Animal Experiment Supervisor		Safety Office		received on	

Animal Experiment Application Form (Synchrotron Radiation Experiment)

Proposal Info.				
Proposal No.	2016A****	Project Leader	Koukido Hanako
Experiment Title	**********	Affiliation	XX Department
		Telephone	***-****-**
Beamline No.	BL****	e-mail	hanako@*********

Date (dd/mm/yy) : _____

To Director, Japan Synchrotron Radiation Research Institute

（Chief Animal Experiment Researcher） [1] Affiliation: XX University, Graduate School

Title: XX Department XX Assistant

Name (Print) Koukido Hanako

Name (Signature)

（Head of Department） [2] Title: XX University, Graduate School

Name (Print) Koukido Tarou

Name (Signature)

[3] Director of SPring-8 Users Office (print)

To carry out the following animal experiment, I hereby apply for approval of the animal committee.

Chief Animal Experiment Researcher contact information	Address(ZIP code) 1-1-1 Koto, Sayo-cho, Sayo-gun, Hyogo, Japan Tel(extension/PHS) 0791-58-**** FAX 0791-58-**** E-mail hanako@*******
Person to act in Chief Animal Experiment Researcher's absence [4]	Affiliation XX University, Graduate School XX Department Researcher Name XXX Saburo Address(ZIP code) 1-1-1 Koto, Sayo-cho, Sayo-gun, Hyogo, Japan Tel(extension/PHS) 0791-58-**** FAX 0791-58-**** E-mail Saburo@********

Chief Animal Experiment Researcher(CAER)／Other Animal Experiment Researchers(AERs)
(All researchers must be registered as an animal experiment researchers) [5)]

Name	Affiliation, Division	The registration number as an animal experiment researcher
CAER Koukido Tarou	XX University, Graduate School	00001**
AERs Saburo Shikakuta	XX University, Graduate School	00002**
Shiro Maruyama	XX University, Graduate School	00003**

Status in your institute [6)]	Has this experiment been approved in your institute? (✓) Yes ( ) No ( ) Submitted ( ) Not a requirement
Purpose of the experiment [7)]	Purpose: As a basic experiment on the radiation of synchrotron light for treatment purposes, brain tumors in rats are irradiated with an X-ray micro beam and we pathologically examine the effect of the radiation. Significance: Micro beam radiation has little impact on healthy cells and selectively exerts lethal effects on cancer cells, which leads to the development of novel cancer treatment with fewer side effects.
Method of the experiment [8)]	(✓)Irradiation ( )Small-angle scattering ( )Angiography ( )CT ( )Imaging ( )Sample collection ( )Others( )
Reasons why animals are needed [9)]	(✓)There is no alternative method of investigation. ( )Sensitivity/accuracy of alternative methods is insufficient ( )Others ( )

Description of the experiment [10)]

Animals to be used			
Species	rat		
Strain	SD (✓)non-transgenic ( )transgenic	SD (✓)non-transgenic ( )transgenic	( )non-transgenic ( )transgenic
Descriptions	Age; 10 weeks Gestation; weeks	Age; 10 weeks Gestation; weeks	Age; weeks Gestation; weeks
Microbiological level	(✓)SPF ( )Germ free ( )Semi-clean ( )Others( )	(✓)SPF ( )Germ free ( )Semi-clean ( )Others( )	( )SPF ( )Germ free ( )Semi-clean ( )Others( )
Number to be used	Female; 40 Male; Number of cages; 20	Female; 10 Male; Number of cages; 5	Female; Male; Number of cages;
Justification of the number to be used (give details of the calculation based on the groups, protocols, statistical power, etc.)	Significant number; 2 (Reason; At least 2 samples are needed to achieve reproducibility in an experiment) Condition number 1; 5 (Content; Condition number of exposure dose. The most important condition in this experiment.) Condition number 2; 4 (Content; Condition number of days before tissue preparation and observation)	Significant number; 1 (Reason; 1 samples because it's a target group and control without tumor induction) Condition number 1; 5 (Content; Condition number of exposure dose. The most important condition in this experiment.) Condition number 2;2 (Content; Condition number of days before tissue preparation and observation)	
Pre-treatment on the animal before shipping [11)]	Location: XX University Treatment: ( )Surgical (✓)Induction of tumor ( )Drug administration Others( )	Location: Treatment: ( )Surgical ( )Induction of tumor ( )Drug administration Others( )	Location: Treatment: ( )Surgical ( )Induction of tumor ( )Drug administration Others( )
Supplier of the animal	CLEA Japan. Temporarily kept in the animal facility of Koto University for treatment.	Japan SLC	
Method of transportation	( )By courier (✓)Carry-in	(✓)By courier ( )Carry-in	( )By courier ( )Carry-in
Delivery location			

What you do to the animal after the experiment [12)]	1)Method of euthanasia (✓)Administration of anesthetics (Chemical name penetobarbital Dose, method: 0.2g infusion with phleboclysis) ( )Destruction of CNS(e.g. cervical dislocation) ( )Other methods( ) 2)Disposal of the corpse (organs/tissues) ( )Bring back to the institute of the applicant and entrust to a disposal contractor ( )Leave in a freezer and request a SPring-8 staff to entrust to a disposal contractor (✓)Others(Live samples are brought back to Koto Univ.) 3)Others(continue observation, used in other experiment, etc.) (Live samples are brought back to Koto Univ. After the fellow-up examination, we perform euthanasia then prepare tissue samples. The samples are observed under a microscope.)
Other safety hazards [13)]	( )none (✓)yes (fill the column below) ( )Use of a transgenic animal(reception number;) (✓)Use of psychotropic drugs (pentobarbital etc.), poison, drastic (✓)Use Pentobarbital stored at SPring-8 ( )Use Midazolam stored at SPring-8 ( )Bring( ) from other institute ( )Others( )

Description of the experiment [Pre-treatment of the animals] [14]		
What you do to the animal	a. Category [15]	( )none ( )Sample collection ( )Surgical operation (✓)Induce tumor ( )Induce disease( ) ( )Drug administration ( )Others( )
	b. Level of pain and distress (SCAW classification) [16]	( )A ( )B (✓)C ( )D ( )E Reason; Transplantation of tumor cells through surgical treatment under anesthesia results in discomfort due to tumor growth.
	c. Location [17]	( )Animal Housing Facility, operation room (✓)Medium-length Beamline Facility Experiment building, operation room ( )Mobile operation house (used at BL) ( )BL Experiment hutch. Optics hutch ( )Others( )
	d. Description of the experiment [18]	I. When animals are treated before carry-in to SPring-8 1. In the animal facility of Koto Univ. Before carry-in to SPring-8, tumor induction is conducted at Koto Univ. Through surgical treatment under anesthesia, we prepare a brain tumor model by implanting tumor cells on the cerebral surface inside the skull. The animals are kept for about a week in the animal facility in Koto Univ. for tumor growth treatment. 2. SPring-8 Animals are carried into the Mouse Room in the Experimental Animal Facility. Only those to be used in the next c.a. 8 hours are transferred to Mobile/Sectional Animal Treatment Room Used at B28B2, where pre-treatment is performed before the radiation experiment. Animals are anesthetized with the anesthesia with hypodermic Nembutal injection into the buttocks. Under anesthesia, they are fixed with a special head-holding device for mouse/rat. After radiation, the animals are released from the device and kept in a special cage or transfer box until they emerge from anesthesia. Then, they are transferred to the Mouse-Rat Room in the Animal Housing Facility. II. When animals are directly carried-in Spring-8 The suppliers directly carry-in the animals into the Mouse Room in the Experimental Animal Facility. Only those to be used in the next c.a. 8 hours are transferred to the Mobile/Sectional Animal Treatment Room Used at BL28B2, where pre-treatment is performed before the radiation experiment. Animals are anesthetized with the anesthesia with hypodermic Nembutal injection into the buttocks. Under anesthesia, they are fixed with a head-holding device for mouse/rat. After radiation, the animals are released from the device and kept in a special cage or transfer box until they emerge from anesthesia. Then, they are transferred to the Mouse-Rat Room in the Animal Housing Facility. Method of restraint (devices, period) Use of special head-holding device for rat/mouse for 1 - 2 hour(s). Prevention of escape Use of special rat guard placed at the entrance. Precautions during transportation Use of special cage or transfer box.
	e. Method of reduction of pain and distress [19]	(✓)Unnecessary because the animal feels little pain ( )Unnecessary because the period retention is short (✓)Use an anesthetic or pain-killer Drug name (pentobarbital) dose, method of administration (40-50 mg/kg, hypodermic injection) ( )No way to reduce pain and/or stress without affecting the scientific purpose (Reason;) ( )No way to avoid retention of animals for a long period (Reason;) ( )Apply a humane endpoint (criteria of decision) ( )Others( )

Description of the experiment [SR experiment] [14]		
SR experiment	a. Category [15]	( )No SR experiment (✓)Irradiation ( )Others( )
	b. Level of pain and distress (SCAW classification) [16]	( )A (✓)B ( )C ( )D ( )E Reason;
	c. Location [17]	( )Animal Housing Facility, operation room ( )Medium-length Beamline Facility Experiment building, operation room ( )Mobile operation house (used at BL) (✓)BL28B2 No.2 Optics Experiment hutch. Optics hutch ( )Others( )
	d. Description of the experiment [18]	1. Animals with tumor Under anesthesia, the animals in the head-holding device are placed on a transfer mechanism. The mechanism moves the animals in order to center the radiation beam spot on the implanted tumor. White synchrotron radiation ranging from 1 to 500 Gy is applied to the tumor part. After radiation, animals are moved to the Mobile/Sectional Animal Treatment Room. 2. Healthy animals Under anesthesia, the animals in the head-holding device are placed on a transfer mechanism. The mechanism moves the animals in order to center the radiation beam spot on the same part of the animals with tumor. White synchrotron radiation ranging from 1 to 500 Gy is applied to the part. After radiation, animals are moved to the Mobile/Sectional Animal Treatment Room. Method of restraint (devices, period) Use of special head-holding device for rat/mouse for c.a. 15 min. Prevention of escape Use of special rat guard placed at the entrance. Precautions during transportation Under anesthesia, the animals in the head-holding device are transported.
	e. Method of reduction of pain and distress [19]	( )Unnecessary because the animal feels little pain ( )Unnecessary because the period retention is short (✓)Use an anesthetic or pain-killer Drug name (pentobarbital) dose, method of administration (40-50 mg/kg, hypodermic injection) ( )No way to reduce pain and/or stress without affecting the scientific purpose (Reason;) ( )No way to avoid retention of animals for a long period (Reason;) ( )Apply a humane endpoint (criteria of decision) ( )Others( )

Description of the experiment [Post-treatment] [14]		
What you do to the animal	a. Category [15]	( )none ( )Sample collection ( )Surgical operation ( )Induce tumor ( )Induce disease( ) ( )Drug administration ( )Others( )
	b. Level of pain and distress (SCAW classification) [16]	( )A ( )B ( )C ( )D ( )E Reason;
	c. Location [17]	( )Animal Housing Facility, operation room ( )Medium-length Beamline Facility Experiment building, operation room ( )Mobile operation house (used at BL) ( )BL Experiment hutch. Optics hutch ( )Others( )
	d. Description of the experiment [18]	Method of restraint (devices, period) Prevention of escape Precautions during transportation
	e. Method of reduction of pain and distress [19]	( )Unnecessary because the animal feels little pain ( )Unnecessary because the period retention is short ( )Use an anesthetic or pain-killer Drug name ( ) dose, method of administration ( ) ( )No way to reduce pain and/or stress without affecting the scientific purpose (Reason;) ( )No way to avoid retention of animals for a long period (Reason;) ( )Apply a humane endpoint (criteria of decision) ( )Others( )

(Notices)

1) Chief Animal Experiment Researcher (CAER) is required to have more than one year's experience in animal experiments and to engage in this experiment at SPring-8. The CAER does not have to be the Project Leader of the SR experiment. Students are not suitable as CAER.
2) Signature of the head of faculty, school or institute of CAER is required.
3) Leave empty.
4) A person to be contacted when CAER is not available.
5) List all researchers who will engage in this animal experiment at SPring-8. All should be registered as animal experiment researchers at SPring-8. For the registration, fill Form17-6 and send it to the Users Office.
6) Specify whether or not this experiment at SPring-8 has been approved by the animal care and use committee in your institute.
7) Describe the scientific aims, benefits and significance of the experiment.
8） Mark the appropriate method.
9) Mark the appropriate reason.
10) "Description of the experiment" should be filled for each species that is subjected to an experiment at SPring-8. For example, if the experiment involves use of mouse, guinea-pig and rat, three sets (each including "Pre-treatment", "SR experiment" and "Post-treatment") of the form are required. The forms should be copied when necessary.
11) Describe the treatment done in your own (or other) institute or in the laboratory of the supplier before shipping the animal to SPring-8. Mention the ethics approval by the institute or laboratory. Details should be described in “Pre-treatment”.
12) Check the appropriate box and fill the necessary details.
13) Fill this when a transgenic animal or a psychotropic drug is used. Use of formalin or other poisonous chemicals for chemical fixation should be described.
14) Describe what you do to the animal in each step of "Pre-treatment", "SR experiment" and "Post-treatment". If a step is not included in this experiment, check "none" in "a. Category" and leave b-e empty.
15) Check the appropriate box.
16) The SCAW categories are defined by "Scientists Center For Animal Welfare" (see below).
17) Describe where the procedure specified by "a. Category" is done.
18) Describe the details of the procedure specified by "a. Category".
19) Check the appropriate box and give required details. Use of an anesthetic for euthanasia should be indicated in "What you do to the animal after the experiment" of "Description of the experiment".

Scientists Center for Animal Welfare (SCAW)

Consensus Recommendations on Effective Institutional Animal Care and Use Committees (Laboratory Animal Science, Special Issue pp.11-13. Jan. 1987)

Category A

Experiments involving either no living materials or use of plants, bacteria, protozoa, or invertebrate animal species. Biochemical, botanical, bacteriological, microbiological, or invertebrate animal studies, tissue cultures, studies on

tissues obtained from autopsy or from slaughterhouse, studies on embryonated eggs. Invertebrate animals have nervous systems and respond to noxious stimuli, and therefore must also be treated humanely.

Category B

Experiments on vertebrate animal species that are expected to produce little or no discomfort. Mere holding of animals captive for experimental purposes; simple procedures such as injections of relatively harmless substances and blood sampling; physical examinations; experiments on completely anesthetized animals which do not regain consciousness; food/water deprivation for short periods (a few hours) ; standard methods of euthanasia that induce rapid unconsciousness, such as anesthetic overdose or decapitation preceded by sedation or light anesthesia.

Category C

Experiments that involve some minor stress or pain (short-duration pain) to vertebrate animal species. Exposure of blood vessels or implantation of chronic catheters with anesthesia; behavioral experiments on awake animals that involve short-term stressful restraint; immunization employing Freund's adjuvant; noxious stimuli from which escape is possible; surgical procedures under anesthesia that may result in some minor post-surgical discomfort. Category C procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.

Category D

Experiments that involve significant but unavoidable stress or pain to vertebrate animal species. Deliberate induction of behavioral stress in order to test its effect ; major surgical procedures under anesthesia that result in significant post-operative discomfort; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible; prolonged periods (up to several hours or more) of physical restraint; maternal deprivation with substitution of punitive surrogates; induction of aggressive behavior leading to self-mutilation or intra-species aggression; procedures that produce pain in which anesthetics are not used, such as toxicity testing with death as an end point; production of radiation sickness, certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold, i.e. the point at which intense emotional reactions occur. Category D experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.

Category E

Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanesthetized, conscious animals. Use of muscle relaxants or paralytic drugs such as succinyl choline or other curariform drugs used alone for surgical restraint without the use of anesthetics; severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior; killing by use of microwave ovens designed for domestic kitchens or by strychnine; inescapably severe stress or terminal stress. Category E experiments are considered highly questionable or unacceptable irrespective of the significance of anticipated results. Many of these procedures are specifically prohibited in national policies and therefore may result in withdrawal of federal funds and/or institutional USDA registration.