INSTITUT NATIONAL DE LA
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TENDER TECHNICAL SPECIFICATIONS

GMO90+ project:

Six-month genetically modified maize feeding in rats, experimental study

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE

CENTRE DE TOULOUSE

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1 – GÉNÉRAL POINTS

1.1 Background

INRA in collaboration with INSERM and ANSES aims at developing an experimental design on the potential effect of GMO feeding in rats.

The present program considers that tests samples and analysis performed in the current rodent 90-days (90d) study according to OECD and EFSA guidelines may be able to evolve by exploiting the most advanced concepts and technologies, in order to optimize their predictive character. These changes include taking into account advances in biological and physiological testing and systems analysis in recent years. Based on these advances, the program aims at identifying early biomarkers of toxicity to improve the predictability of the rodent 90d studies applied to Genetically Modified Plants (GMP).

For this purpose, the program will conduct a **rat study during six months on groups of animals fed two GM maizes compared to groups of negative control animals** (animals fed with genetically close non-GMO maize). The animals will be exposed for a period of 6 months to MON 810 (Bt resistance) or NK603 (glyphosate resistance) treated or not by the glyphosate herbicide. The followed approach, namely the exploration of new parameters for monitoring animals will be to identify variations between groups. Meanwhile, the animal trial will have to be as close as possible to subchronic oral toxicity rodent: 90 days study in rats according to OECD guideline 408 and EFSA guidance on conducting repeated dose 90 days oral toxicity study in rodents on whole food/feed.

1.2 Indicative procedure timetable

The beginning of experiment wanted is april 2015 and the experiment will last 6 months for each rat. The contract period is one year or more if necessary as agreed between the parties.

1.3 Accordance with legislation

1.3.1 Animal welfare and ethics

The study will be conducted in accordance with the EU Directive 2010/63/EU of the European Parliament and the Council of 22nd September 2010 on the protection of animals used for scientific purposes as transposed in national law and approved in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.
The tenderer will be in charge of ethical approach in line with his country and European legislations.

1.3.2 Test facilities, human resources, time scheduling

The tenderer should be qualified ISO 9001. The present study does not need to be a GLP study but a quality insurance system close to the GLP recommendation will be strongly appreciated.

1.4 Project Resources

The tenderer will provide animal, material and human resources needed to conduct this project.

The consortium is an organization created for this research program regrouping several national French institutes and universities and private partners. INRA will be the mediator between the consortium and the performer.
This program is a 3-years project supported by the French Ministry of Ecology, Sustainable Development and Energy. It is coordinated by Bernard Salles (INRA), who will be the scientific referent for the performer.
The consortium's laboratories will proceed to a part of sample and data analysis and the other part will be borne by the performer.
During the experimental phase, the consortium will provide animal food and specific training for some organ sampling.

1.5 Tenderer's tasks

When tenderer applies, these elements have to be described:

- Test facilities will have to be identified and localized. Precisions will be provided concerning health status, veterinary agreement of the animal facility.
- Individual identification system of rats
- Precise localization and conditions of animals housing (number of rooms, health status, temperature, pressure relative humidity, light/dark cycle, recording, type of cages,…). Precision on cleaning procedures of the rooms of the animal facility as well as housing and feeding materials will be provided.
- Receipt and acclimatization conditions. In particular, time of acclimatization, housing conditions, health status evaluation, weighing will be provided.
- Provide the methodology to record feed and water consumption for 6 months
- The frequency and the type of observations, as well as the procedure in case of clinical signs, moribund or dead animals (isolation, observation frequency, necropsy…).
- The frequency and the type of observations, as well as the procedure in case of deviations from normal will have to be provided for physical and functional examination of rats
- Sample collection and tissue processing (number of rats per day, euthanasia process, type of sampling, animal transport, weighing, sample storage,…). In particular, euthanasia procedure will have to take into account specific samplings such as blood sampling for hormonal studies.
- personal disposition for conducting the sampling: how to proceed, number of rats per day, randomization procedure, tagging…
- analysis processing for hematology, biochemical analysis and histology (required volumes, duration, equipment)
- Procedure to make sure that analysis will be in a double blind manner and sampling traceability
- Steps taken to minimize variability, to optimize quality
- Training capacity for acquiring new skills
- A detailed experimental plan with deadlines and number of person involved will be provided
- Study preparation and reporting plans will be provided (form, number, costs)
- A detailed estimate of costs for at least 10 rats/sex/diet and the total.

- The tenderer, as an advisor, will propose solutions to save money and time or to improve the experimental design.

During experimental phase, the tenderer will:
- Provide animal management and sampling and a part of analysis
- Note all events that are not in line with the present procedures
- Send samples of diets to the analytical laboratories contracted for the analysis of the composition after T90 and T180.
- Send samples immediately after sampling at T0, T90, T135 and T 180
- Do a preliminary report 3 months after the beginning of the experiment

After experimental phase, the tenderer will:
- Provide a part of sample analysis
- White a final report
- Send raw data and historical control data to the consortium

1.6 Scope of the service

Laboratories composing the consortium will analyze blood and urine samples, some organs and other data. Samples and data will be prepared as asked by them. These samples will be sent to laboratories from the consortium (see Annexe 2 for details, contact's details will be given at the beginning of the study).
INRA is in charge of this program and the scientific referent is B Salles assisted by B Broux. Slight changes may appear in the protocol by the beginning of experiment.

2. TECHNICAL SPECIFICATIONS

2.1 ANIMAL MANAGEMENT

2.1.1 Species and strain

In order to compare results of the present program with European programs GRACE and G-TwYST, we will use rats Wistar Rcc Han /Specific Pathogen Free (SPF). It will be interesting in the process of interaction between the programs that the rats are provided by HARLAN .

2.1.2 Number of animals

Based on the number of parameters analyzed and statistical requirements, the number of animals has been set to at least 480 divided into 8 groups including 30 males and 30 females. Ten males and 10 females will be sacrificed 90 days after the beginning of treatment (T90), and samples will be taken at this time (group 1). Ten males and 10 females will be sacrificed at T180 and samples will be taken at T0, T90, T135 and T180 (group 2a). The remaining 10 males and 10 females will be sacrificed at T180 but sampling will occur at this time only (group 2b). Females will be nulliparous and non-pregnant. A sentinel survey will have to be planned and explained.

2.1.3 Approximate weight and age

Upon arrival, the animals will weigh between 100-120g and will be 5 weeks old. The animals will be 6 weeks old at the start of the study and will weigh between 110-140g. Ideally, they should be born the same day ± 2 days and be of uniform weight (± 20% of the mean). Due to the experimental time required for urine, blood and tissues sampling, all the rats cannot start at the same T0.

2.1.4 Identification

Within the frame of treatment groups, each rat will be individually marked by appropriate system, ideally by microchip implant tagging.

2.1.5 Animal housing

It is required to house 2 rats per cage and to use separate rooms for male and female.
In order to avoid mistakes, we ask that cages of the same treatment groups will be clustered in vertically arranged groups, which will be rotated on a regular basis (once per week). Each vertical row of cages (within the same dose group) will be rotated from top to bottom. Racks will be rotated clockwise every two weeks within the original room configuration. Only one experimenter, always the same, will be involved in that process.

2.1.6 Rodent feeding

The production and feed formulation will be done in February-march 2015 and provided to the test facility. The diet for the 6 months experimental time will be provided and should be stored at +4°C. A storage at -20°C will be appreciated.
During acclimatization, all rats will be fed with a control diet without GMO provided by the tenderer.
After acclimatization period (one week), eight diets (pelleted-diets) will be compared, integrating in their composition kernels from MON810 and its control, kernels from NK603, NK603 with glyphosate and NK603 control: 8 diets (*Figure 1*).

Figure 1: Group of rats fed with 8 different diets

	Dose (% w/w feed)					No of animals	
group	non GM isogenic 1	MON810	non GM isogenic 2	NK603	NK603+ glyphosate	male	female
1	33	0	0	0	0	30	30
2	22	11	0	0	0	30	30
3	0	33	0	0	0	30	30
4	0	0	33	0	0	30	30
5	0	0	22	11	0	30	30
6	0	0	0	33	0	30	30
7	0	0	22	0	11	30	30
8	0	0	0	0	33	30	30
Total						**240**	**240**

2.2 EXPERIMENTAL DESIGN

see *Figure 2*

2.2.1 Human resources

Human resources involved in the project will have to be namely identified as well as their function in the study.
Une des personnes du consortium réalisera une visite à chaque étape critique du protocole, avant et pendant son exécution.

An external auditor from the consortium will be involved in each critical phase before and during execution.

2.2.2 Animal receipt and acclimatization

After arrival in experimental facility rats will become acclimatizated to their new conditions of life. Food (GMO free) and water will be supplied *ad libitum*. A sample of diet will be sent to the analytical laboratories contracted for the analysis of the composition.

2.2.3 Group allocation

Prior to the start of treatment on study day T0, parameters of the detailed examination of all animals will have to be provided. Rats should be randomly allocated to experimental groups. Before the sampling procedure, mean weight differences between each group (same gender) should be less than 10%.

2.2.4 Animal treatments: diets

After group allocation, each group will be fed with one of the eight diets. Food and water will be supplied *ad libitum*.
Diets are coded in a “double blind” manner by the diet-producing company. Samples of the diets are coded with different codes than the diets themselves. The coding scheme is shared with the study monitor and Bernard Salles (the company’s contact within the GMO90+ consortium). It is to be kept confidential and therefore not to be distributed further among. Diets will be encoded from 1 to 8 by the food supplier.
A detailed table will be provided to explain general experimental design (blind identification of group, reference diet, GM, non GM, conventional, number of male and female animals per group). Samples of diets will be sent to the analytical laboratories contracted for the analysis of the composition after 3 and 6 months experimental time.

2.3 MEASUREMENTS AND SAMPLING

2.3.1 Health status observations

Rats’ health status will be checked everyday. It includes:
- changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions as well as activity level and change in behavior.
- morbidity and mortality
- clinical signs
- functional assessment

Moreover, feed and water consumption will have to be recorded weekly per each cage (2 rats) for the treatment period (6 months).

Each animal will have to be weighed at the following times: 1) 48 hours after arrival, 2) on the first day of feeding, 3) weekly during the study period, 4) at the termination of the study, 5) in the event of an early death or sacrifice in extremis.

2.3.2 Procedures for sample collection

Sampling will take place at T0, T90, T135 and T180 of the treatment period (see Figure 2).
Sampling procedure will be performed during the shortest period of time. Rats will be fasted 3-4 h before sampling procedure or euthanasia.
Samples will include blood, urine and organs and analysis will include hematology, blood and urine chemistry, omics, histology and pathology. Each type of sample should be taken at the same time during the day for every sampling day.

- Blood samples will be divided for haematology, clinical chemistry and omics.
- Urine samples will be collected for omics
- Tissues and organs will be removed and evaluated by histology and omics.

From each group of 30 rats per sex: 10 will be euthanized at T90 (group 1). The 20 other rats will constitute the group 2 subdivided into 2a and 2b, each of 10 rats/sex.

Sampling will be as follows:
Group 1 (10 rats/sex/diet): at T90: blood, urine, histology and organs collection
Group 2a (10 rats/sex/diet): at T0, T90, T135: blood and urine and at T180: blood, urine, histology and organs collection
Group 2b (10 rats/sex/diet): at d180: blood, urine, histology and organs collection

2.3.2.1 Blood

Blood samples will be collected for omics and hormonal assays for the consortium and for the tenderer in charge of hematology and clinical biochemistry.
For the consortium: at least 1 ml at T0 (or 1,5 ml at T90 and T135 and 5 ml during the euthanasia process) will be collected in heparinized vials which will be centrifuged at 3000 g at 4°C for 15 min.
For each animal, after collecting blood in one heparinized vial, samples will be kept not more than 15 min at 4°C before starting centrifugation to separate red cells from plasma. Plasma will be aliquoted in 500 µL eppendorff tubes under 100 µL volume each plus the remaining plasma in the last tube.
Blood will also be collected for hematology and clinical biochemistry under the minimal volume.
Aliquots will be kept at -80°C and sent to INSERM U1124, U1149 at Paris, to Profilomic at Gif-sur-Yvette and to INSERM-IRSET U1085 at Rennes in dry ice (Annexe 2).

2.3.2.2 Urine

Urine samples will be collected for hormonal assays, omics, and biochemistry.

Twenty-four hour urines will be collected for animals of group 2a. After urine collection, samples will be conditioned in Falcon type tubes of appropriate size, under 1 ml volume and stored at -80°C. For transportation to consortium's laboratory Axiom (Toulouse), the Laberca laboratory (Rennes) and INSERM U1149 (Paris), samples will be packed in dry ice (Annexe 2).

2.3.2.3 Organs for Omics and histology

All organs listed in Annexe 1 (Gross necropsy) will be systematically taken, weighed and prepared for conservation. Organs will be taken up (after pathology and histology processes from the CRO) by one laboratory of the consortium (Annexe 2).

- **Liver** must be taken first. It will be excised and weighed. The right lateral lobe will be collected, cut in 3 equivalent pieces, 2 of them immediately snap frozen in liquid nitrogen, the 3rd being processed for histology examination by the tenderer. The tissues will be preserved in the fixative medium (neutral buffered 10% formalin) for histopathological examination for gross lesions.
  Four fragments of 50-100 mg each will be dissected from the center of the liver large lobe, placed in individual 1.5 or 2 mL eppendorf tubes (or equivalent), snap-frozen in liquid nitrogen and stored at -80°C until shipment on dry ice to consortium's laboratory INSERM 1124 unit. Liver samples (50-100 mg fragment) for RNA extraction will be treated in high priority, within 2-5 min following euthanasia, to avoid RNA degradation.
- Then, **both kidneys** will be dissected and weighed. The right kidney will be cut transversally (cross-section) in 2 pieces of ~150-250 mg for the upper part and the rest for the lower part. The upper section will be cut transversally in 2 equivalent pieces and all 3 samples (lower part + 2 sections of the upper part) will be identified and placed in individual 1.5 or 2 mL tubes, snap frozen in liquid nitrogen and stored at -80°C until shipment on dry ice to INSERM 1124 unit. Histology will be done on one half of the left kidney. The other half will be snap frozen in liquid nitrogen and stored at -80°C until shipment on dry ice to INSERM 1149 unit. Kidney samples will be stored in appropriate vials.
  The dissection of kidneys will be done by the same person to ensure consistency and reproducibility. Kidney samples for RNA extraction will be treated with high priority, within 3-5 min following euthanasia to avoid RNA degradation.

2.3.2.4 Organs for histology examination only

- **Gut** should be rapidly taken after death. A special training will be done by a consortium member to take these samples.
  Two kinds of samples are needed and sent to consortium's laboratory INRA Toxalim unit (Toulouse):
  - samples of jejunum and colon (1 cm, rinsed with saline), to be frozen immediately after sacrifice. They will be used for western blot.
  - other samples of jejunum and colon (2cm, rinsed with saline) will be treated as follows :
    - Fixation with buffered paraformaldehyde 4%, at 4°C for 4 to 6 hours according to the size of the sample

- Impregnation, 1 h then one night at 4°C in a solution of sucrose 30%. The sample should sink at the bottom of the tube, indicating good impregnation
- Freezing:
    - Fill the tin with Neg50 medium
    - Place the sample inside the tin and give it a good orientation for future slicing
    - Put in contact the tin with nitrogen-frozen isopentane, without immersion
    - When the medium turns white, immerse the tin in isopentane
    - Freeze at -80°C

A sample of each part of the gut (duodenum, jejunum, ileum, and colon) will be preserved in neutral buffered 10% formalin and will be examined for histopathological evaluation.

This will be done by the same person to ensure consistency and reproducibility.
Testis and ovaries: a special training will be done by a consortium member to take these samples. The right one will be fixed in Bouin's fluid for 2h and prepared in paraffin. The left one will be frozen at -80°C. This will be done by the same person to ensure consistency and reproducibility.
Epididymides: taken and rapidly frozen at -80°C. A special training will be done by a consortium member to take these samples. This will be done by the same person to ensure consistency and reproducibility.

- **The following organs will be prepared for histology only, analysis done by the tenderer:** remaining parts of liver, left kidney, stomach, adrenals and pancreas.

  The following organs will be prepared for conservation: some parts fixed in formalin and the remain frozen at -80°C: remaining parts of liver, heart, lungs, spleen, pancreas, uterus, vagina, urinary bladder, stomach, remaining parts of gut, adrenals, thymus, brain, thyroid gland and parathyroid glands, sternum with bone marrow.

Figure 2 : experimental design

régime *diet*	variété non OGM 1 (MON 810) *non GMO variety 1*	MON 810	variété non OGM 2 (NK 603) *non GMO variety 2*	NK 603	NK 603 + glyphosate	groupe *group*	nb rats(M+F) *rat number*		sevrage *weaning*		T0	T90	T135	T180
								âge des rats *rat's age*	21j *21d*					
1	33%					1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
2	22%	11%				1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
3		33%				1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
4			33%			1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
5			22%	11%		1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
6				33%		1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
7			22%		11%	1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
8					33%	1	20		acclimatation *acclimatization*			sang*, urine, sacrifice		
						2a	20				sang*, urine	sang*, urine	sang*, urine	sang*, urine, sacrifice
						2b	20							sang*, urine, sacrifice
						tous *all rats*			acclimatation *acclimatization*		Suivi hebdomadaire : poids vif, consommation alimentaire et eau *weekly: live weight, feed and water consumption*			
											Suivi quotidien : état santé *daily: health status*			

régime témoin, sans OGM *control diet without GMO*

traitement : régimes maïs OGM / non OGM *treatment: GMO maize / non GMO maize*

prélèvements *sampling*

Les Temps (T0, T90, T135 et T 180) sont le nombre de jour par rapport à l'introduction des régimes expérimentaux.
Times (T0, T90, T135 and T 180) are in number of days after the beginning of treatment.
*"sang" is blood

2.4 RÉCEPTION

All the samples will be sent according to the table in Annexe 2. Biological samples will be sent under dry ice and all aliquots will not be sent at the same time.
The raw data will be given using the data entry form produced by the statistical work package of the project.
A report at 3 months will be written and the final report is expected before 10 months (from T0).

2.5 GUARANTEED RESULTS

Guaranties on results

In application of the initial offer to make as stated in article 1.5, the tenderer must describe in the offer guaranties to obtain data and samples for at least 10 rats per sex per diet in the conditions previously described and according to the experimental plan (Figure 2).

OPTION 1:

In order to obtain 10 samples per experimental condition the tenderer may propose 12 rats/sex/diet for the sampling at 6 months and in such case mention it the cost in option 1.

In case of wrong results, tenderer has to begin again experimentations on their own expenses.

Annexe 1: Analysis to be performed by the tenderer

Animal health status

changes in skin – fur – eyes - mucous membranes - ococcurrence of secretions and excretions - activity level -change in behavior- morbidity and mortality - clinical signs - functional assessment - feed and water consumption – live weight

Hematology

Erythrocyte Count (RBC) - Red blood cell Distribution Width (RDW) - Haematocrit (HT) - Haemoglobin (Hb) - Mean Corpuscular Haemoglobin (MCH) - Mean Corpuscular Haemoglobin Concentration (MCHC) - Mean Cell Volume (MCV)- Reticulocyte count - Leukocyte Count (WBC) - Differential Leukocyte Count - Platelet Count (PLT) - Activated Partial Thromboplastin Time - Prothrombin Time (PT) from citrate-treated plasma.

Clinical biochemistry (blood samples)

Parameters will include total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), gamma glutamyl transferase, glutamate deshydrogenase, bile acids, creatinine (CREA), urea, fasting blood glucose, total bilirubin (TBIL), total cholesterol, triglycerides, ions (Na, K, Ca, Cl, PO43-) - inhibin B.

Urine parameters

Urine volume (24h) – urine appearance - hematuria – osmolarity or conductivity – pH – glucose – urine glucose volume – specific gravity.

Pathology

Organs and tissues preserved in neutral buffered 10% formalin for histopathological evaluation. Complete microscopic examination of the organs listed in the paragraph 2.3.2.4. will be performed in accordance with the OECD TG 408 on all the animals from each group.

Gross necropsy

A complete necropsy will be performed on all animals at study termination on T 90 and T 180. The weight of organs will be recorded in line with OECD guideline 408 and organs/tissues will be examined macroscopically for any deviations from normal (in accordance with ŠPP / TOX / V005). A specific training for some organ sampling (epididymides, ovaries, testes, seminal vesicles, prostate, gut) will be provided by members of the consortium.

The weight of each animal before euthanasia will be recorded. The wet weight of the following organs will be recorded:

liver, heart, lungs, kidney, spleen, pancreas, testes, uterus, ovary, vagina, urinary bladder, epididymides, stomach, gut, adrenals, thymus, brain, thyroid gland and parathyroid glands, sternum with bone marrow.

Anterior part of the prostate and seminal vesicles will be weighted and this practice will be taught (if necessary) by the INSERM group from Rennes.
Histological evaluation of tissue specimens will be done on each animal.

Additional tissues may need to be investigated based on clinical or any other findings.
Also any organs/tissues that are likely to be considered as target organs based on the known toxicological properties of the test material should be preserved.
Parts of specific organs will be snap frozen in liquid nitrogen in order to allow additional examinations.

Histopathology

Organs and tissues preserved in neutral buffered 10% formalin will be examined for histopathological evaluation. Complete microscopic examination of the tissues listed below will be performed on all the animals from each group in accordance with the OECD TG 408:
liver, kidney, testis, ovary, stomach, gut (duodenum, jejunum, ileum, colon), adrenals, pancreas

Data analysis

The statistical analysis will be done by the statistical team (WP3 and WP5).
As a first step, the data will be screened for any obvious errors and outliers. Outliers will be checked against the original paper records. Outliers which are not due to transcription or other obvious types of error will be retained, but noted.
The clinical data and the corresponding annotations will be inserted using the data entry form produced by the statistical work package of the project.
Historical data from previous control group will be provided in order to check that control groups samples in the present experiment are in the normal range of data.

Annexe 2: Sample destination (2 pages)

échantillon	catégorie de l'échantillon	type d'échantillon	moment du prélèvement	analyse	groupes de rats	nombre de rats	nombre d'échantillons envoyés	volume minimum requis/rat	conditions envoi	moment de l'envoi	destination
sample	sample category	sample type	sampling time	analysis	rats group	number of rats	number of samples	minimum volume/rat	sending conditions	time of sending	destination/recipient
plasma	blood biochemistry	raw data	T0, T90, T135, T180	CRO	all	480	960		e-mail	after T180	Bernard Salles, INRA T
plasma	omics	tubes	T0, T90, T135, T180	Profilomic	2a	160	640	0.1 mL	dry ice	after each sampling	Profilomic, Gif-sur-Yve
plasma	hormonal assays	tubes	T90, T180	INSERM IRSE	1;2a	320	960	0.3 mL	dry ice	after T180	INSERM IRSET U1085, R
plasma	blood biochemistry	tubes	T90, T180	Inserm U114	1;2a	320	320	0.1 mL	dry ice	after T180	Inserm U1149, Paris
plasma	reserve	tubes	T0, T90, T135, T180		all	480	X		dry ice	after T180	Inserm U1124, Paris
blood	haematology	raw data	T0, T90, T135, T180	CRO	all	480	960		e-mail	after T180	Bernard Salles, INRA T
rat individual data		raw data	from reception to sacrifice	CRO	all	480	480		e-mail	after each sampling	Bernard Salles, INRA T
feed & water consur	feeding	raw data	from reception to sacrifice	CRO	all	480	X		e-mail	after T180	Bernard Salles, INRA T
rats growth	growth	raw data	from reception to sacrifice	CRO	all	480	X		e-mail	after each sampling	Bernard Salles, INRA T
organs weight	growth	raw data	T90, T180	CRO	all	480	X		e-mail	after each sampling	Bernard Salles, INRA T
liver sections	histopathology	slides/pictur	T90, T180	CRO	all	480	X			after T180	Bernard Salles, INRA T
liver samples	omics	tubes	T90, T180	Inserm U112	1;2a	320	960	50-100 mg*3	dry ice	after each sampling	Inserm U1124, Paris
liver samples (edge	reserve	tubes	T90, T180		1;2a	320	X		dry ice	after T180	Inserm U1124, Paris
intestine sections	histopathology	slides/pictur	T90, T180			480	X			after T180	INRA Toxalim, Toulous
colon samples	biochemistry	tubes	T90, T180		1;2a	320	640		dry ice	after each sampling	INRA Toxalim, Toulous
jejunum samples	biochemistry	tubes	T90, T180		1;2a	320	640		dry ice	after each sampling	INRA Toxalim, Toulous
upper quarter of the	omics	tubes	T90, T180	Inserm U112	1;2a	320	320	50-100 mg	dry ice	after each sampling	Inserm U1124, Paris
other upper quarter	reserve	tubes	T180		2b	160	160		dry ice	after T180	Inserm U1124, Paris
other 3 quarters of t	reserve	tubes	T90, T180		all	480	1440		dry ice	after T180	Inserm U1124, Paris
half left kidney	histopathology	slides/pictur	T90, T180	CRO/Inserm	1;2a	320	X			after T180	Inserm U1149, Paris Bernard Salles, INRA Toxalim, Toulouse
half left kidney	reserve	slides/pictur	T180		2b	160	X			after T180	Bernard Salles, INRA T
other half left kidne	biochemistry	tubes	T90, T180	Inserm U1149, Paris	1;2a	320	320		dry ice	after T180	Inserm U1149, Paris
other half left kidne	reserve	tubes	T180		2b	160	160		dry ice	after T180	Bernard Salles, INRA T
right ovary	histopathology	slides/pictur	T90, T180	CRO/IRSET-I	all	240	X			after T180	IRSET-INSERM U1085, Rennes Bernard Salles, INRA Toxalim, Toulouse
left ovary	hormonal assays	tubes	T90, T180	IRSET-INSER	1;2a	160	160		dry ice	after T180	IRSET-INSERM U1085, F
left ovary	reserve	tubes	T180		2b	80	80		dry ice	after T180	Bernard Salles, INRA T
right testis	histopathology	slides/pictur	T90, T180	CRO/IRSET-I	all	240	X			after T180	IRSET-INSERM U1085, Rennes Bernard Salles, INRA Toxalim, Toulouse
left testis	hormonal assays	tubes	T90, T180	IRSET-INSER	1;2a	160	160		dry ice	after T180	IRSET-INSERM U1085, F
left testis	reserve	tubes	T180		2b	80	80		dry ice	after T180	Bernard Salles, INRA T
epididymides		tubes	T90, T180	IRSET-INSER	1;2a	160	320		dry ice	after T180	IRSET-INSERM U1085, F
epididymides	reserve	tubes	T180		2b	80	160		dry ice	after T180	Bernard Salles, INRA T

échantillon	catégorie de l'échantillon	type d'échantillon	moment du prélèvement	analyse	groupes de rats	nombre de rats	nombre d'échantillons envoyés	volume minimum requis/rat	conditions envoi	moment de l'envoi	destination
sample	sample category	sample type	sampling time	analysis	rats group	number of rats	number of samples	minimum volume/rat	sending conditions	time of sending	destination/recipient
heart	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
spleen	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
pancreas	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
uterus	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
vagina	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
urinary bladder	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
stomach	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
gut	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
adrenals	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
thymus	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
brain	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
thyroid gland	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
parathyroid glands	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
sternum with bone r	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
one lung	reserve histopathology		T90, T180		all	480	X				Bernard Salles, INRA T
heart	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
the other lung	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
spleen	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
pancreas	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
uterus	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
vagina	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
urinary bladder	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
stomach	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
gut	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
adrenals	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
thymus	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
brain	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
thyroid gland	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
parathyroid glands	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
sternum with bone r	reserve frozen		T90, T180		all	480	X				Bernard Salles, INRA T
urine	urine biochemistry	raw data	T0, T90, T135, T180	CRO	all	480	X		e-mail	after each sampling	Bernard Salles, INRA T
urine	omics	tubes	T0, T90, T135, T180	INRA Toxalin	2a	160	640	1 mL	dry ice	after each sampling	INRA Toxalim AXIOM,
urine	urine biochemistry	tubes	T90, T180	Inserm U1149, Paris	1;2a	320	640	0.5 mL	dry ice	after T180	Inserm U1149, Paris
urine	reserve	tubes	T0, T90, T135, T180		all	480	X		dry ice	after T180	Bernard Salles, INRA T
urine	hormonal assays	tubes	T0, T90, T180	LABERCA, Nantes	2a	160	480	1 mL	dry ice	after T180	LABERCA, Nantes
hematuria	urine biochemistry	raw data		Inserm U1149, Paris	1;2a	320	320	0.02 mL	e-mail	after T180	Inserm U1149, Paris
hematuria	urine biochemistry	raw data	T180	CRO	2b	160	160	0.02 mL	e-mail	after T180	Inserm U1149, Paris