PTC THERAPEUTICS

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February 4, 2009

Tom Thompson
U. S. Nuclear Regulatory Commission
Nuclear Materials Safety Branch 2
Region I
475 Allendale Road
King of Prussia, PA 19406-1415

03035590

RECEIVED REGION I 2009 FEB -6 PM 12: 30

RE: License No. 29-~~306032~~ 30602-01

Dear Mr. Thompson:

I would like to respectfully request the following amendment to PTC Therapeutics NRC license:

1. Please remove the following name from the license:

   S. Paushkin

   Dr. Paushkin is no longer employed by PTC Therapeutics.

2. Please increase tritium limits from 30 mCi to 60 mCi with radioactivity usage license. The purpose for proposed increase is to screen an essential bacterial enzyme for potential inhibitors from our chemical library that can serve as a starting point to discover new bacterial antibiotics.

Please see the proposed experiment SOP attached.

I trust this information is sufficient for you to expedite processing this request. If you have any questions, please contact Olga Pergament at 908-222-7000, ext. 9354 or 732-991-4205

Sincerely,

Richard Lipman

R. Lipman, Ph.D.
Radiation Safety Officer
PTC Therapeutics

143335

NMSS/RGN1 MATERIALS-002

PTC THERAPEUTICS

January 29, 2009

Re: Increase tritium limits from 30 mCi to 60 mCi with radioactivity usage license.

Purpose:
To screen an essential bacterial enzyme for potential inhibitors from our chemical library that can serve as a starting point to discover new bacterial antibiotics. A radioactive assay using tritium is available now and will be used to screen this enzyme target. A non-radioactive assay will be used as soon as it is available. Our "Validation" library of 3,520 compounds will be screened in this manner. The screen itself utilizes approximately 12 mCi of commercially available tritium-labeled substrate and will be conducted once. After recently purchasing 12 mCi of tritium for the screen, the total amount of tritium housed in our facility is about 24 mCi. Raising our tritium limits from 30 to 60 mCi allows continued tritium use for follow-up experiments and additional experiments without requiring commercial radioactive waste disposal immediately following the screen. Volumes of containers to store solid and liquid wastes from this screen can be confined to about 40 and 5 gallons, respectively. Our existing radioactive waste storage areas are suitable for the additional tritium capacity requested herein. Commercial radioactive waste disposal will be utilized before we reach our limit of tritium capacity. Raising our limit from 30 to 60 mCi extends the time until commercial disposal is required and thus helps to keep costs low.

Below is a representative example of the screening assay procedure:

1. Obtain from the HTS department, 384-well assay plates containing 2 uL of each test compound and controls (in 10% DMSO) in each well.
2. Prepare 2.4X buffer, enough for both Enzyme master mix and Substrate master mix.
3. Prepare Enzyme master mix, aliquot 80uL to each well of a 384-well plate, centrifuge the plate and give it to HTS to pipette 5 uL to each well of the 384-well assay plates, centrifuge plates.
4. Anneal tRNA (70°C for 5 minutes then let cool for 20 min on ice).

All subsequent steps are carried out in the radioactive laboratory.

5. Prepare Substrate master mix with tritium-labeled amino acid and pipette 5 uL to each well of each assay plate using the Apricot pipette with 30 uL Matrix tips. Cover plates with plate tape and centrifuge plates to initiate the reaction and incubate plates for 2 hours at room temperature. Dispose of used tips in solid waste for tritium.
6. After 2 hours of incubation, stop the reaction with the addition of 5% TCA. Use the Apricot pipette equipped with the 96 EZ-Load 125 uL tips to transfer 64 uL 5% TCA to the 12 uL reaction in each well of the 384-well plate, one quadrant at a time. Transfer 72 uL of the quenched reaction to a 96-well plate filterplate (Millipore Multiscreen HTS HV plates - MSHVN4B50) with catch plate underneath and centrifuge at 1000 rpm for 1 min to collect

the tritium-labeled tRNA in the filterplate. The unincorporated tritium washes through. Dispose of used tips and assay plates in solid waste for tritium.

7. Wash each 96-well filterplate twice with 100 uL of 5% TCA and centrifuge as above. After each spin transfer filtrate into tritium liquid waste recepticle.
8. Wash each 96-well filterplate twice with 100 uL of 95% ethanol and centrifuge as above. After each spin transfer filtrate into tritium liquid waste recepticle.
9. Remove bottom filterplate gasket, air-dry plates for 5 min and tape bottom of each plate. Dispose of filterplate gasket in solid waste for tritium.
10. Add 100 uL of liquid scintillation cocktail to each well, seal plate with plate tape and count in LS Counter using protocol #18 on the MicroBeta to count tritium in 96-well format. Dispose used plates in solid waste for tritium containing liquid scintillation cocktail.
11. Conduct a post-experiment survey for potential contamination. Swipe all surfaces in proximately to tritium usage. Read swipes, decontaminate if necessary and reswipe to document decontamination.

Prepared by: Richard Lipman
Radiation Safety Officer
PTC Therapeutics
100 Corporate Court
South Plainfield, NJ 07080

This is to acknowledge the receipt of your letter/application dated

2/4/2009, and to inform you that the initial processing which includes an administrative review has been performed.

AMEND. 29-30602-01

☑ There were no administrative omissions. Your application was assigned to a technical reviewer. Please note that the technical review may identify additional omissions or require additional information.

☐ Please provide to this office within 30 days of your receipt of this card

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A copy of your action has been forwarded to our License Fee & Accounts Receivable Branch, who will contact you separately if there is a fee issue involved.

Your action has been assigned **Mail Control Number** 143335.
When calling to inquire about this action, please refer to this control number.
You may call us on (610) 337-5398, or 337-5260.

NRC FORM 532 (RI)
(6-96)

Sincerely,
Licensing Assistance Team Leader