

Animal Experiment Supervisor		Safety Office		received on		Permission number	
------------------------------	--	---------------	--	-------------	--	-------------------	--

Animal Experiment Application Form (Synchrotron Radiation Experiment)

Proposal Info.	Proposal No.	2016A****	Project Leader	Koukido Hanako
	Experiment Title	*****	Affiliation	XX Department
	Beamline No.		Telephone	***-****-**
			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) _____

Name (Signature) _____

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 ³⁾	Affiliation <u>XX University, Graduate School XX Department Researcher</u> Name <u>XXX Saburo</u> Address(ZIP code) 1-1-1 Koto, Sayo-cho, Sayo-gun, Hyogo, Japan Tel(extension/PHS) 0791-58-**** FAX 0791-58-**** E-mail <u>Saburo@*****</u>

Description of the experiment 9)

Animals to be used			
Species	rat		
Strain	SD (<input checked="" type="checkbox"/>)non-transgenic ()transgenic	SD (<input checked="" type="checkbox"/>)non-transgenic ()transgenic	()non-transgenic ()transgenic
Descriptions	Age; 10weeks Gestation; weeks	Age; 10weeks Gestation; weeks	Age; weeks Gestation; weeks
Microbiological level	(<input checked="" type="checkbox"/>)SPF ()Germ free ()Semi-clean ()Others()	(<input checked="" type="checkbox"/>)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 (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)	
Supplier of the animal	CLEA Japan. Temporarily kept in the animal facility of Koto University for treatment.	Japan SLC	
Method of transportation	()By courier (<input checked="" type="checkbox"/>)Carry-in	(<input checked="" type="checkbox"/>)By courier ()Carry-in	()By courier ()Carry-in
Delivery location			
What you do to the animal after the experiment ¹⁰⁾	1)Method of euthanasia (<input checked="" type="checkbox"/>)Administration of anesthetics (Chemical name pentobarbital Dose, method: 0.2g infusion with phlebotoclysis) () Pithing under anesthesia (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 (<input checked="" type="checkbox"/>)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 ¹²⁾	()none (<input checked="" type="checkbox"/>)yes (fill the column below) ()Use of a transgenic animal(reception number;) (<input checked="" type="checkbox"/>)Use of psychotropic drugs (pentobarbital etc.), poison, drastic (<input checked="" type="checkbox"/>)Use Pentobarbital stored at SPring-8 ()Use Midazolam stored at SPring-8 ()Bring() from other institute ()Others()		

Description of the experiment [SR experiment]		
SR experiment	a. Category ¹⁴⁾	<input type="checkbox"/> No SR experiment <input checked="" type="checkbox"/> Irradiation <input type="checkbox"/> Others()
	b. Level of pain and distress (SCAW classification) ¹⁵⁾	<input type="checkbox"/> A <input checked="" type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D <input type="checkbox"/> E Reason;
	c. Location ¹⁶⁾	<input type="checkbox"/> Animal Housing Facility, operation room <input type="checkbox"/> Medium-length Beamline Facility Experiment building, operation room <input type="checkbox"/> Mobile operation house (used at BL) <input checked="" type="checkbox"/> BL 28B2 No.2 Optics Experiment hutch. Optics hutch <input type="checkbox"/> Others()
	d. Description of the experiment ¹⁷⁾	<p>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.</p> <p>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.</p> <p>Method of restraint (devices, period) Use of special head-holding device for rat/mouse for c.a. 15 min.</p> <p>Prevention of escape Use of special rat guard placed at the entrance.</p> <p>Precautions during transportation Under anesthesia, the animals in the head-holding device are transported.</p>
	e. Method of reduction of pain and distress ¹⁸⁾	<input type="checkbox"/> Unnecessary because the animal feels little pain <input type="checkbox"/> Unnecessary because the period retention is short <input checked="" type="checkbox"/> Use an anesthetic or pain-killer Drug name (Pentobarbital) dose, method of administration (40-50 mg/kg, hypodermic injection) <input type="checkbox"/> No way to reduce pain and/or stress without affecting the scientific purpose (Reason;) <input type="checkbox"/> No way to avoid retention of animals for a long period (Reason;) <input type="checkbox"/> Apply a humane endpoint (criteria of decision) <input type="checkbox"/> 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) A person to be contacted when CAER is not available.
- 4) 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.
- 5) Specify whether or not this experiment at SPring-8 has been approved by the animal care and use committee in your institute.
- 6) Describe the scientific aims, benefits and significance of the experiment.
- 7) Mark the appropriate method.
- 8) Mark the appropriate reason.
- 9) "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.
- 10) 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". Specify the location of treatment and describe details in "Treatment of laboratory animals".
- 11) Check the appropriate box and fill the necessary details.
- 12) 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.
- 13) In "Description of the experiment," fill in all fields from "a. Category" to "e. Method of reduction of pain and distress" for each step from "Pretreatment", "Treatment of laboratory animals at SPring-8", and "SR experiment", to "Post-treatment". For steps not applicable, check "none" in "a. Category" and ignore the fields from "b. Level of pain and distress" to "e. Method of reduction of pain and distress".
- 14) Check the appropriate box.
- 15) The SCAW categories are defined by "Scientists Center For Animal Welfare" (see below).
- 16) Describe where the procedure specified by "a. Category" is done.
- 17) Describe the details of the procedure specified by "a. Category".
- 18) Check the appropriate box and give required details. Method of 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.