UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Division of Environmental Health and Safety

102 Environmental Health and Safety Building
101 South Gregory Street
Urbana, IL 61801-3070

August 21, 2001

Dr. Amy T. Patterson, M.D.
Director of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Rockledge 1, Suite 750
Bethesda, Maryland 20892

IN RE: Rendering of Pigs Containing the Microinjected Bovine α-LA/IGF-I Gene Construct

Dear Dr. Patterson:

Thank you for taking the time to respond to the information we provided you in regards to the inadvertent rendering of two, approximately 40 pound, male transgenic pigs, by the University of Illinois at Urbana-Champaign (University) on January, 4, 2001. Please find below additional information in response to your follow-up letter I apologize for the delay in replying but I had tried to call you to enable us to discuss your concerns verbally. However, after many unsuccessful attempts, I spoke with Dr. Rosenthal, who suggested I directly respond to you in writing.

The University takes the responsibility for safety and for compliance with the NIH Guidelines very seriously. The commitment to compliance is evident in several ways. First, letters sent to Principal Investigators after research approval from the campus Institutional Biosafety Committee (IBC) have always stated, "Please be advised that you must comply with the responsibilities outlined for the Principal Investigator in Section IV-B-7 of the NIH Guidelines." However, we will now modify this section of the approval letter to include the requirements for reporting significant problems or violations. Secondly, as another proactive step, we have planned on reviewing the responsibilities of the institution and the IBC at the next IBC meeting. Thirdly, the Biological Safety Section (BSS) of the Division of Environmental Health and Safety (DEHS) will begin offering regularly scheduled training seminars this Fall to Principal Investigators and their personnel, a portion of which will explain the requirements of the NIH Guidelines. Fourthly, the website for DEHS is being updated to include a link to the NIH Guidelines and a Facts Sheet concerning research that involves recombinant DNA that is conducted at, or sponsored by the University.

Our understanding of the Guidelines is that the work involving the transgenic pigs in question is actually exempt from the NIH Guidelines. Our reasoning was as follows:

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SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES*

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see Section III-A), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see Section III-B), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see Section III-C), (iv) those that require Institutional Biosafety Committee approval before initiation (see Section III-D), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-E), and (vi) those that are exempt from the *NIH Guidelines* (see Section III-F).

Note: *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed.* If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.

Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required:

Section III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

For the two pigs in question, the only part of the gene construct that does not have Food and Drug Administration (FDA) approval for rendering was a synthetic portion that is identical to the porcine gene for IGF. Although this experiment could fall into two Sections of the Guidelines, (Section III-D-4 Experiments Involving Whole Animals, and Section III-F Exempt Experiments), given the note under Section III (If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.), we considered this portion of the experiment to be exempt because pig DNA was being put into a pig.

To alleviate any possible misunderstandings, disposition of any transgenic animal used for research at the University is by incineration, except for the one line of transgenic pigs that does have FDA approval for rendering. The only break in this standard procedure concerned the two pigs we informed you of in my previous letter, dated May 18, 2001. According to National By-Products, the rendering company for the University, all animal carcasses are used for animal feed only. Furthermore, the Principal Investigator has submitted an application to the FDA for rendering of the line from which the two pigs were derived. This application was submitted on February 21, 2001 (letter dated February 5, 2001) to the FDA. Although no confirmation has been received to date, approval is anticipated. As soon as documentation is received, a copy will be sent to you.

A system of easy visual identification for transgenic animals has been a priority for the laboratory creating these animals. The original ear marking protocol has been replaced with one that should

leave no doubt in any laboratory or farm personnel's mind as to the disposition of a carcass. Please see the attached description. If you have any additional suggestions concerning this system, we will gladly embrace them.

Please feel free to contact me by phone (217-244-7801) or e-mail (i-cooke@uiuc.edu) should you have any questions concerning the incident or if the University needs to take additional actions.

Respectfully,

Irene Cooke

Irene Cooke, D.V.M., Ph.D.
Director

Attachment

c: Van Anderson
Matthew Wheeler
Paul Bohn

Visual identification system for transgenic pigs. The information below is posted on signs in the transgenic pig barns.

TRANSGENIC PIG EAR TAG CODE
(tags will have a "T" for transgenic or a "C" for controls, "T's" CANNOT be sold under any circumstances)

These can be rendered:
RED - Big Al (Line α-LAC-I)
BLUE- Alice (Line α-LAC-II)

These MUST be INCINERATED:
GREEN- Iggy (Line α-LAC-IGF-I)
YELLOW- Biggy (Line α-LAC-IGF-II)
PURPLE- Piggy (Line α-LAC-IGF-III)
BLACK- Twiggy (Line α-LAC-IGF- IV)
SPECIAL INSTRUCTIONS:

PINK -(crosses between Line α-LAC-I and
Line α-LAC-IGF-I) -since these are crosses there are four(4) possibilities:

1) If the animal is transgenic for α-LAC-I they will have both a blank pink tag and red "T" tag and will be rendered.

2) If transgenic for Line α-LAC-IGF-I they will have both a blank pink tag and green "T" tag and MUST be INCINERATED.

3) If transgenic for both they will have a pink "T" tag and MUST be INCINERATED.

4) If they are controls they will have a pink "C" tag and can be sold, rendered or incinerated.

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AT URBANA-CHAMPAIGN

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102 Environmental Health and Safety Building
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May 18, 2001

CONFIDENTIAL

Dr. Amy T. Patterson, M.D.
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National Institutes of Health
6705 Rockledge Drive
Rockledge 1, Suite 750
Bethesda, Maryland 20892

IN RE: Rendering of Pigs Containing the Microinjected Bovine α-LA/IGF-I Gene Construct

Dear Dr. Patterson,

I am writing to confirm the information given today to Dr. Cheryl McDonald, of your office, in regards to the inadvertent rendering of two, approximately 40 pound, male transgenic pigs, by the University of Illinois at Urbana-Champaign (University) on January, 4, 2001. Per our conversation, please find below the information that we discussed.

The University has approval from the Food and Drug Administration (FDA) to render transgenic pigs that contain the bovine gene for α-lactalbumin (α-LA) under the Investigational New Animal Drug (INAD) permit number 010-519. The two transgenic pigs that were inadvertently rendered contain the same α-LA gene construct that has FDA rendering approval, but the construct also contains the porcine gene for insulin-like growth factor-I (IGF-I). A request for rendering of the α-LA/IGF-I has subsequently been submitted to the FDA, and approval of the request is anticipated.

The α-LA/IGF-I gene construct is only expressed in the mammary gland and the gene is only expressed during lactation. To prove this, four pigs of varying weights were euthanized and tissue samples were taken for RNA analysis. Three pigs were female and one was male. RNA was analyzed from the following tissues: heart, stomach, spleen, muscle, liver, kidney, intestine and mammary gland. No RNA encoding the IGF-1 transgene was detected in any of the samples from the four pigs, except the lactating mammary gland. Therefore, no gene expression is expected in male pigs. Furthermore, polymerase chain reaction (PCR) analysis and expression data had confirmed that the gene construct used to create the transgenic pigs has the same structure as the integrated transgene. To identify pigs carrying the transgene, PCR is performed on DNA extracted from the right and left ear and tail biopsies, using two different primer sets specific for the gene construct, on each animal.

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Upon recognition of the rendering occurrence, the Principal Investigator (PI) contacted the University Institutional Biosafety Committee, and the University then contacted the FDA, the agency that had issued the INAD for the α-LA construct, to inform them of the event. Subsequently, the FDA visited the University on April 3 through April 6, 2001 to investigate the incident and to learn more about the processes involved in creating and containing transgenic food animals. The FDA audit team has verbally informed the University that they are satisfied with corrective measures taken by the University. The FDA audit team also verbally informed the University that they were satisfied that the incident produced no public risk. Furthermore, as the FDA begins the process of developing regulations and guidance documents for transgenic food animals, they indicated they may call on the University for our input. Also, the FDA did state that they felt it would be appropriate for the University to notify NIH, and the telephone call and this follow-up letter are our notification to you.

The PI has sought approval for producing transgenic pigs in the past and has approval from the University Biological Safety Committee (BSC) to create these transgenic pigs. The BSC has determined that the work involving the insertion of the bovine α-LA into the pig genome is subject to the Guidelines under Section III-D-4-b (*For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-D-1, Experiments Using Human or Animal Pathogens (Risk Group 2, risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector systems), or III-D-4-a, Experiments Involving Whole Animals, the appropriate containment shall be determined by the Institutional Biosafety Committee*), and recommended Animal Biosafety Level 1-N (Appendix Q-II-A) for this research. The work involving the insertion of the porcine IGF-1 into the pig genome is exempt from the NIH Guidelines under Section III-F-4 (*Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species)* and Animal Biosafety Level 1-N was again recommended.

Please be advised that the inadvertent rendering incident was approached from a very serious standpoint by the PI and the University, and corrective actions were immediately taken by both the PI and the University to prevent a recurrence. These completed, and continuing, actions include:

- After discussion with the Institutional Animal Care and Use Committee (IACUC) and the research group, a secondary ear notching system and additional ear tags will be used to increase ability to quickly and accurately identify transgenic pigs.
- Procedures for procurement and transfer of traditional laboratory animals already exists and the Division of Animal Resources (DAR) must be notified of any procurement or transfers. Currently, no similar procedures exist for farm animals, but the issue is being discussed for implementation by DAR.
- The IACUC and DAR continue to make a concerted effort to notify the Division of Environmental Health and Safety (DEHS) of all recombinant DNA (rDNA) and transgenic animal use protocols. DAR has recently modified the IACUC request form to clarify transgenic animal use. Checkboxes for both rDNA and trangenic animal use are included on the form. If either box is marked, the form is sent to DEHS for further review. DEHS sends a request to the investigator to register with the Institutional Biosafety Committee.

- A draft Fact Sheet on Transgenic Animal Research, Transgenic Plant Research, and Recombinant DNA Research was provided to the BSC members at the March 28, 2001 meeting for their review. After approval, these sheets will be distributed to investigators through the DEHS website, during project registration, and upon request.
- Signs specifying requirements for incineration and appropriate procedures have been posted on the walls of the incinerator/rendering cooler.
- The Vice Chancellor for Research, the Vice Chancellor for Administration and Human Resources, the IBC, and the IACUC will make a concerted effort to inform investigators for their responsibility to register research involving transgenic animals; and,
- The IBC and the IACUC will work with investigators to ensure that all research personnel are informed of the proper handling and disposal of transgenic animals.
- A request for rendering of the α-LA/IGF-I has been submitted to the FDA, and approval of the request is anticipated.

Please feel free to contact me by phone (217-244-7801) or e-mail (i-cooke@uiuc.edu) should you have any questions concerning the incident or actions taken by the University.

Respectfully,

Irene Cooke

Irene Cooke, D.V.M., Ph.D.
Director, Division of Environmental Health and Safety

c: Charles Colbert
Van Anderson
Susan Kingston
Melanie Loots
Joseph Thulin
Tony Waldrop
Matthew Wheeler

Lanman, Robert (OD)

From: McDonald, Cheryl (OD)
Sent: Friday, May 18, 2001 7:35 PM
To: Patterson, Amy (OD)
Cc: Fennington, Kelly (OD); Lanman, Robert (OD); Shih, Tom (OD); Shipp, Allan (OD)
Subject: Another Transgenic Pig Disposal Problem

Amy,

Today I received a telephone call from Susan Kingston, DVM the Head of Biological Safety of the Division of Environmental Health and Safety at the University of Illinois. She was reporting an incident that happened in January and has been reported to the institutional IBC and the FDA. The situation is as follows:

A researcher at the Urbana-Champaign campus of U of I has appproval for disposal by rendering of transgenic pigs with the bovine protein alpha-lac-albumin gene which is only expressed in the lactating mammary glands. This investigator also has an INDA for swine with porcine insulin-like growth factor 1 gene and the bovine alpha-lac-albumin genes. But these animals are to be incinerated only. The PI allowed a different investigator to use 2 male pigs (each less than 40 pounds) to be used in a totally different study. This was a feeding study.

At the end of the study the graduate student put them in the cooler for rendering rather than incinerating them. The mistake was recognized approximately 1 week later. The INDA-holding PI was notified; Environmental Health and Safety officials at the university were notified; the IBC was notified; the rendering company was notified; and the FDA was notified. The renderer assured the Dr. Kingston that no products from the rendering would enter the human food or consumable goods chain (e.g. soaps, candles, etc.), rather the by-products were only for animal feeds. The renderer can track were the materials went if necessary, but has not been asked to do so. The FDA inspected/investigated the site on April 3rd, 4th, and 5th, 2001. They(FDA) were satisfied that the appropriate steps had been taken, according to Dr. Kingston.

The dates/events are outlined below. Dr. Kingston will be sending documentation of this information by USPS today. Her contact information is also given below.

Events
January 4, 2001- Pigs put into the rendering cooler rather than incinerated
About 1 week later- Principal Investigator and Environmental Health and Safety Officer notified
January 22, 2001-The Rendering Company was notified.
February 5, 2001-Dr. Woodrow Knight of the CVM/FDA was notified.
April 3rd, 4th, 5th, 2001- Four FDA officials visit site. One official was from Human Food Safety; one was from Biometrics; one from the Division of Compliance; and one was a local FDA Field Officer from the local area.
May 18th, 2001-NIH OBA was notified of the events.

Contact Information
Susan Kingston, DVM
Division of Environmental Health and Safety
University of Illinois
101 South Gregory Street
Urbana, Illinois 61801
Phone 217-244-1939
Fax 217-244-6594

I will get the appropriate paper work in order for a letter to Dr. Kingston and OLAW, etc. I have sent a message to Tom Shih and Bob Lanman for the background information that I can reference.

Cheryl McDonald