank toxicity nor biologically significant increases in methemoglobin production were seen at the initial dose levels studied (1.0, 2.5 and 6.0 mg/kg/day), the dose levels were increased in animals receiving 6.0 mg/kg/day to 12.0 mg/kg/day (starting Day 8) and for the animals receiving 2.5 mg/kg/day to 24.0 mg/kg/day (starting Day 15). The primary toxic effect of WR269410 was hemolytic anemia, supported by reticulocytosis, nucleated RBCs, Heinz bodies and secondary splenic extramedullary hematopoiesis. Dose-related anemia, methemoglobinemia and microscopic changes in the liver (swollen hepatocytes and cholestasis) were observed at the 6.0/12.0 and 2.5/24.0 mg/kg/day dose levels. On the basis of these findings and after consultation with the Sponsor, the following dose levels have been selected for use in the four week oral toxicity study: 2, 7 and 24 mg/kg/day.

Study No. 136 (Task Order UIC-7)

This study evaluated the toxicity of WR269410 in dogs following four weeks of daily administration by gelatin capsule. Dose levels studied were 0, 2, 7 and 24 mg/kg/day. The primary toxic effect of WR269410 was hemolytic anemia. The diagnosis of anemia was supported by anisocytosis, polychromasia, macrocytosis, reticulocytosis, nucleated

RBCs, Heinz bodies, splenomegaly, secondary splenic extramedullary hematopoiesis and bone marrow hyperplasia in mid and high dose animals. Additionally, decreased haptoglobin levels observed in mid and high dose animals further supported hemolysis as the origin of the anemia. A slight, statistically insignificant decrease in body weight (-0.7 kg) was also seen in high dose females but not males. Methemoglobinemia, the desired pharmacologic effect, accompanied by clinical signs of cyanosis (blue gums, tongue and sclera and white/grey gums and tongue), was primarily limited in mid and high dose animals. Increases in serum total bilirubin levels in mid and high dose animals, not accompanied by corresponding histopathologic changes, suggests that WR269410 induced marginal hepatobiliary changes. Thrombocytosis was observed in mid dose males and in high dose animals. Because the aforementioned toxic responses were limited to the mid and high dose levels, the no-observed effect level (NOEL) of WR269410 was determined to be 2.0 mg/kg/day.

Study No. 137 (Task Order UIC-7)

This dose range-finding study evaluated the developmental toxicity of WR242511 tartrate in time-mated New Zealand White (Pasteurella Free) female rabbits. Doses were 0, 0.5, 1, 2.5, 6 and 14 mg base/kg/day administered by gavage during gestation days (GD) 6 - 18 (GD0 = day of observed mating). The doses were based on a preliminary dose range-finding study of WR242511 in non-pregnant rabbits and a dose range-finding developmental toxicity study in rats. All animals in the 6 and 14 mg base/kg/day doses were dead by GD12. Changes in their reproductive indices (e.g. % total loss, % preimplantation loss) were a reflection of early maternal mortality. In the 2.5 mg base/kg/day dose, marginal maternal toxicity was indicated by biologically, but not statistically, significant decreases in food consumption at GD15 and GD18 (i.e. only towards the end of dosing), accompanied by a marginal loss of weight in one of these pregnant rabbits. The 2.5 mg base/kg/day dose was therefore considered at or near the low observable adverse effect level (LOAEL) for maternal toxicity.

Fetal toxicity was apparent in the 2.5 mg base/kg/day dose, and included one non-viable fetus. Biologically significant decreases in fetal body weights were also observed in this dose and in 1 mg base/kg/day female fetuses. This decrease was also statistically significant in the female fetuses at 2.5 mg base/kg/day. No other test article-related differences were observed in any other fetal parameters across groups. The 1 mg base/kg/day dose was considered at or near the low observable adverse effect level (LOAEL) in the fetuses. Accordingly, the following doses are recommended for the definitive developmental toxicity (Segment II) study in rabbits: 0, 0.5, 1.3 and 3.5 mg base/kg/day.

Study No. 138 (Task Order UIC-7)

This developmental toxicity (Segment II) study evaluated the developmental toxicity of WR242511 in time-mated rabbits. Doses were 0, 0.5, 1.3 and 3.5 mg base/kg/day administered by gavage during gestation days (GD) 6-18 (GD0 = day of observed mating). In addition, retinol palmitate (75,000 IU/kg/day) was administered on GD 9 and 10 in a fifth group of rabbits (i.e. positive control group). The results of maternal and fetal toxic responses are currently being evaluated.

In Vitro Mutagenicity Testing of WR242511 Tartrate (Task Order UIC-7)

WR242511 Tartrate was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests (Ames Test, Chromosome Aberration Test using Chinese Hamster Ovary cells and Mouse Lymphoma Assay). WR242511 Tartrate was shown to be negative in the three assays utilized.

In Vitro Mutagenicity Testing of WR269410 (Task Order UIC-7)

WR269410 was tested for point mutations and chromosomal aberrations in three *in vitro* mutagenicity tests. WR269410 was shown to be negative in the Ames Test and the Chromosome Aberration Test using Chinese Hamster Ovary cells, but induced point mutations in the Mouse Lymphoma Assay when a 9000 g rat liver preparation was included.

Study No. 143 (Task Order UIC-7)

This dose range-finding study evaluated the developmental toxicity of WR242511 tartrate in time-mated CD[®] female rats. Doses were 0, 0.5, 1, 2, 4 and 8 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). Maternal toxicity was observed at the high dose as a significant decrease in total weight gain. In addition, significant decreases in mean daily food consumption were seen during the treatment period. Rough coat was also observed in three females during GD13-15. The 4 mg base/kg/day dose was considered near or at the maternal no observable effect level (NOEL).

Fetal toxicity was apparent at 8 mg base/kg/day as significant decreases in body weights were seen. At the 4 mg base/kg/day dose, a biologically significant decrease in fetal mean body weights was observed, but was only statistically significant in female fetuses. However, at 1 mg base/kg/day a statistically significant decrease was present in both sexes. No biological differences in any other fetal parameters were observed at the high dose or mid high dose groups vs. the control group. The absence of an effect on fetal

body weights at 2 mg base/kg/day could not be explained. Fetal body weight changes at 8 mg base/kg/day were considered due to and/or associated with maternal toxicity. The 1 mg base/kg/day dose was considered at or near the low observable adverse effect level (LOAEL) for fetal toxicity. Accordingly the following doses are recommended for the definitive developmental toxicity (Segment II) study in rats: 0, 0.5, 2 and 8 mg base/kg/day.

Study No. 144 (Task Order UIC-7)

This developmental toxicity (Segment II) study evaluated the developmental toxicity of WR242511 tartrate in time-mated CD[®] female rats. Doses were 0, 0.5, 2, and 8 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug). In addition, retinol palmitate (250,000 IU/kg/day) was administered on GD 9 and 10 in a fifth group (i.e. positive control group). Preliminary findings suggest that the drug is not developmentally toxic.

Study No. 129 (Task Order UIC-8)

This study evaluated the toxicity in dogs of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC) in the formulation used in Atropen autoinjector following one week of daily administration by intramuscular injection. The dose levels studied were 70 mg/kg/day of HI-6 dm co-administered with ATC dose levels of 0.10, 0.25, 0.50 or 1.0 mg base/kg/day. No animals died during the study. Treatment with the test article dosing solutions produced a painful stimulus as judged by escape behavior and vocalization. The noxious stimuli may account for the occasional emesis observed, possibly stress-induced, following treatment. Decreased activity was observed in the three highest ATC dose levels. The ATC high dose female was also observed to be lethargic. Because these clinical signs of toxicity were not seen after day 3, it appeared that tolerance developed to these CNS effects. Mydriasis, a pharmacologic (anti-muscarinic) action of atropine, was generally observed throughout the study at all dose levels. Slight decreases in body weights accompanied by no apparent decreases in food intake were observed in males. Only the high dose female had body weight loss, which was accompanied by decreased food intake. Based on these findings and the premise that tolerance develops to decreased activity, following consultation with the Sponsor, a dose level of 1.0 mg base/kg/day ATC was selected for use in a two week intramuscular toxicity study of co-administered HI-6 dm and ATC.

Study No. 130 (Task Order UIC-8)

This study evaluated the toxicity in dogs of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC), the formulation used in the Atropen autoinjector, following two weeks of daily administration by intramuscular injection. The dose levels

of HI-6 dm and ATC were 0, 35 or 70 mg/kg/day and 0 or 1 mg base/kg/day, respectively. The control group received the vehicle (ATC placebo) which was the Atropen autoinjector formulation without atropine. The administration of the various dosing solutions produced significant pain as evidenced by vocalization and escape behavior and required the use of two technicians to forcefully restrain the dogs while a third person administered the injection. Ataxia, lethargy, decreased activity, tremors and unsteady gait were observed in groups receiving atropine alone or in combination with HI-6 dm. Because these CNS effects were only seen in groups receiving ATC, they were considered related to atropine's central anti-muscarinic actions. Emesis was observed in high dose HI-6 dm animals and in low and high combination dose animals. Significant decreases in body weights and/or weight gains accompanied by decreases in food consumption were observed in males receiving atropine alone or in combination with HI-6 dm. Although not statistically significant, decreases in body weights or weight gains accompanied by decreases in food consumption were also seen in females receiving atropine alone and the low dose combination. Decreased food consumption was also observed in high dose HI-6 dm males.

Slight positive chronotropic and dromotropic effects were seen in animals which received atropine with or without HI-6 dm. These ECG changes were associated with atropine's pharmacologic action and were not considered adverse effects. Minimal, but significant increases in serum ALT and/or AST in high dose HI-6 dm animals and high dose combination females, not accompanied by corresponding histopathologic changes, suggests that the high dose of HI-6 dm may be marginally hepatotoxic.

Myotoxicity (subacute inflammation) was observed at the last injection site in all groups including ATC placebo-treated animals (vehicle controls). Increases in serum CK levels were observed in treatment groups receiving HI-6 dm alone or in combination with ATC, but not in vehicle-treated controls or in ATC-treated animals. However, based upon the results of the previously conducted two week co-administration study of HI-6 dm and ATC in rats (UIC/TRL Study No. 132), the myotoxicity observed appears to be mainly resulting from the acidic dosing solutions and not a direct toxic effect of either test article. The addition of HI-6 dm to either the ATC placebo or the ATC dosing formulation notably decreases the pH of the dosing solutions. The observation of myofiber necrosis in one high dose HI-6 dm male and hemorrhage in all high dose HI-6 dm males and in one high dose HI-6 dm female may also be related to the decreased pH seen in dosing solutions of high HI-6 dm alone compared to those also containing ATC (UIC/TRL Study No. 132).

These findings indicate that the anti-cholinergic action of atropine was largely responsible for the majority of the biologic effects (CNS depression with subsequent decreases in body weights and food intake, and ECG changes) observed in combination drug-treated animals. The addition of HI-6 dm to ATC did not appear to increase these anti-cholinergic responses, except in the case of tremors where a slight increased occurrence was seen in dogs receiving the drug combination compared to ATC alone. Emesis, increased myotoxicity (hemorrhage and myofiber necrosis) and marginal hepatotoxicity

were observed in high dose HI-6 dm treated animals. Likewise, the addition of ATC to HI-6 did not appear to potentiate these apparent HI-6 dm-induced effects. Therefore, the combination of ATC and HI-6 dm generally did not potentiate the biologic effects seen from each drug given separately, except possibly increased tremors as previously noted.

Study No. 131 (Task Order UIC-8)

This study assessed the toxicity in rats of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC) in the formulation used in the Atropen autoinjector following one week of daily administration by intramuscular injection. The dose levels studied were 0 (ATC placebo), and 150 mg/kg/day of HI-6 dm co-administered with ATC dose levels of 1, 2, 4, 7 or 14 mg base/kg/day. Treatment with the test article dosing solutions produced a painful stimulus as judged by escape behavior and vocalization. The noxious stimuli may possibly account for the observance of rough coat in a few animals in most of the test article-treated groups. Possible decreases in body weight gains were seen in test article-treated males, but not females in an ATC dose-independent manner without accompanying decreases in food consumption. At necropsy, no test article-related gross lesions were observed. One early death (male; HI-6 150 mg/kg/day and ATC 7 mg base/kg/day) occurred on day 4. At necropsy, the animal's airway was obstructed by a large bolus of food, possibly related to atropine's anti-cholinergic effect, i.e. reduction of salivary and esophageal secretions thereby inhibiting the movement of food through the esophagus. Based upon these findings and after consultation with the Sponsor, an ATC dose level of 14 mg base/kg/day was selected for use in a two week intramuscular toxicity study of co-administered HI-6 dm and atropine citrate in rats.

Study No. 132 (Task Order UIC-8)

This study evaluated the toxicity in rats of co-administered HI-6 dichloride monohydrate (HI-6 dm) and atropine citrate (ATC), the formulation used in the Atropen autoinjector, following two weeks of daily administration by intramuscular injection. The dose levels of HI-6 dm and ATC were 0, 17, 50, or 150 mg/kg/day and 0 or 14 mg base/kg/day, respectively. The control group received the vehicle (ATC placebo) which was the Atropen autoinjector formulation without atropine.

Three deaths in the study (an ATC-treated male, a mid dose combination male and a high dose combination female) were associated with the anti-cholinergic action of atropine producing dysphagia which resulted in asphyxiation due to obstruction of the larynx by boluses of food. Dysphagia was also observed in other animals receiving atropine with or without HI-6 dm, but not in any animal receiving HI-6 alone or in control animals. The administration of the dosing solutions including the ATC placebo produced a painful stimulus as judged by escape behavior and vocalization, resulting in stress-induced poor grooming and slight leukocytosis in all drug-treated and control groups. A significant decrease in body weight gains accompanied by a decrease in food consumption was

observed once in high dose combination males, but did not result in statistically significant decreases in body weights or total weight gains, and was not observed in females. Hunched posture was also observed in one high dose combination male.

Myotoxicity (subacute inflammation, myofiber degeneration and hemorrhage) was observed at the last injection site in ATC placebo-treated animals (vehicle controls) and in high dose combination animals to a similar extent. These lesions were considered to be related to the large dosing volume being repeatedly administered and/or the physical properties of the dosing solutions (pH or tonicity). Mild thrombocytosis was seen in all groups including vehicle-treated control animals, and was apparently secondary to the myotoxicity (hemorrhage) induced by the intramuscular injections.

Minimal, but significant increases in serum ALT, AST and/or LDH in mid and high dose combination females, not accompanied by corresponding histopathologic changes, suggests that joint test article treatment may be marginally hepatotoxic. The increased AST and LDH levels may also be related to the observed myotoxicity. It is not known why these changes were not apparent in HI-6 dm alone-treated animals, as previously seen (UIC/TRL Study No. 072). Additionally, increased serum total bile acids were seen in ATC-treated animals, either alone or in combination with HI-6 dm (mid dose combination animals and high dose combination males but not females), but not in low dose combination animals or any group treated with HI-6 dm alone. These apparent changes in hepatobiliary function may be related to atropine's anti-spasmodic action on the smooth muscle of the bile ducts. Because toxicity was observed in mid and high dose combination groups, the drug combination no observed effect level (NOEL) in the present study was determined to be 17 mg/kg/day HI-6 dm/14 mg base/kg/day ATC.

Study No. 153 (Task Order UIC-10)

This dose range-finding study evaluated the developmental toxicity of WR238605 Succinate in time-mated CD® female rats. Doses were 0, 1, 2.5, 6, 15 and 35 mg base/kg/day administered by gavage during gestation days (GD) 6 - 15 (GD0 = day of vaginal plug).

At the 35 mg base/kg/day dose, body weights were significantly decreased starting on GD9. This was reflected as a significant decrease in total weight gain by GD20. A marginal biological decrease in body weights was also observed at the 15 mg base/kg/day dose. Food consumption was significantly decreased at the intervals GD6-10, 10-15 and 15-20 at the 35 mg base/kg/day dose, while at the 15 mg base/kg/day dose, significant decreases were only observed during the second half of dosing i.e. GD10 through 15. No mortality was observed at any group, however rough coat was seen in two animals in the high and in two animals in the midhigh doses. Accordingly, the 15 mg base/kg/day dose was considered at or near the maternal low observable adverse effect level (LOAEL).

In fetuses, decreases in body weights were observed in the 15 and 35 mg base/kg/day doses. These decreases were statistically significant in females, while males showed statistical significance only in the high dose. With the exception of one low dose fetus demonstrating spurious anomalies, no significant change in the incidence of external anomalies was observed in any dose group when compared to the control. Normal variations (hematomas and petechial hemorrhages) were observed in all the dose groups without any dose-response relationship. The numbers of early and late resorptions in various groups were not representative of any test article-related effect. The 15 mg base/kg/day was considered a marginal developmentally toxic dose. Accordingly, the following doses are suggested for the definitive developmental toxicity (segment II) study in rats: 0, 3, 10 and 30 mg base/kg/day.

Study No. 154 (Task Order UIC-10)

This developmental toxicity (Segment II) study evaluated the developmental toxicity of WR238605 Succinate in time-mated CD® female rats. Doses were 0, 3, 10 and 30 mg base/kg/day administered by gavage during gestation days (GD) 6-15 (GD0 = day of vaginal plug). In addition, retinol palmitate (250,000 IU/kg/day) was administered on GD 9 and 10 in a fifth group of rats (i.e. positive control group). The results of maternal and fetal toxic responses are currently being evaluated.

Study No. 168 (Task Order UIC-11)

This study evaluated the toxicity of halofantrine hydrochloride in B6C3F1 mice following four weeks of daily oral (gavage) administration. Dose levels studied were 0 (vehicle control), 4, 20 and 100 mg/kg/day. Beginning day 13, clinical signs of toxicity (rough coat, hunched posture, decreased activity and/or lethargy) were seen in high dose males. Clinical signs of toxicity were not observed in other groups until day 20 and were limited to rough coat, hunched posture and decreased activity in one high dose female. In the second week of treatment, a significant decrease in body weight without an accompanying decrease in food consumption was observed in high dose males. A decrease in body weight gain accompanied by decreased food consumption was not seen until the final week of treatment in high dose females. In the beginning of week 3 (days 14 and 15), one high dose male was found dead and the four other high dose animals were sacrificed moribund. Splenic lymphocytic necrosis was observed in all high dose males and three out of five high dose females, but not in lower dose levels. Splenic granulopoiesis secondary to the splenic lymphocytic necrosis was observed in one high dose female. Increased serum ALT and cholesterol levels in high dose females and increased serum ALT in mid dose males, not accompanied by corresponding histologic changes, suggests that halofantrine may be marginally hepatotoxic. Decreases in serum alkaline phosphatase levels were also observed in high dose females, and may be related to reductions in food intake. Dose-related, mild, microcytic, apparent iron-deficiency anemia was seen in high dose females and to a lesser extent in mid dose animals.

Marginal, statistically significant RBCs changes were also seen in low dose females. Leukocytosis, consisting of elevated mature neutrophils and monocytes, and thrombocytosis (possibly secondary stress-responses) were also seen in high dose females, but not in lower dose levels. Marginal leukopenia was paradoxically seen in mid dose males and consisted of decreases in mature neutrophils and lymphocytes. The purpose of the study was to select dose levels for a three month toxicity study in rats. It is anticipated that significant toxicity would occur at the high dose, marginal or no toxicity at the mid dose, and no toxicity at the low dose. On this basis, the following dose level ranges are suggested: 1 - 2, 4 - 8 and 15 - 30 mg/kg/day.

Study No. 170 (Task Order UIC-13)

This dose range-finding study evaluated the developmental toxicity of WR6026 Dihydrochloride in time-mated CD[®] female rats. Doses were 0, 1.5, 3, 6, 12 and 24 mg base/kg/day administered by gavage during gestation days (GD) 6-15 (GD0 = day of vaginal plug). The results of maternal and fetal toxic responses are currently being evaluated.

3. Comments on administrative and logistical matters during the reporting period.

No problems occurred.