ORD CLEARANCE FORM

1. EPA Pub. No. (if applicable)	2. Laboratory/Center/Office Tracking Number	3. Copyright Permission: __ Yes (attached) __ No (not applicable)

4. Title

Development of a 3D co-culture model for studying embryonic palatal fusion.

5. Author(s), Affiliation, and Address. Include either Telephone Number or Email Address (required) to provide unique identifiers for non-EPA authors.

Cynthia J. Wolf, Carrie Becker, Kaberi Das, Andrew Watkins, David Belair, Barbara D. Abbott
ORD/NHEERL/TAD/DBB Watkins: ORD/NHEERL/TAD/IO
Research Triangle Park, NC 27711

6. Internet Address (If the product is posted on a website, provide the URL for linking to the publication.

A PDF file of the final publication and a WordPerfect or Word file of the product abstract (approximately 200 words) should be submitted with the final package for uploading to the Technical Information Management (TIMS) Database.)

7. Enter these numbers:	8. Project Officer/Principal Investigator Name and Telephone Number
OMIS Task # CSS 17.02	Barbara D Abbott, 919-541-2753
APG FY & # Virtual Tissues	9 . Cooperative Agreement, Contract, Grant, Interagency Agreement Number
APM FY & # Task1 Morphogenetic Fusion	
Multi-Year Plan (MYP)	

10. Product Type w/o Subtype

Assessment Document
Criteria Document
ETV Document
Internal Report
IRIS Assessment
Newsletter
Risk Assessment Guidelines
Summary
Unpublished Report
Book
Book Chapter

10a. Product Type w/ Subtype	
Published Report	EPA Proceeding Guidance Document Handbook Manual Methodology Report Tech Transfer User's Guide
Communication Product	Announcement Brochure External Fact Sheet Flyer General Public Videotape
Data Product	Database Map Model Scientific Data Software Spreadsheet
Extramural Document	Interagency agreement Contract Grant Cooperative Agreement
Journal	Peer-Reviewed Non-Peer Reviewed
X Presentation	X Abstract Exhibit Extended Abstract Paper X Poster
Site Document	Bulletin Capstone Doc Capsule Project Report/Summary

11. Bibliographic Citation (For presentations, give name, place and date)
Teratology Society Annual Meeting in San Antonio, TX on June 25-29, 2016

12. Technical Information Manager Signature and Date

Signature Date

13. Laboratory/Center/Office Recommending Approval Signatures

Dates
2/8/16
2/9/16

14. Comments

15a. Peer Review Categories
1 Major Agency Scientific or Technical Work Product
2 Important, Highly Visible Scientific
3 General Scientific or Technical Work Product
4 Non-major Scientific or Technical Work Product

16a. Hierarchical Keywords

1			
2			
3			

15b. Contact/location for peer review comments

17. This Product enunciates new policy or affects existing policy: ___ Yes X No

Technical Manuscript Review Form

Title	Author(s)
Development of 3D co-culture model for studying embryonic palatal fusion.	Cynthia Wolf, Carrie Becker, Kaberi Das, Andrew Watkins, David Belair, Barbara Abbott
Date Review Requested: 2/04/16 / **Date Review Required**: 2/09/16	**Project Officer/Organization/Address**: Barbara Abbott, USEPA/ORD/NHEERL/TAD/DTB
Type of Publication/Audience: Abstract for Teratology Society scientific meeting	**Reviewer/Organization/Address**: Sid Hunter, USEPA/ORD/NHEERL/ISTD/SBB
Review Coordinator (e.g., PO, TIM, Supervisor)	

You are asked to review and comment on the attached manuscript. Feel free to make notations on the manuscript as well as in the comments section below, particularly regarding your recommendations for revisions. if you are unable to review the manuscript by the required date, please return it now. You suggestions for alternate or additional reviewers will be welcomed.

SUMMARY RATING			RECOMMENDATIONS
Please rate the manuscript as follows:	Satisfactory	Unsatisfactory	
Content & scope	X		G (1) Acceptable as is
Organization & presentation	X		G (2) XXX Acceptable after minor revisions
Quality of data & validity of analytical techniques	X		G (3) Acceptable after major revisions
Soundness of Conclusions	X		G (4) Not acceptable
Editorial Quality	X		If you have checked either 3 or 4, please specifically state reason(s) in the comments space below.
Other (specify)			
			I have read and reviewed the document. e-signature E Sidney Hunter, III 5 February 2016 Reviewer's Signature Date

Comments: (Use extra sheets if needed):

This is exciting research. I have minor comments in the word document.
ESH^3

Development of a 3D co-culture model for studying embryonic palatal fusion.
Wolf, C., Becker, C., Das, K., Watkins, A., Belair, D., Abbott, B.

Morphogenetic tissue fusion is a critical and complex event in embryonic development and failure of this event leads to birth defects, such as cleft palate. Palatal fusion requires adhesion and subsequent dissolution of the medial epithelial layer of the mesenchymal palatal shelves, and is regulated by growth factors, EGF, TGF_{β}, and others, although the complete regulatory mechanism is not understood. Three dimensional (3D) organotypic models allow us to mimic the native architecture of human tissue to facilitate the study of tissue dynamics and their responses to developmental toxicants. Our goal was to develop and characterize a cell-spheroidal model of palatal fusion to investigate the mechanisms regulating fusion with exposure to growth factors and ToxCast chemicals known to disrupt this event. We present a spheroidal model using human umbilical-derived mesenchymal stem cells (hMSC) spheroid cores, coated with MaxGel™ basement membrane and a mantel of human progenitor epithelial keratinocytes (hPEKp) added on day 13. We characterized the growth, differentiation, proliferation and fusion activity of the model. Spheroid diameter was dependent on hMSC seeding density, size of the seeding wells, time in culture, and type of medium. hMSC spheroid growth was enhanced with osteogenic differentiation medium. Alkaline phosphatase activity in the hMSC spheroid, indicating osteogenic differentiation, increased in intensity throughout culture to day 14. Preliminary results showed EGF exposure at 2 or 4 ng/ml increased cell proliferation in multicellular spheroids by almost 2-fold. In initial observation, hMSC spheroids when placed in contact began to merge within 8 hrs, while epithelial-layered spheroids began to fuse at a later time point, 40-48 hrs, and completely merged at ~ 4 days. This model will enable us to study the regulation of fusion by manipulation of spheroid activity with growth factors and to evaluate the effects of exposure to ToxCast chemicals associated with cleft palate. Additionally, this model can be implemented in the study of other embryonic fusion events that involve mesenchymal and epithelial tissues. This abstract does not necessarily reflect USEPA policy.

Commented [HS1]: Do you mean fuse, in contrast to merge?
You said merge for MSC spheroids and fusion for PEK covered spheroids. Are you drwing attention to the differences?

1. May want to leave out ToxCast in Hbstract since it was not defined – do people know what it is?
2. May want to clarify ~~days~~ time (e.g. 13 day) – is it 13 days after initiation of incubation? When was EGF added to culture? → fusion completed 4 days later

Development of a 3D co-culture model using human stem cells for studying embryonic palatal fusion.

Wolf, C., Becker, C., Das, K., Watkins, A., Belair, D., Abbott, B.

Morphogenetic tissue fusion is a critical and complex event in embryonic development and failure of this event leads to birth defects, such as cleft palate. Palatal fusion requires adhesion and subsequent dissolution of the medial epithelial layer of the mesenchymal palatal shelves, and is regulated by the growth factors EGF and TGF_{β}, and others, although the complete regulatory mechanism is not understood. Three dimensional (3D) organotypic models allow us to mimic the native architecture of human tissue to facilitate the study of tissue dynamics and their responses to developmental toxicants. Our goal was to develop and characterize a spheroidal model of palatal fusion to investigate the mechanisms regulating fusion with exposure to growth factors and chemicals in the ToxCast program known to disrupt this event. We present a spheroidal model using human umbilical-derived mesenchymal stem cells (hMSC) spheroid cores cultured for 13 days and then coated with MaxGel™ basement membrane and a layer of human progenitor epithelial keratinocytes (hPEK) (hMSC+hPEK spheroids). We characterized the growth, differentiation, proliferation and fusion activity of the model. Spheroid diameter was dependent on hMSC seeding density, size of the seeding wells, time in culture, and type of medium. hMSC spheroid growth was enhanced with osteogenic differentiation medium. Alkaline phosphatase activity in the hMSC spheroid, indicating osteogenic differentiation, increased in intensity throughout culture to day 14. Preliminary results showed EGF exposure at 2 or 4 ng/ml in hMSC+hPEK spheroid cultures increased cell proliferation by almost 2-fold. In a pilot fusion study, hMSC spheroids when placed in contact began to merge within 8 hrs, while hMSC+hPEK spheroids began to fuse at a later time point, 40-48 hrs, and were completely merged at ~ 4 days. This model will enable us to study the regulation of fusion by manipulation of spheroid activity with growth factors and to evaluate the effects of exposure to ToxCast chemicals associated with cleft palate. Additionally, this model can be implemented in the study of other embryonic fusion events that involve mesenchymal and epithelial tissues. This abstract does not necessarily reflect USEPA policy.