

**Integrated Formulation, Testing and Analysis Program
for Trans Sodium Crocetinate (TSC)**

ONR Contract No: N00014-05-C-0257
Quarterly Report
February 1, 2006 - April 30, 2006

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ABSTRACT

This Quarterly Report for ONR Contract No: N00014-05-C-0257, Integrated Formulation, Testing and Analysis Program for Trans Sodium Crocetin (TSC), covers the period February 1, 2006 through April 30, 2006. The purpose of the research under this contract is to support the transition of the novel drug *trans sodium crocetin* (TSC) from preclinical to clinical use, especially as to advanced drug formulation, stability testing and chemical characterization and data documentation and analysis tasks required by the U.S. FDA.. TSC was invented at the University of Virginia by Professor John Gainer, who has received funding from the Office of Naval Research (ONR) since 1995 to advance the development of TSC for the treatment of hemorrhagic shock.

ONR is currently supporting Diffusion Pharmaceuticals LLC, Charlottesville, VA in development of TSC for clinical testing. An important step is the development of a drug product formulation that has the required solubility, stability, pH and osmolality characteristics for use both in humans and in high-dosage animal toxicology tests. As previously reported, manufacture of the basic API (active pharmaceutical ingredient), including five related (“analog”) molecules, has been completed by CarboGen to Good Manufacturing Practice (GMP) standards. During this quarter, stability tests were conducted by CarboGen (of Switzerland) on the API (active pharmaceutical ingredient) up through the 9-month time point, and stability tests were conducted on the final, formulated drug product through the 6-month time point by Aptuit of Kansas City. Both the API and the final drug product show good stability in these tests, which suggests that TSC can be safely stored under various conditions, both as a raw drug and as a final, formulated drug product. The improved formulation to support animal toxicology testing shipped in March, 2006.

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REPORT

Drug Stability

A standard concern about any drug is its stability during storage. Most of the TSC itself (the API) is stored in Switzerland, and they are doing tests to determine its stability when stored under various conditions. The conditions of those studies involve temperatures varying from 5 to 40 °C (41 to 104 ° F.) and the relative humidity varying from 40 to 75%. It was felt that this would cover the conditions in much of the world where TSC might need to be stored when being used on the battlefield.

Data have now been received from CarboGen for up to 9 months of storage, and the results are shown on the next page as Table 1. The storage temperatures and relative humidity (RH) are given at the top of the table. The API is stored in glass bottles which are placed inside sealed metal cans. It can be seen that TSC can be stored for up to at least 9 months at all temperatures and relative humidities tested. Stability will also be tested at 12 months storage, 18 months storage and 24 months storage. When stored at "accelerated conditions" (40 °C and 75% RH), it looks as though the API may be beginning to degrade at a time of 9 months; however, it will take the data at later times to definitely know that since there is some natural variation in the measurements.

Stability of the formulated drug product is being conducted by Aptuit of Kansas City. We have results from their tests up through 6 months, and they are given in Table 2. Note that the acceptable level is 90% to 110% of the standard.

Table 1: Storage of API

Time Point	5°C	25°C 60% RH	30°C 65% RH	40°C 75% RH	40°C 75% RH (with desiccant)
0 month	98.6%w/w	98.6%w/w	98.6%w/w	98.6%w/w	98.6%w/w
1 month	97.0%w/w	98.1%w/w	98.4%w/w	98.2%w/w	97.4%w/w
3 months	100.2%w/w	97.6%w/w	99.9%w/w	97.9%w/w	Not analyzed
6 months	99.1%w/w	98.5%w/w	97.7%w/w	97.0%w/w	97.4%w/w
9 months	99.8 % w/w	98.7 % w/w	99.2 % w/w	92.3 % w/w	Not Analyzed

Table 2. Stability of Formulated Drug Product

Time Point	5°C	25°C 60% RH	40°C 75% RH
0 month	100.5 %	100.5%	100.5%
1 month	100.9%	99.6%	96.8%
3 months	95.4%	95.1%	93.1%
6 months	96.1%	95.9%	95.3%

Formulation Studies

As previously reported, the final drug formulation consists of 20 mg/ml of TSC dissolved in sterile water made by Abbott Laboratories, North Chicago, IL, 8% w/v of β -cyclodextrin (Cavamax from Wacker Chemicals), 2.3% w/v mannitol (for tonicity purposes) and 50 mM glycine (as a buffer). The final drug product has been produced to GLP quality, and was shipped

in March, 2006, to the toxicology laboratories. GMP manufacture of formulated TSC (clinical trials supplies) will take place in the near future.

Documentation and Analysis Activities

Chris Dvergsten, PhD, Senior Director of Scientific Affairs, and Kelly Hoy, Director of Regulatory filings, are continuing the extensive formulation documentation activities required for the transition from preclinical testing. These activities will ensure that all requirement for solubility, stability, quality, and packaging study data are met. They will also ensure compliance with all Good Laboratory Practices, Good Manufacturing Practices, and the requirements set forth in the Guideline for the Format and Content of the Chemistry, Manufacturing, and Controls Section of an Application, February 1987, including individual guidance document requirements.

Over the next quarter, Dr. Dvergsten and Ms. Hoy will review completed pharmacology and toxicology study data, including pharmacodynamic, mechanism of action, interaction with other drugs, adverse reaction, and all animal toxicology study data to ensure that they meet all requirements according to the Guideline for the Format and Content of the Nonclinical Pharmacology/Toxicology Section of an Application, February 1987.

RECOMMENDATIONS

As reported, research conducted in the Diffusion Pharmaceuticals laboratory suggest that TSC might also be administered other than via intravenous injection. It appears to be quickly absorbed via other administration routes, such as intramuscular, inhalation, oral and transdermal. Such alternative routes may be of use in treating actual human combat casualty victims. This should be investigated more in the future, since this new knowledge may bring about easier methods for use under battlefield conditions.

In addition, as previously reported, the total transition requirements to complete the tasks necessary to move TSC from the preclinical to the clinical stage are larger than originally anticipated due to technical considerations in 1) preparing and validating the use of a cyclodextrin compound to achieve required levels of solubility and stability, and 2) the introduction by the cyclodextrin compound of unacceptable levels of endotoxins during production, leading to the necessity for an additional filtration step and repeat of certain previously performed production processes activities, including lyophilization and fill. The Company is currently taking steps to find the necessary resources to bridge shortfalls which may occur as a result.